[Frontiers in Bioscience 17, 65-89, January 1, 2012]

Differentiating human stem cells into neurons and glial cells for neural repair

Vimal Selvaraj[1], Peng Jiang[2], Olga Chechneva[2], U-Ging Lo[2], Wenbin Deng[2,3]

[1]Department of Animal Science, Cornell University, Ithaca, NY 14853, [2]Department of Cell Biology and Human Anatomy, University of California at Davis, Sacramento, CA 95817, [3]Institute of Pediatric Regenerative Medicine, Shriners Hospitals for Children, Sacramento, CA 95817

TABLE OF CONTENTS

1. ABSTRACT

Research on the biology of adult stem cells, embryonic stem cells and induced pluripotent stem cells, as well as cell-based strategies for treating nervous system disorders has begun to create the hope that these cells may be used for therapy in humans after injury or disease. In animal models of neurological diseases, transplantation of stem cells or their derivatives can improve function not only due to direct replacement of lost neurons or glia, but also by providing trophic support. Despite intense research efforts to translate these studies from the bench to bedside, critical problems remain at several steps in this process. Recent technological advancements in both the derivation of stem cells and their directed differentiation to lineage-committed progenitors have brought us closer to therapeutic applications. Several preclinical studies have already explored the behavior of transplanted cells with respect to proliferation, migration, differentiation and survival, especially in complex pathological disease environments. In this review, we examine the current status, progress, pitfalls, and potential of these stem cell technologies, focusing on directed differentiation of human stem cells into various neural lineages, including dopaminergic neurons, motor neurons, oligodendroglia, microglia, and astroglia, and on advancements in cell-based regenerative strategies for neural repair and criteria for successful therapeutic applications.

2. INTRODUCTION

In the adult mammalian central nervous system (CNS), there remains only an insignificant capability for regeneration. Therefore, most CNS injuries and diseases result in permanent damage and loss of function. During embryonic development, CNS progenitors proliferate and acquire regional identities, progressively losing their differentiation repertoire (1). This process is known to happen in response to local stimuli within the developing brain, generating various classes of specialized cells. These CNS progenitors or neural stem cells (NSCs) have the ability to self-renew when cultured *in vitro* and generate different classes of neurons, oligodendrocytes and astrocytes. *In vivo*, the developmental potential of NSCs is known to progressively change in response to complex environmental cues that allow the generation of different cell types during the course of development (2).

In the adult brain, NSCs persist in two neurogenic niches, the subventricular zone (SVZ) and the hippocampal dentate gyrus (DG) (1). Similar to embryonic NSCs, SVZ and DG cells can self-renew and generate neurons, oligodendrocytes and astrocytes in culture. The lineage of these adult NSCs can be traced back to the so-called radial glial cells that are NSCs critical for neocortical development (3-5). In these niches, SVZ and DG cells asymmetrically self-renew and contribute to a

heterogeneous differentiating population of cells that give rise to neurons in the olfactory bulb and hippocampus, respectively (6-8). NSCs in the SVZ can also generate oligodendrocyte progenitor cells (OPCs), most of which migrate into the neighboring corpus callosum to form myelinating oligodendrocytes (9-11). Factors surrounding the maintenance and self-renewal of these NSCs in the neurogenic niche *in vivo* are not completely understood (2). Moreover, these NSCs are only minimally responsive to injury with limited potential for ectopic migration to injured sites. Therefore, SVZ and DG cells do not significantly contribute to regeneration and recovery of the injured brain. This could be due to the fact that cells originating from adult NSCs encounter a very different environment compared to embryonic development with the added factor of a pathological milieu in conditions of CNS injury. In contrast to the brain, there is no evidence for the presence of neural progenitors in the adult spinal cord, implying that there could be no or little regeneration after spinal trauma or injury. As a result, translational research on regeneration has mainly been focused on cell replacement strategies for regaining CNS function.

Experiments grafting tissue from fetal brains as a strategy to replace damaged cells in the adult hosts attracted a lot of attention in the late 1970s. It was demonstrated that these fetal grafts could provide functional improvement in different pathologic disease models (12, 13). Careful examination of these studies revealed that beneficial effects were due to replacement of damaged neurons, establishment of new neural circuitry, and release of trophic factors (neuroprotective factors and inflammatory modulators) by the grafts (12-18). However, practical human treatment using this method was not straightforward due to rarity of human fetal tissue and immune incompatibility and thus rejection of grafts. In 1998, isolation of human embryonic stem cells (hESCs) opened up a novel possibility for obtaining NSCs for transplantation (19). The potential of hESC in regenerative medicine has fueled research in CNS repair for the past decade. However, we are yet to achieve reliable methods for clinical stem cell-based therapy for human patients. In this review, we will discuss the recent advancements in deriving and differentiating stem cells for neural repair. We will also examine the current status of transplantation-based therapeutic strategies for several major neurological disorders.

3. TYPES OF STEM CELLS

Stem cells are undifferentiated cells characterized by their ability to self renew and differentiate into a variety of specialized cell types. Depending on their developmental potential, stem cells have been classified into several categories: (1) *Totipotent*, when the cell can give rise to all cell types of the body, including those that make up the extra-embryonic tissues such as the placenta. The zygote (fertilized oocyte) is considered to be totipotent. (2) *Pluripotent*, when the cell can give rise to all the different cell types of the three germ layers (ectoderm, mesoderm and endoderm) in the body. ESCs are considered pluripotent. (3) *Multipotent*, when the cell can develop into more than one cell type in the body. Tissue-specific adult somatic stem cells that are endogenous lineage-committed progenitors are considered multipotent. Within these classes, stem cells from different sources have been utilized for CNS regeneration with variable outcomes.

3.1. Adult stem cells

The existence of stem cells or progenitors has been identified in a wide range of adult tissues: these include the bone marrow (20, 21), liver (22), brain (23, 24), skin (25, 26), skeletal muscle (27), adipose tissue (28) and testes (29-31). Although several types of these adult stem cells have demonstrated pluripotency/multipotency and regenerative potential, only bone marrow, the source of hematopoietic stem cells and mesenchymal progenitors, is considered a practical resource for clinical treatments (32). This is mainly because of the ease of derivation and purification of these cells from human patients. In addition to the benefits of isogenic use in the same individual, there is also a reduced risk of teratoma formation compared to hESCs. The population of "mesenchymal stromal cells" in the bone marrow is heterogeneous and expresses a number of surface markers, such as CD105, CD73, CD106, CD54, CD29, CD44, CD90, STRO-1 (21, 33, 34). A recent report suggests that human mesenchymal stem cells (hMSCs) in the bone marrow also express the hESC marker SSEA-4 (35). Umbilical cord blood has also been identified as an alternate source of somatic progenitors (36). With potential for their autogenic use, hMSCs have already made a clinical impact in repairing bone defects in patients (37-40). Studies are currently underway to explore their potential in regeneration of other tissues and organ systems. It has been shown that these progenitors in the bone marrow can convert into multiple tissue types including neural cells (41, 42) and therefore could be a potential model for neural regeneration and repair. However, many questions remain as to the validity of a number of these studies owing to loose criteria used to define neural differentiation (43).

3.2. Embryonic stem cells

ESCs are pluripotent cells derived from the inner cell mass of blastocyst embryos with the ability to proliferate over prolonged periods while remaining undifferentiated and maintaining a stable karyotype. Derivation of hESCs held significant promise in regenerative medicine as they formed a source of cells with the potential to differentiate into derivates of all three germ layers (19). Culture conditions for the differentiating hESCs into neural cells (44-46), cardiomyocytes (47, 48), hematopoietic cells (49, 50), smooth muscle cells (51), pancreatic β cells (52, 53), endothelial cells (54, 55), osteoblasts (56, 57), and hepatocytes (58) have been well documented. In principle, hESC-derived cell therapy approaches could facilitate repair of any injured and/or degenerated tissue. As hESCs could be maintained in culture as undifferentiated cells in the presence of factors that promote self-renewal, their therapeutic potential was further amenable to gene therapy manipulations prior to transplantation (59). Such *ex vivo* gene transfer of therapeutic genes could present strategies to improve cellular host integration. Although these attributes have captured the imagination of the lay public and scientists

alike, formidable challenges exist before hESCs could be used for clinical therapeutics. For example, the destruction of embryos required for the derivation of hESCs has generated ethical concerns (60). Besides ethics, strategies transplanting hESC-derived cells are prone to immune rejection due to unmatched histocompatibility antigens (61, 62). Moreover, there is also risk of teratoma formation by contaminating undifferentiated cells after transplantation (63-65). An approach that can circumvent immune rejection by the host is the generation of hESCs from embryos derived from somatic cell nuclear transfer (SCNT) or "cloning." However, therapeutic cloning by SCNT is equally linked to ethical controversies. An alternate way to address immune rejection of transplanted hESC-derived cells is to select lines with compatible human leukocyte antigen (HLA) alleles (61, 66). However, through this approach, it would be possible to establish a hESC bank compatible for only 60% of the human population (67). Due to all these shortcomings, there is continued interest in developing hESC-based strategies that will overcome immune rejection or generating patient-specific pluripotent cells. Meanwhile, sources of isogenic adult stem cells are currently considered more practical for therapeutic applications in regenerative medicine.

3.3. Induced pluripotent stem cells

A recent breakthrough in stem cell biology is the success of converting differentiated human somatic cells into ESC-like cells, termed induced pluripotent stem cells (iPSCs), by using defined "reprogramming factors". The iPSC technology has opened up a new field of research almost overnight and holds out hope of life-saving medical advances. iPSCs were generated by epigenetic reprogramming of somatic cells through the forced exogenous expression of specific transcription factors. Trials based on the hypothesis that factors responsible for maintenance of pluripotency in ES cells might induce pluripotency in somatic cells led Yamanaka and colleagues to identify four transcription factors, Oct3/4 (also known as Pou5f1), Sox2, Klf4 and c-Myc, that could reprogram murine fibroblasts to iPSCs (68). These iPSCs were indistinguishable from ESCs in their developmental potential. They could differentiate into cells of all three germ layers and generate chimeras when injected into blastocyst embryos (68). It was subsequently shown that human fibroblasts could also be reprogrammed to iPSCs (69). Alternate to the use of the above four genes, it was also shown that Nanog and Lin28 could replace Klf4 and c-Myc to achieve pluripotency in human fibroblasts (70). These developments opened up unprecedented potential in iPSC-based regenerative medicine, circumventing the ethical controversies of hESCs and therapeutic cloning. It is anticipated that iPSCs could replace many applications in regenerative medicine suggested for hESCs. However, clinical use of these first-generation iPSCs is limited because of risks imposed by insertional mutagenesis by these transcription factors some of which are oncogenes. Efforts to eliminate these concerns have led to experimentation using transient expression of the reprogramming factors with plasmids (71), adenoviruses (72), transposon vectors (73), and even purified recombinant proteins (74). Although these methods have proven experimentally successful for iPSC generation, their reprogramming efficiency is prohibitively low for practical applications. Moreover, there are emerging concerns about their quality of epigenetic reprogramming in different iPSC clones (75). Therefore, with future improvements in the derivation, quality and validation, we can be optimistic that an improved version iPSCs will become available for cell therapy-based regenerative medicine in the times ahead.

4. DIFFERENTIATION OF STEM CELLS INTO NEURAL LINEAGES

Progressive steps of directed differentiation with specific stimuli orchestrate neural differentiation of hESCs. This procedure involves the generation of lineage-restricted neural progenitor cells followed by specific differentiation into neurons, oligodendrocytes or astrocytes. Although this process appears straightforward, the response of cells to the different stimuli is far from an expected "all or none" outcome. This heterogeneity resulting in low purity of the desired lineage-restricted cell types has complicated and impeded the optimal use of these precursor cells for therapy. Therefore, there have been several studies that examine and improvise existing methods to produce more pure cells for transplantation. In this section, we examine the current understanding and strategies utilized for the differentiation of different stem cells into transplantable neural cell types.

4.1. Neural progenitors derived from stem cells

In vitro, neural differentiation appears to be a primary default lineage for hESC differentiation under conditions that do not maintain pluripotency. Therefore, earliest methods for generating NSCs from hESCs, albeit with very low efficiency, were by spontaneous differentiation in the absence of conditions that promote self-renewal (44). Subsequent studies utilized the addition of specific stimuli to mimic embryonic neurogenesis to improve the yield of NSCs derived from hESCs. For murine ESCs (mESCs), retinoic acid (RA) provided reliable signaling for generating NSCs (76-78). However, RA-based signaling in hESCs appeared to be involved in a later stage of differentiation that specifies spinal cord progenitors rather than neural induction (79). Therefore, a reverse strategy blocking bone morphogenic protein (BMP) and/or Smad signaling pathways has been developed to efficiently generate NSCs from hESCs (80). Signaling by BMPs activated the Smad1 pathway in hESCs and promoted their differentiation into primitive endodermal cells (80). Inhibition of Smad signaling by noggin induced a large population of neural progenitors from hESCs that expressed early neuroectodermal markers Pax6 and nestin (80). The efficiency of this approach was significantly improved by dual Smad inhibition by using both noggin and a small molecule SB431524 that blocks downstream signaling of Smad 2/3 (81). However, the synchronous differentiation response of hESCs largely depends on the culture format used during the procedure; cellular response to factors in the medium is more or less uniform in monolayer cultures compared to cells grown as aggregates/multilayered colonies.

hESCs have traditionally been cultured on mouse embryonic feeder (MEF) layers. Initial studies on differentiation of hESCs involved the generation of suspended cellular aggregates called embryoid bodies (EBs) by plating detached hESC colonies in suspension culture on low adhesion plates. These EBs were capable of forming multilayered structures that could contain several cell types representing all three germ layers, recapitulating aspects of cell differentiation that occurs during early embryogenesis (82). It was suggested that this three-dimensional organization of cells as EBs was important for organized hESC differentiation (83). Neural induction of EBs using RA or noggin resulted in a mosaic of neural progenitors at different stages of differentiation (84). These cells could eventually be dissociated and enriched by selection and purification methods. This heterogeneity in differentiation was mainly because cells of the inner layers of the EB do not have access to specific growth factors or morphogens added to the culture medium. Recent developments in hESC culture circumvent this hurdle by using reagents that allow hESC growth on feeder-free conditions using matrigel as a substrate (85). In this adherent culture system, neural induction could be directed in a synchronous fashion by noggin resulting in a homogenous population of NSCs from hESCs (81, 85, 86). In an analogous strategy, synchronous differentiation of hESCs could be achieved by co-culture with cells that produce specific factors that direct the development of a specific cell type. It is well established that mesodermal signaling is required for neural induction (87). Therefore, hESCs co-cultured with bone marrow-derived stromal cell lines promoted neural differentiation (88-90). Based on studies in mouse ESC (mESC) differentiation, this co-culture method appears to generate neural cells with superior *in vitro* neuroectodermal patterning (91). However, isolation and purification of neural cells from any co-culture system presents an added complication for clinical use.

Techniques have also been developed for the derivation of NSCs from adult stem cells. Adult human MSCs from bone marrow and umbilical cord have been shown to differentiate to putative NSCs after treatment with a combination of RA and growth factors, such as, brain-derived neurotrophic factor (BDNF), neural growth factor (NGF) and neurotrophin-3 (NT3) (92, 93). Moreover, induction of MSCs using a combination of chemicals: β-mercaptoethanol, dimethylsulfoxide and butylated hydroxyanisole, has also been reported to generate cells that express NSC markers (94). Using a similar experimental approach, adult stem cells from skin (95) and adipose tissue (96) were also demonstrated to generate putative NSCs. However, all the above cases, the differentiation potential of these putative NSCs were not completely characterized and the resulting neuronal cell types were not functionally evaluated. Although adult stem cells could be an attractive source of autologous cells for transplantation, their potential remains to be definitively scrutinized.

hESC-derived NSCs resemble primary cultures of neuroectodermal columnar cells and form neural rosettes (90, 97). Cells forming rosettes expressed early neuroectodermal markers such as Pax6 and Sox1 (79, 90, 98). These NSCs from neural rosettes were capable of multiplying by symmetrical division over extended periods in culture. Substrates such as fibronectin promoted undifferentiated expansion of adherent cultures of NSCs in the presence of fibroblast growth factor 2 (FGF2) (85, 99-102). Epidermal growth factor (EGF) (44), laminin (103) and ascorbic acid (104) have also been used in combination with FGF2 for NSC expansion in culture. Non-adherent suspension cultures of NSCs as "neurospheres" have also been optimized with similar growth conditions but with a greater potential for expansion (105). Accumulating evidence suggests that the multipotent differentiation potential of NSCs was limited to early rosette stage cells and progressively diminished when expanded *in vitro* (79, 106, 107). This phenomenon mimics *in vivo* neural development as only neural precursors at neural plate stage exhibited broad patterning potential compared to neural precursors emerging after neural tube closure (108). Elkabetz et al. showed that neural rosettes that expressed anterior markers of the nervous system, such as *Forse1*, had the broadest differentiation potential (106). These cells were able to differentiate to neural cell types of anterior-posterior central and peripheral nervous system. *Forse1* expression was consistently observed in early NSCs derived from EB-based or stromal cell co-culture methods (106, 109). Few other studies also corroborated that hESC-derived NSCs were unable to develop midbrain dopaminergic neurons, spinal motor neurons and oligodendrocyte progenitor cells after expanded in cultures even in the presence of growth factors (106, 110-112). Based on this observation, it can be concluded that only early NSCs were found more responsive to "caudalizing factors" such as RA (98, 110, 111). Maintenance of *Forse1*-expressing neural rosettes required activation of sonic hedgehog (SHH) and Notch signaling pathways for self-renewal and maintenance (106). However, the same study showed that this maintenance was possible only when NSCs were grown at a high density, suggesting that yet unidentified autocrine factors may be required for proliferating multipotent NSCs. Therefore, future studies need to develop methods for reliable expansion of NSC without any loss of potential. This would be critical for cell therapy-based clinical use that necessitates access to a homogenous and considerably large population of NSCs.

4.2. Generation of dopaminergic neurons

Based on patterning signals that occur during embryonic development, hESC-derived NSCs can be directed towards specific neuronal lineages. In the developing neural tube, rostral or forebrain features are acquired first, followed by gradual caudalization. BMP, FGF, RA and Wnt signaling events play major roles in rostro-caudal patterning. After rostrocaudal patterning, dorso-ventral features appear in the neural tube, controlled by dorsal BMP signals from the roof plate and ventral SHH signals from the notochord (113). Dopaminergic (DA) neurons of the substantia nigra arise from progenitors in the ventral midbrain (ventral mesencephalon). These neurons

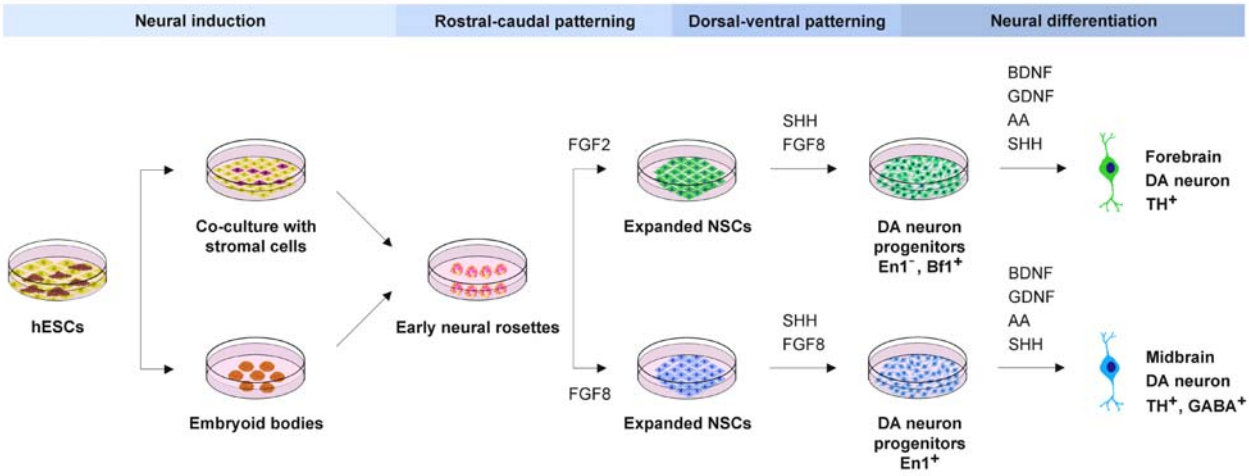

Figure 1. Differentiation of hESCs into dopaminergic neurons. After neural induction from hESCs either using co-culture with stromal cells or formation of embryoid bodies, SHH, FGF8 and FGF2 are used to specify the NSCs to DA neurons. The different timing of exposure to FGF2 and FGF8 leads to differentiation of NSC to neural progenitors with either forebrain or midbrain characteristics. When cultured with factors BDGF, GDNF, AA and SHH, these DA progenitors further mature into forebrain or midbrain DA neurons.

are characterized by expression of transcription factors Pitx3, Lmx1a, Nurr1, En1 and Foxa2 (114-119) and expression of tyrosine hydroxylase (TH) (120). DA neurons of the forebrain develop from another distinct group of progenitors. These neurons are characterized by the expression of transcription factor Bf1, a forebrain cell marker (121), but not midbrain DA neuron marker En1 (122). Forebrain DA neurons express γ-aminobutyric acid (GABA) as well as TH (123, 124). Progressive loss of DA neurons in Parkinson's disease (PD) occurs primarily in the substantia nigra of the midbrain. Therefore, strategies to derive DA neurons have mainly focused on generating ventral midbrain DA neuron progenitors for transplantation in PD (125).

To obtain midbrain DA neurons, hESC-derived NSCs were exposed to SHH, FGF8 and FGF2 to instruct differentiation (90, 112, 126) (Figure 1). This strategy was designed mainly based on knowledge of *in vivo* developmental signaling. SHH in the developing CNS specifies ventral cell types of the neural tube (127, 128). FGF8 is involved in the patterning of the isthmus, a region that divides the midbrain and hindbrain regions during brain development (129). In addition to its involvement in NSC expansion, FGF2 is also suggested to be involved in neural patterning (113, 130). However, the timing of exposure to these factors was important for directing NSC differentiation. After NSC expansion with FGF2, treatment with FGF8 and SHH mainly generated forebrain DA neurons that co-express GABA and TH (131). Without prolonged NSC expansion with FGF2, exposure to FGF8 and SHH generated midbrain DA neurons that co-express TH and En1 (112). This effect suggested that expansion of NSCs in culture made them lose their broad differentiation potential including their ability to become caudalized.

To increase efficiency of DA neuron differentiation, the fundamental strategy using FGF2, SHH and FGF8 has been modified in several studies. The culture of EBs in medium conditioned with a human hepatocarcinoma cell line showed increased DA neuron differentiation (102, 132). The use of Noggin in neural induction and subsequent differentiation in stromal cell co-culture methods increased the yield of DA neurons (133). Co-culture of hESC-derived NSCs with telomerase-immortalized fetal midbrain astrocytes also promoted their differentiation to DA neurons (65).

As an autologous source of cells for transplantation, hMSCs have also been differentiated into DA neurons that express TH upon exposure to SHH and FGF8 or glial-derived neurotrophic factor (GDNF) (134, 135). In another approach, induced overexpression of recombinant Notch intracellular domain in bone marrow hMSCs and subsequent treatment with bFGF, forskolin, and ciliary neurotrophic factor also induced NSCs that could be differentiated to DA neurons with GDNF treatment (134). In another study, DA neurons were derived from umbilical cord hMSCs through a stepwise culture process in neuron-conditioned medium followed by SHH and FGF8 treatment (135).

4.3. Generation of motor neurons and oligodendrocytes

During CNS development, spinal motor neurons (MNs) and majority of oligodendrocytes (OLs) sequentially arise from the ventral fringe of the embryonic neural tube (136), which is termed pMN domain (137). Studies in mice show that MNs develop from the pMN domain starting at about embryonic day 9-12 and OLs first appear at about embryonic day 12.5-13 (138). The progenitors from pMN domain are characterized by the expression of transcription factor Olig2 (137). In conditions such as spinal cord injury, amyotrophic lateral sclerosis and spinal muscular atrophy, there is loss of motor neurons. In addition, trauma that occurs during spinal cord/brain injury leads to OL loss and demyelination. Moreover, specific conditions like multiple

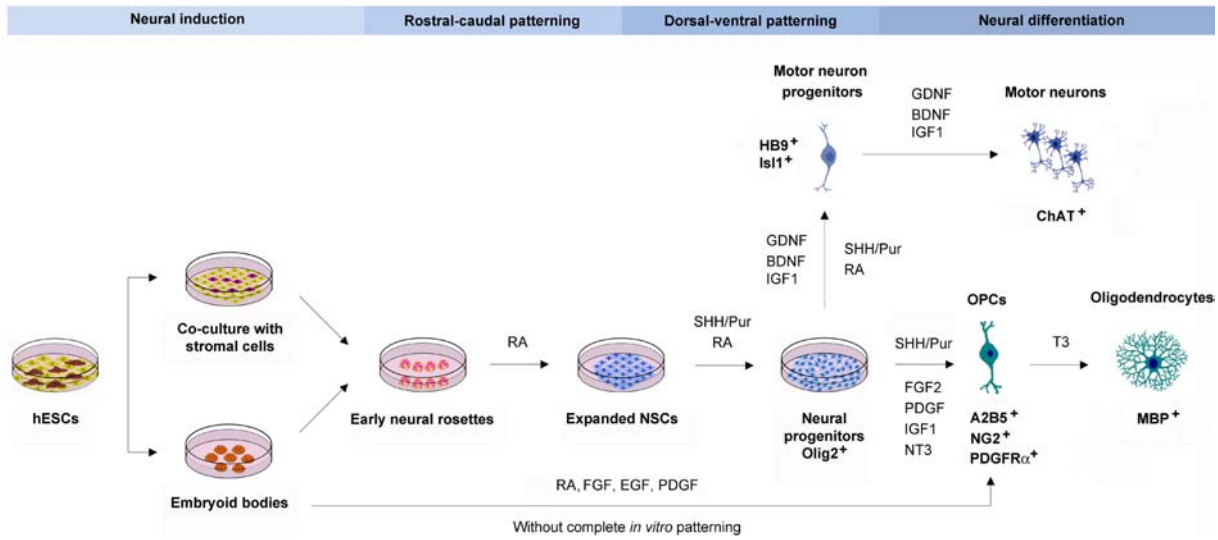

Figure 2. Differentiation of hESCs into motoneurons and oligodendrocytes. After neural induction, NSCs are sequentially caudalized and ventralized using patterning factors RA and SHH/Pur, respectively. The Olig2$^+$ progenitors could be differentiated to MNs in the presence of factors GDNF, BDNF and IGF1. Removal of RA and addition of factors FGF2, PDGF, IGF1 and NT3 leads to the differentiation of Olig2$^+$ progenitors into OPCs. These OPCs are capable of maturing to OLs *in vitro* in the presence of T3. Without full exposure to the patterning factors, OPCs can also be generated when hESC-derived EBs are cultured with a regiment of growth factors, including FGF2, EGF and PDGF.

sclerosis results in demyelination that leads to axonopathy and motor neuron disease. In treating these injuries, stem cell therapy-based strategies for CNS regeneration hold significant promise for replacing lost MNs and OLs.

To derive MNs from hESCs, neural rosettes were exposed to RA and SHH to trigger caudal and ventral patterning, respectively (79, 88, 139) (Figure 2). Consistent with embryonic pMN development, the progenitors obtained after RA and SHH treatment also expressed transcription factor Olig2. A small molecule agonist of SHH signaling, purmorphamine, has been shown to be potent in inducing Olig2 expression in NSCs and can be used as a superior substitute for SHH (46, 140). Treatment of Olig2$^+$ progenitors with BDNF, GDNF and insulin-like growth factor 1 (IGF1) further differentiated them into MNs characterized by the expression of transcription factors such as HB9, HOXB4 and ISL1, and mature MN markers such as choline acetyltransferase (ChAT) (108). These fundamental stimuli for the derivation of MNs from hESCs have also been successfully adapted for the differentiation of human iPSCs to MNs (111, 141). MNs have not been derived from MSCs. Although NSCs can be derived from human MSCs (92, 93), studies have not examined the differentiation of MSC-derived NSCs to MNs.

The Olig2$^+$ progenitors derived from hESCs can also give rise to OL progenitor cells (OPCs) in the presence of growth factors FGF2 combined with platelet-derived growth factor (PDGF), IGF1 and NT3 (110) (Figure 2). These OPCs are characterized by the expression of cell surface markers including the ganglioside A2B5, the proteoglycan NG2, and platelet-derived growth factor receptor α (PDGFRα) (142). These hESC-derived OPCs were capable of maturing to myelinating OLs when transplanted either into the rat spinal cord (143) or in the spinal cord of myelin-deficient *Shiverer* mutant mice (107). Interestingly, myelination competent OPCs could be differentiated from hESCs even without full exposure to patterning factors. In one method, OPCs were produced from EBs cultured with only a combination of FGF-2, EGF and RA (144). OPCs were also differentiated from mechanically isolated neural rosettes and neural tubes that were cultured with FGF2 and EGF followed by N2 medium containing FGF2 and PDGF (145).

To increase the efficiency of hESC differentiation to OPCs and obtain cells with better myelination potential, the fundamental differentiation protocol has been improvised in several studies. Use of the BMP antagonist noggin after induction of NSCs by RA treatment could improve differentiation and produce an enriched population of OPCs (146). This treatment of cells with noggin also significantly increased their potential for myelination *in vivo* (146). Moreover, use of the extracellular matrix protein vitronectin that shows developmental expression in the ventral neural tube enhanced the efficiency of differentiating hESCs to OPCs (147).

Although putative NSCs could be derived from human MSCs (92, 93), studies have not completely examined their differentiation potential and the functional properties of the resulting neuronal cells. A subpopulation of human umbilical cord blood cells that resemble the properties of NSCs has been shown to be able to differentiate into OLs *in vitro* (148). A recent study also suggested that cord blood cells could be differentiated to

OL-like cells after transplantation into an animal model of spinal cord injury (149). However, methods for the directed differentiation of hMSCs into OPCs or OL have not been well defined.

4.4. Generation of astrocytes

Astrocytes perform important roles in the brain to maintain functional homeostasis. These include regulating extracellular ion concentrations, detoxifying xenobiotics, modifying synaptic efficacy, inactivating neurotransmitters, inducing and maintaining the blood–brain barrier and providing nutrients and trophic support for neurons and oligodendrocytes (150). In the developing CNS, astrocytes arise from a variety of progenitors based on anatomic regional differences (151-153). Embryonic CNS progenitors, the so-called radial glial cells (154-156), express a number of markers that are shared by immature astrocytes (157, 158). This property is also seen in cells of the adult neurogenic zones, the SVZ and DG of the brain (159). Recent evidence suggests that under conditions of CNS injury, explant cultures of cortical astrocytes can gain multipotency and give rise to neurons and oligodendrocytes (160). Therefore, astrocyte populations could develop progenitor properties under conditions of injury or stress. Although these properties of astrocytes remain greatly understudied cell in the CNS, the recent discovery that astrocyte pathology could be a major contributor of neuronal death in amylotrophic lateral sclerosis (ALS) (161) has injected new interest in this cell type. Cell therapy using glial-restricted precursor cells is an emerging focus for preventing motor neuron deterioration in clinical cases of ALS.

Very few studies have examined astrocyte differentiation from different stem cell types. Astrocytes were reported merely as by-products in most methods for differentiating hESCs to neurons or oligodendrocytes (162). The first study that directed hESC differentiation into $GFAP^+$ cells reported a protocol that involves the inhibition of SHH signaling (163). Treatment of adherent hESCs with cyclopamine, a known SHH inhibitor, induced expression of markers of radial glia and astrocytes including glutamate aspartate transporter (GLAST). Continued culture resulted in nearly 70% $nestin^+$ and 78% $GFAP^+$ cells, characteristic of immature astrocytes. In another study, rat MSCs, after a threshold number of passages, also expressed nestin (164). When co-cultured with brain-derived NSCs, these MSC-derived $nestin^+$ rat cells differentiated to $GFAP^+$ cells. There are no reports on astrocyte differentiation from hMSCs.

Multipotent neural progenitors generated by several different approaches can spontaneously differentiate into astrocytes among other neural cell types either *in vitro* or after transplantation *in vivo* (97). However, limited information is known regarding specific signaling events required to promote astrocyte differentiation from hESC-derived NSCs.

4.5. Generation of microglia

Microglia are the resident immune cells of the CNS. Unlike neurons, oligodendrocytes and astrocytes that are derived from the neuroectoderm, microglia are derived from the mesoderm. Microglia first populate the nervous system during embryonic development (E11-15) and are found widely dispersed in the adult CNS (165, 166). In addition to these "resident" microglia in the brain, there is evidence that myelioid progenitors in the bone marrow can migrate across the blood-brain barrier and repopulate microglia of the CNS throughout adult life (167, 168). Microglial activation is a major cellular event in response to acute neuroinflammation in the CNS. However, there is an emerging debate in the field on whether microglial activation is beneficial or detrimental to the outcome of acute CNS inflammation (169). It has recently become evident that microglia are "primed" to respond to the nature and context of different stimuli effectively generating diversity of phenotypes of which some could be beneficial and others destructive (170). Therefore, the use of microglia in cell therapy for CNS disorders is an emerging field that is in fact multifaceted. Studies have shown that microglial transplantation could curb an immune response leading to recovery in models of spinal cord injury (171); they could enhance amyloid-beta clearance in Alzheimer's disease models (172); they could produce trophic factors that can mitigate ischemic white matter injury to the CNS (173). Therefore, efficient generation of "beneficial" microglia from stem cell sources could have widespread applications in neural repair.

Currently, there are no specific protocols for the directed differentiation of microglia from either murine or human ESCs. Instead, a modified neuronal differentiation protocol has been effectively modified (174). Despite their mesodermal origin, microglia appear as a minor subpopulation during the differentiation of mESCs to neurons. Strategies to select and enrich for this microglial population have been used to generate pure populations of microglia from mESCs. This procedure involves the generation of EBs and neural induction with RA; subsequently EBs are dissociated and cultured as a monolayer with FGF2 and laminin (104, 174-176). Microglia appear within neuronal populations at a later time point (16-40 days) of differentiation. Addition of granulocyte macrophage colony stimulating factor (GM-CSF) to this mixed culture promotes microglial proliferation and negatively selects for neurons (174). These cells could be easily passaged and maintained *in vitro*. They expressed MHC class I, MHC class II, CD40, CD80, CD86, and IFN-γ receptors characteristic of microglia (174, 176). The capacity of mESC-derived microglia to be able to migrate into the intact CNS after intravenous transplantation (174), gives them a specific advantage that includes the potential for secondary manipulations to strategize possible delivery of therapeutic molecules into the CNS or in gene therapy of neurological disorders. To date, there have been no reports on the derivation of microglia from hESCs - an area to be developed for potential therapeutic benefit in the future.

5. CELL BASED THERAPIES FOR NERVOUS SYSTEM DISORDERS

Injury to the CNS leads to loss of neurons and glial cells. In acute conditions such as spinal cord injury (SCI) or ischemic stroke, there is death of neurons and glia within a restricted area in the brain. In chronic conditions, there is either death of a specific group of neurons, such as DA neurons in Parkinson's disease (PD) and MNs in amylotrophic lateral sclerosis (ALS), or widespread loss of various types of cells, such as that in multiple sclerosis (MS). In all the above conditions, it might be possible to replace lost neurons and glial cells by transplantation of *in vitro* generated lineage committed progenitors derived from stem cells and therapeutically restore function. Proof of principle for such regeneration has been demonstrated for several CNS disease models (Table 1). In this section, we examine current progress in the development of stem cell-based therapies for specific CNS disorders.

5.1. Cell therapy for spinal cord injury

Most spinal cord injuries (SCI) are caused by an initial trauma to the spinal cord. This leads to blood-brain barrier breakdown, changes in ion balance, lipid peroxidation and glutamate release. The trauma may also result in an acute episode of local ischemia that can contribute to secondary degeneration. At the secondary phase of injury, a cascade of signaling events can increase inflammatory cytokines and chemokines, cause excitotoxicity, induce apoptosis and lead to progressive loss of oligodendrocytes, resulting in demyelination and axonal degeneration (177, 178). The ensuing SCI pathology has been described as a cystic degenerative cavity surrounded by a "glial scar" (179). Patients with SCI experience loss of mobility, sensation and autonomic control below the level of the injured spinal segment. Although there is potential for regenerative events at the vicinity of damaged tissue (180-182), these endogenous responses are inefficient at repair partly due to the hostile inflammatory microenvironment presented after injury (177, 183). Currently, no effective treatments are available for SCI. Therefore, stem cell-based approaches are considered invaluable for regenerative therapy after SCI by aiming to regenerate neurons and ameliorate inflammatory damage.

Use of NSCs for cell replacement in SCI has been considered an appropriate approach to reconstruct the injured spinal cord as they can theoretically give rise to neurons, oligodendrocytes or astrocytes. However, studies showed that injured spinal lesions were not conducive to neuronal differentiation due to inhibitory signals and a lack of survival, growth, and guidance molecules (184). Accordingly, transplantation of naïve NSCs into spinal cords of SCI animal models predominantly generated glial cells (185-188). Although this gliogenesis promoted recovery of motor function in these studies potentially due to trophic effects and myelination brought about by oligodendrocytes (described below), astroglial differentiation of NSCs and aberrant axonal sprouting have been implicated as the major cause of severe side effects like allodynia that is observed in several cases after spinal cord transplantations (189). Therefore, transplantation of a further differentiated fate-restricted progenitor may be superior compared to NSCs for SCI therapy. In agreement with this hypothesis, use of neuron-restricted progenitors for transplantation in SCI improved functional recovery (184, 190). However, these transplanted precursors did not readily differentiate into neurons but rather remained as $nestin^+$ precursors for up to 2 months in the injured spinal cord (184), suggesting that the beneficial effects of transplantation were mainly due to the production of trophic factors by these progenitors. Therefore, manipulation of the microenvironment in the injured spinal cord will likely be necessary to facilitate neuronal regeneration/replacement.

In addition to neurons, progressive loss of oligodendrocytes after SCI leads to demyelination that also contributes to neurological dysfunction following SCI as they impair axonal conduction and can lead to axonopathy (191, 192). Acute transplantation of hESC-derived OPCs after SCI enhanced remyelination and improved of motor function by preventing progressive axonal damage that occurs due to myelin loss (193). However, beyond a 7-day window after injury there was little improvement in functional outcome suggesting permanent damage via axon loss. The transplanted hESC-derived OPCs also conferred neurotrophic benefits to surrounding tissue, perhaps by expressing factors that promote neurite outgrowth and neuronal survival including hepatocyte growth factors (HGFs), BDNF, transforming growth factor beta 2 (TGFβ2), and activin A as observed *in vitro* (194). Based on this fundamental paradigm of cell therapy, Geron Corp., a US-based company has received FDA approval for testing hESC-derived OPCs in clinical studies evaluating regeneration and functional recovery in SCI patients. Although this clinical trial falls within the bracket of all the risks associated with the use of hESCs, preclinical evidence for efficacy and benefits for patients who lack effective treatments for SCI justify these trials.

Stem cell-based approaches using MSCs and primary NSCs, in SCI animal models have been shown to ameliorate the severity of SCI pathology and promote the regeneration (184, 186-189, 195-197). Based on this success, clinical trials using umbilical cord blood and bone marrow-derived MSCs have already been performed with variable outcomes in patients (198-201). Recent studies show that MSCs have a very limited potential to differentiate into neural lineage cells (177, 202). Therefore, the mechanisms underlying the beneficial effects of MSC transplantation is mainly considered neurotrophic (177).

Motor neurons differentiated from hESCs have been shown to survive and integrate into the developing and adult spinal cord (88). However, cell therapy for SCI still suffers from failure to replace lost neurons at the sites of injury. Thus, more studies need to be carried out to understand the hostile environment that prevents neurogenesis in the injured spinal cord. Cell therapy-based efforts to modulate the inflammatory milieu in SCI perhaps using beneficial microglia transplantation need to be carefully examined. Undoubtedly, neuronal regeneration in SCI holds the potential to restore function. Therefore, in

Table 1. Summary of clinical reports on stem cell-based therapy for neural repair

Cell types	Injuries/ Diseases	Methods of transplantation	Outcomes	References
Autologous bone marrow cells	SCI	Intraspinal injection at injury site, followed by GM-CSF treatment.	Improvements were observed in acute and subacute treatment groups. No significant improvement was observed in the chronic treatment group. Increasing neuropathic pain was observed during the treatment.	(197)
Autologous bone marrow cells	SCI	Intraspinal injection at injury site, followed by GM-CSF treatment.	Sensory recovery in the sacral segment and significant motor improvements were observed at 3 to 7 months post-transplantation. MRI showed slight enhancement at the transplantation sites.	(200)
NT2N cells*	Ischemic stroke involving basal ganglia or basal ganglia and cerebral cortex	Intracerebral transplantation into the penumbral area	No significant benefits reported. No cell-related adverse effects 5 years after transplantation.	(222)
NT2N cells*	Ischemic or hemorrhagic stroke involving basal ganglia	Intracerebral transplantation into the penumbral area	No significant benefits in motor function. No cell-related adverse effects.	(223)
Fetal porcine cells	Ischemic stroke involving the striatum	Intracerebral transplantation into the penumbral area	This study was terminated after 2 patients developed adverse effects.	(227)
MSCs	Ischemic stroke in middle cerebral artery territory	Intravenous infusion	No significant benefits reported. No cell-related adverse effects.	(224)
Human fetal cells	Ischemic or hemorrhagic stroke involving the middle cerebral artery territory	Subarachnoidal transplantation	Some patients developed fever and meningism 48 hours after transplantation	(226)
Human ventral mesencephalon tissue	PD	Transplantation into caudate and anterior putamen.	Significant and sustained improvement of motor function. No increase in fluorodopa uptake at the striatum after transplantation.	(232, 234)
Human embryonic dopamine-rich mesencephalic tissue	PD	Transplantation into the putamen.	The reduction of parkinsonian symptoms on the side contralateral to the graft. Fluorodopa uptake increased in the grafted putamen.	(233)
Human embryonic mesencephalic tissue	PD	Transplantation into the caudate and putamen.	Survival of implanted fetal dopamine cells and significant neurologic improvement.	(235, 236)
Human fetal nigral tissue	PD	Implantation of fetal nigral tissue with diffuse distribution.	All patients experienced clinically meaningful benefits. Increased striatal flurodopa uptake was evident in all patients.	(237)
HSCs	ALS	Intravenous infusion	No clinical benefits were evident. Cells migrated to the CNS and engrafted at pathologic lesions.	(252)
MSCs	ALS	Intraspinal injections at thoracic level.	Mild improvements noticed in 4 patients. No significant side effects were evident. No modification of the spinal cord volume or other signs of abnormal cell proliferation were observed.	(253)
HSCs (peripheral blood)	ALS	Intracerebral injection into frontal motor cortex.	Transplantation was well tolerated and the survival of treated patients was statistically higher ($P = 0.01$) than untreated control patients.	(256)
Bone marrow derived HSCs	ALS (Sporadic)	Intraspinal injection of BM-derived HSCs at C1 to C2 level.	Electro-neuro myography (ENMG) results show that the patients became better compared to pre-operative status.	(257)

addition to promoting oligodendrogenesis and trophic support, future studies need to harness the potential of stem cells to regenerate neurons in the injured spinal cord.

5.2. Cell therapy for stroke

Ischemic stroke that occurs due to interruption of blood supply to brain regions is characterized by focal necrosis of neurons, oligodendrocytes, astrocytes and endothelial cells within the infarct. The severity of clinical signs in patients depends on the location of the infarct and varies from mortality to permanent disabilities including paralysis and cognitive impairment. Depending on etiology, acute thrombolytic treatment using tissue plasminogen activators can be beneficial in only a minority of cases to

restore blood supply (203), albeit with significant risk of intracranial hemorrhages (204). In most stroke cases, there is no effective treatment to promote recovery. Although increase in proliferation of neural precursors has been observed in stroke patients, they do not contribute into endogenous repair mechanisms mainly due to the hostile inflammatory microenvironment surrounding the region of infarct (205). Endogenous functional reorganization of neural circuitry partially contributes to bypass lost neurons and provide relief of clinical signs to variable degrees, but most stroke patients never recover completely and require supportive care throughout life. In this regard, stem cell strategies have been investigated to regenerate brain tissue and restore function after ischemic stroke.

Several types of stem cells have been investigated in animal models of stroke for neural regeneration (206, 207). In these studies, adult and fetal-derived NSCs as well as NSCs derived from ESCs could survive, migrate from the site of transplantation and differentiate into mature functional neurons integrating with the existing neuronal networks (206-209). In another study, grafting of hESC-derived NSCs into the infarct boundary zone in a rat model of ischemic stroke improved lost forelimb function (210). Functional electrophysiological recordings on transplanted cells showed that these cells received synaptic input from host neurons (211), suggesting the integration of transplanted human cells with existing neuronal circuitry. Similar results have been obtained from experiments transplanting NSCs derived from human fetal tissues (212, 213).

In addition to replacement of lost neurons, neurotrophic factors secreted by transplanted cells can promote angiogenesis and enhance survival of damaged neurons (214, 215). Studies have shown that stromal-derived factor 1 and vascular endothelial growth factor produced by NSCs after transplantation protect neurons against ischemic damage (216, 217). In addition, these cells brought about reductions in inflammation and glial scar pathology due to ischemic injury (216). In a similar way, transplantation of MSCs also provided anti-inflammatory and angiogenic effects that act to reverse functional deficits after stroke (218-221). Even with intravenous delivery, MSCs-derived from hESCs migrated into the CNS and brought about neuronal and endothelial regeneration (219). This study was performed in a transient focal ischemia rat model and reported both the reduction of infarct size and improvements in functional outcome (219).

Preliminary clinical trials using stem cells for stroke have already been attempted with variable outcomes (222). For example, neuronal cells derived from an immortalized human teratocarcinoma cell line when transplanted into infarcts that affected only the basal ganglia, no significant functional outcome was observed, however PET scans showed increases in local metabolic activity (223, 224). In another study, intravenous transplantation of autologous MSCs into few patients with ischemic lesions via the middle cerebral artery occlusion did not show any functional improvement in patients (225). Following up, the same group recently reported beneficial effects of MSC transplantation from a long-term study with a larger number of patients (226). Trials have also been performed with human fetal cells (227) and porcine fetal cells (228) with no significant benefits. Based on preclinical evidence and functional studies in the rat stroke model, a UK-based company ReNeuron is also performing clinical trials in stroke patients using a conditionally immortalized cell line derived from NSCs isolated from the human fetal cortex (229). The success of this strategy remains to be disclosed.

Stem cell based strategies for neural regeneration in stroke is fairly complex and tends to be patient specific. Cell replacement and regeneration requires intricate understanding of patient-specific disease pathology and affected functional anatomy behavior in addition to graft types and requisites for functional differentiation after transplantation. Therefore, many concerns remain before stem cell-based therapy can be harnessed for clinical treatment of stroke.

5.3. Cell therapy for Parkinson's disease

Primary pathology of PD is the degeneration of DA neurons in the substantia nigra. This results in clinical symptoms that are characterized by muscle rigidity, hypokinesia, tremor, and postural instability. PD is both chronic and progressive with a relatively late middle age of onset. However, there are cases of "early-onset" Parkinson's disease in which patients develop symptoms as early as 30 years of age. In most patients, PD is idiopathic thereby allowing only supportive symptomatic therapy to alleviate clinical symptoms. Although motor symptoms can be treated to some extent using L-3,4-dihydroxy phenylalanine (L-DOPA), DA agonists, enzyme inhibitors and deep brain stimulation (230), effective treatments are not available for non-motor manifestations like dementia (231). In either case, neurodegeneration and disease progression is not slowed or stopped by these treatments. As a result, patients become dependent and experience severe side effects to L-DOPA treatment after a few years. Therefore, cell replacement using DA neuron precursors has been studied as a viable therapeutic option for PD.

Early evidence from the transplantation of fetal mesencephalic tissue, a progenitor source for DA neurons, provided the first evidence that cell replacement therapy for PD is feasible and could be beneficial (232). DA neurons formed from the transplants became functionally integrated are restored striatal dopamine release and brought about symptomatic relief in some patients (233-238). The donor-derived DA neurons were significantly resistant to PD pathology and were functional for up to a decade after transplantation (239, 240). However, the scarcity of embryonic tissue and dramatic variability in the patient responses (241, 242), is mainly due to difficulty in standardizing embryonic mesencephalic material, warrant the development of alternative sources of cells for transplantation.

As described earlier, hESCs and hMSCs have been differentiated into functional DA neurons *in vitro* (90, 112, 126, 135). It has also been shown that hESC-derived

DA neuron precursors can survive and mature in animal models of PD (65, 126). However, properties of re-innervation and restoration of dopamine release after transplantation have not been reported in hESC-derived DA neurons in these preclinical studies. Therefore, further studies are required to establish cellular property requisites for CNS integration of hESC-derived DA neurons before these cells can be considered for treatment of human patients. Despite their differences in gene expression profile, functional differences between forebrain DA neurons and midbrain DA neurons remains to be elucidated. Neuroblast differentiation into either one of the DA neuron subtype needs to be optimized for reliable and homogenous functional effects after transplantation.

In order to be practical for clinical PD therapeutics, hESC-based cell therapy need to show significant success providing more than 50% amelioration of motor symptoms without posing any risks. As the life expectancy of patients affected by PD is not significantly reduced, even a minor risk of teratoma formation can be considered unacceptable (208). Moreover, for long-term function of donor DA neurons in PD patients, supplemental neuroprotective therapy may be required to hamper disease progression (243).

5.4. Cell therapy for amyotrophic lateral sclerosis

ALS is characterized primarily by progressive dysfunction and degeneration of motor neurons in the cerebral cortex, brainstem and spinal cord. As a result, there is rapid progression of muscle weakness that causes death within a few years after diagnosis. The vast majority of ALS cases are sporadic; a minor incidence (approximately 5%) has also been reported for familial cases due to superoxide dismutase 1 mutation (244). Despite the selective functional loss due to motor neuron loss, recent evidence has implicated astrocytes and microglia as contributors to this neuron death (161, 245). Although several mechanisms have been proposed to likely contribute to sporadic disease pathogenesis, the etiology of selective death of motor neurons in this disease remains elusive. As a result, there exists no effective treatment for ALS. Recently, much attention has been placed on stem cell-based regeneration strategies as a promising new treatment for ALS.

Cell therapy for ALS aims to regenerate MNs and additionally provide a favorable microenvironment by replacing astrocytes and microglia. MNs have been generated *in vitro* from several stem cell sources. However, to be clinically successful in ALS, transplanted MN precursors should extend axons across long distances and integrate with the existing neural circuitries and innervate target muscle fibers. Preclinical studies in rat models showed that spinal transplantation of MN progenitors could extend axons to ventral roots (246, 247), and innervate host muscles resulting in partial recovery from paralysis (247). Although this sounds promising, several finer characteristics need to be defined before clinical translation of this technology. For example, MN progenitors need to be delivered at multiple sites along the spinal cord and they should be directed to cervical, thoracic or lumbar phenotypes (79, 248). It should also be evaluated whether corticospinal neurons (249) that degenerate in ALS could be effectively replaced. Perhaps the most important hurdle in MN regeneration would be to ensure that the hostile microenvironment for this cell type in the spinal cord is also favorably modified.

During early signs of ALS, counteracting motor neuron loss by transplantation of supporting glial cell types to release neurotrophic molecules or by modifying the inflammatory milieu is a more realistic clinical goal for ALS treatment. Several lines of preclinical data have provided the rationale for this approach. For example, in a rodent models for ALS, glial restricted precursors extensively differentiated into mature astrocytes in grafts, and prevented motor neuron loss and reduced microgliosis (250); transplantation of human vascular endothelial growth factor (VEGF)-overexpressing NSCs conferred protection on motor neurons and delayed disease progression (251); transplanted human embryonic germ cells into the cerebrospinal fluid also mitigates motor injury by growth factor-mediated neuroprotection (252). Based on these principles, NeuralStem Inc., a US-based company has received FDA approval for ALS clinical trials by transplantation of human fetal-derived NSCs into the spinal cord. At the present time, fetal NSC transplantation is considered safer than using of hESC-derived NSCs due to risk of teratoma formation.

Several clinical studies have examined the effect of transplanting hMSCs to alter the inflammatory microenvironment in the spinal cord of ALS patients. Intravenous allo-transplantation of HSCs showed migration of the cells to the pathologic lesions (253). Intraspinal injections of autologous MSCs reduced inflammation and motor neuron loss in some ALS patients (254-257). Cervical intraspinal injections of autologous HSCs led to significant improvement in 9 out of 12 ALS patients (258). Although, these studies show that ALS patients can benefit from hMSC transplantation, continued fundamental research to understand the specific effects mediated by these transplanted cells will be necessary to improve therapeutic directions. This understanding will also facilitate optimizing hESCs or patient specific iPSCs protocols for ALS treatment.

5.5. Cell therapy for multiple sclerosis

Multiple sclerosis (MS) is an autoimmune disease characterized by a self-reactive attack on CNS myelin by T-lymphocytes. This results in demyelination and subsequent axonal degeneration leading to functional deficits in the brain and spinal cord. The form of neurological symptoms of MS varies in severity depending on age and ranges from changes in sensation to difficulty in moving, visual and speech problems, fatigue and depression (259). Based on disease pattern among different individuals, MS has been classified as either being progressive or relapsing/remitting.

Relapsing/remitting MS that can develop at a relatively young age (20-30 years) often turns into a secondary progressive form. Both primary and secondary

progressive MS are characterized by a gradual increase in disease severity. Although immunomodulatory therapies available today decrease the frequency of new plaques in relapsing/remitting MS, they are often not satisfactory for primary or secondary progressive MS (260). Moreover, immunosuppressive therapy also brings about significant side effects. Therefore, stem cell therapy-based strategies have been explored as means to regulate inflammation and facilitate functional CNS regeneration in MS patients.

The potential of HSC transplantation to reconstitute immune cells after immune elimination in recipients led to experiments designed to "reset" the immune system and eliminate autoreactive T cells. In murine models of MS (experimental autoimmune encephalomyelitis, EAE), HSC-based immune reconstitution resulted in reduced disease severity in several studies (261, 262). In human patients, autologous transplantation of HSCs after irradiation or chemotherapy-induced elimination of self-reactive lymphocytes resulted in the generation of self-tolerant lymphocytes (263). As a result, many patients with relapsing remitting MS experienced disease-free remissions (263). Although, HSC transplantation could not significantly reverse the disability in patients with progressive forms of MS it was able to halt further progression of the disease (263-265).

The main therapeutic effect of MSC transplantation has been identified to be due to immunosuppression and inhibition of inflammation in the recipient. After transplantation, MSCs migrate to lymphoid organs and reduce the proliferation of autoreactive T cells (266). In addition, it has also been shown that MSCs prevent host dendritic cell maturation (267), and decrease the production of immunoglobulins specific for the encephalitogenic myelin antigen proteolopid protein (268). Preclinical studies transplanting MSCs showed that these cells were able to ameliorate the clinical signs of EAE, reduce inflammation and demyelination when transplanted either intravenous or intracerebroventricular at the onset or peak of disease (262, 269). However MSCs failed to improve EAE clinical signs when transplanted after the disease is in progress past the peak of clinical signs (262). MSCs also have a direct CNS repair-inducing potential by migrating into the injured CNS and producing trophic factors that promote oligodendrogenesis and myelination in the white matter lesions (261). Autologous HSCs and MSCs are currently in phase I/II clinical trials in MS patients; however, preliminary results of clinical benefits from these treatments have been largely variable (270).

Similar to MSCs, NSCs have also demonstrated therapeutic potential in MS. Studies have shown that NSCs were able to ameliorate clinical signs of experimental autoimmune encephalomyelitis (EAE, a mouse model for MS) by migrating into foci of disease and differentiating into myelin-forming oligodendrocytes (271, 272). Additional evidence in a primate model of MS suggests that transplanted hESC-derived NSCs also alleviate clinical signs of MS (273). These cells only made a negligible contribution to remyelination but had potent immunosuppressive and anti-inflammatory effects in the animal. Although NSCs could differentiate into neurons, oligodendrocytes or astrocytes, the fate of transplanted NSCs in these experiments were not completely characterized.

The benefits of microglial transplantation for MS therapy are greatly understudied. There is emerging evidence that microglia could in fact help "clean up" and restore a physiological microenvironment in CNS lesions. Emerging evidence suggests that resident microglia associated with CNS lesions could be "primed" to local stressors resulting in a destructive phenotype (170). Therefore, "beneficial" microglia derived from exogenous sources could have the potential to reduce inflammation, restore the tissue homeostasis and provide regenerative signals in the injured CNS. In support of this hypothesis, it was observed that transplantation of bone marrow-derived myeloid cells overexpressing triggering receptor expressed on myeloid cells 2 (TREM-2) reduced demyelination and ameliorated the progression of EAE (263). In the CNS, these exogenous myeloid cells were homologous to microglia as they expressed microglial markers and functioned to phagocytose debris at the CNS lesions (263). Although the concept of "beneficial" microglia is recent and still controversial, we believe that this is a promising area with a direct clinical link to autoimmune diseases such as MS and requires further exploration.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

Intense basic and clinical research accomplished during the recent years on stem cells has constituted a revolution in regenerative medicine for neurological disorders that currently lack effective treatments. There has been continuous progress on the generation of pure populations of multiple therapeutically useful cell types and methods to decrease the risk of stem cell therapies. As a result, approved clinical trials are currently underway for patients with ALS and stroke with human fetal tissues that are rich in progenitors. Although fetal tissue avoids the risk of teratomas, rarity and inconsistency of fetal material available make this approach less practical for mainstream applications. Therefore, future therapeutics is better accomplished by using renewable cell resources.

Despite ethical concerns, tremendous focus has been placed on hESCs as a source of pluripotent therapeutic cells. Based on preclinical evidence, there is an undeniable risk of teratoma formation in using hESC-derived cell transplants that arise from reminiscent pluripotent cells and inherent heterogeneity of their *in vitro* differentiation properties (274, 275). Advances in understanding of the mechanisms of hESC differentiation in recent years have led to improvements but these have not yet eliminated this concern. The recent FDA approval for clinical trials using hESC-derived OPCs marked a milestone for hESC-based cell replacement therapies. However, prevailing concerns on the characteristics of the differentiated cell populations led to a clinical hold on this initially approved project with requests for additional characterization and preclinical trials in animal models.

Owing to the roadblocks in hESC research, lot of clinical emphasis has also been placed on adult stem cells such as MSCs that can be derived from the bone marrow. These cells are relatively easy to access, and offer the possibility of autologous transplantation. More importantly, adult stem cells show a high degree of genomic stability during culture (276, 277), and do not result in tumor formation after transplantation (277). However, most benefits from the use of MSCs that are of clinical value appear to be derived through immunomodulation, trophic actions and neuroprotection rather than direct phenotypic regeneration (179, 256, 262, 278-280). This limits the potential for functional recovery when using MSCs compared to direct cell replacement strategies attempted with hESC-derived cells. That being said, it is becoming increasingly clear from recent studies that the characteristics of the pathological CNS microenvironment can affect the survival, differentiation and function of transplanted cells. Even after hESC-derived cell transplants in injuries like SCI, the lineage-committed neuronal progenitors failed to differentiate and remained in an undifferentiated state for prolonged periods (184).

Therefore, harnessing the clinical potential of stem cells requires more basic research in two fronts: [i] understanding and controlling the mechanisms regulating the proliferation, migration, differentiation, survival and functional integration of transplanted cells, and [ii] understanding the characteristics of the pathological CNS microenvironment and the potential for the brain's self-repair mechanisms. These studies should be complemented with, developing better *in vitro* methods to handle, maintain and differentiate stem cells with adequate knowledge of in vitro patterning properties that could facilitate better integration into existing anatomically relevant circuits. Emerging evidence suggests that *in vitro* patterning properties may be crucial for generating functional ESC-derived neural cells (91). In addition, identification of specific biomarkers for each progenitor cells relative to their more committed and mature neural progeny is important for the characterization of their specific physiological functions and regenerative potential.

Finally, progress toward clinical translation of stem cell therapies would also tremendously benefit from the development of patient-specific pluripotent cells. The recent generation of iPSCs (68, 69), allows for cautious optimism that use of this technology in CNS regeneration may just be around the corner. Although the first generation of iPSCs could not be used for human therapies due to the viral methods used for deriving them, they opened up the possibility of studying patient-specific hereditary diseases using their own cells (281-284). Intense research and development in this area brought about the second (72), third (74), and fourth (285) generation of iPSC production using non-genome integrating methods albeit with very low efficiency. Improving this technology, a recent report describes the highly efficient production of a fourth generation clinically "safe" iPSCs using synthetic RNA taking us a step closer to regenerative therapies using iPSCs (286). Although perfectly plausible, it remains to be established that protocols developed for differentiating hESCs would be identical for iPSC differentiation. Overall, the field of stem cell-based regeneration requires multidisciplinary expertise from basic scientists, pathologists and clinicians alike for developing translational approaches that would be specific for each neurological disorder. With accelerating developments in this field, clinical therapy may not remain an unrealized goal for long.

7. ACKNOWLEDGEMENT

Vimal Selvaraj (vs88@cornell.edu) and Peng Jiang (pjiang@ucdavis.edu) contributed equally to this manuscript. Wenbin Deng is supported by grants from the National Institutes of Health (R01 NS059043 and R01 ES015988), Roche Foundation for Anemia Research, National Multiple Sclerosis Society, Feldstein Medical Foundation, and Shriners Hospitals for Children. The authors declare no conflicts of interest related to this article.

8. REFERENCES

1. A Kriegstein and A Alvarez-Buylla: The glial nature of embryonic and adult neural stem cells. *Annu Rev Neurosci* 32, 149-84 (2009)

2. A Alvarez-Buylla, M Kohwi, TM Nguyen and FT Merkle: The heterogeneity of adult neural stem cells and the emerging complexity of their niche. *Cold Spring Harb Symp Quant Biol* 73, 357-65 (2008)

3. FT Merkle, AD Tramontin, JM Garcia-Verdugo and A Alvarez-Buylla: Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc Natl Acad Sci U S A* 101, 17528-32 (2004)

4. TE Anthony, C Klein, G Fishell and N Heintz: Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron* 41, 881-90 (2004)

5. SC Noctor, V Martinez-Cerdeno, L Ivic and AR Kriegstein: Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci* 7, 136-44 (2004)

6. A Gritti, L Bonfanti, F Doetsch, I Caille, A Alvarez-Buylla, DA Lim, R Galli, JM Verdugo, DG Herrera and AL Vescovi: Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. *J Neurosci* 22, 437-45 (2002)

7. Z Liu and LJ Martin: Olfactory bulb core is a rich source of neural progenitor and stem cells in adult rodent and human. *J Comp Neurol* 459, 368-91 (2003)

8. MA Hack, A Saghatelyan, A de Chevigny, A Pfeifer, R Ashery-Padan, PM Lledo and M Gotz: Neuronal fate determinants of adult olfactory bulb neurogenesis. *Nat Neurosci* 8, 865-72 (2005)

9. B Nait-Oumesmar, L Decker, F Lachapelle, V Avellana-Adalid, C Bachelin and AB Van Evercooren: Progenitor

cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci* 11, 4357-66 (1999)

10. N Picard-Riera, L Decker, C Delarasse, K Goude, B Nait-Oumesmar, R Liblau, D Pham-Dinh and AB Evercooren: Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *Proc Natl Acad Sci U S A* 99, 13211-6 (2002)

11. B Menn, JM Garcia-Verdugo, C Yaschine, O Gonzalez-Perez, D Rowitch and A Alvarez-Buylla: Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci* 26, 7907-18 (2006)

12. MJ Perlow, WJ Freed, BJ Hoffer, A Seiger, L Olson and RJ Wyatt: Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* 204, 643-7 (1979)

13. N Sunde, S Laurberg and J Zimmer: Brain grafts can restore irradiation-damaged neuronal connections in newborn rats. *Nature* 310, 51-3 (1984)

14. RD Lund and SD Hauschka: Transplanted neural tissue develops connections with host rat brain. *Science* 193, 582-4 (1976)

15. A Bjorklund and U Stenevi: Experimental reinnervation of the rat hippocampus by grafted sympathetic ganglia. I. Axonal regeneration along the hippocampal fimbria. *Brain Res* 138, 259-70 (1977)

16. LG Nygren, L Olson and A Seiger: Mnoaminergic reinnervation of the transected spinal cord by homologous fetal brain grafts. *Brain Res* 129, 227-35 (1977)

17. MM Oblinger, BH Hallas and GD Das: Neocortical transplants in the cerebellum of the rat: their afferents and efferents. *Brain Res* 189, 228-32 (1980)

18. F Haun and TJ Cunningham: Cortical transplants reveal CNS trophic interactions in situ. *Brain Res* 317, 290-4 (1984)

19. JA Thomson, J Itskovitz-Eldor, SS Shapiro, MA Waknitz, JJ Swiergiel, VS Marshall and JM Jones: Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145-7 (1998)

20. GJ Spangrude, S Heimfeld and IL Weissman: Purification and characterization of mouse hematopoietic stem cells. *Science* 241, 58-62 (1988)

21. Y Jiang, BN Jahagirdar, RL Reinhardt, RE Schwartz, CD Keene, XR Ortiz-Gonzalez, M Reyes, T Lenvik, T Lund, M Blackstad, J Du, S Aldrich, A Lisberg, WC Low, DA Largaespada and CM Verfaillie: Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418, 41-9 (2002)

22. SH Oh, HM Hatch and BE Petersen: Hepatic oval 'stem' cell in liver regeneration. *Semin Cell Dev Biol* 13, 405-9 (2002)

23. FH Gage: Mammalian neural stem cells. *Science* 287, 1433-8 (2000)

24. S Temple: The development of neural stem cells. *Nature* 414, 112-7 (2001)

25. JG Toma, M Akhavan, KJ Fernandes, F Barnabe-Heider, A Sadikot, DR Kaplan and FD Miller: Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* 3, 778-84 (2001)

26. T Tumbar, G Guasch, V Greco, C Blanpain, WE Lowry, M Rendl and E Fuchs: Defining the epithelial stem cell niche in skin. *Science* 303, 359-63 (2004)

27. X Shi and DJ Garry: Muscle stem cells in development, regeneration, and disease. *Genes Dev* 20, 1692-708 (2006)

28. PA Zuk, M Zhu, P Ashjian, DA De Ugarte, JI Huang, H Mizuno, ZC Alfonso, JK Fraser, P Benhaim and MH Hedrick: Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 13, 4279-95 (2002)

29. S Conrad, M Renninger, J Hennenlotter, T Wiesner, L Just, M Bonin, W Aicher, HJ Buhring, U Mattheus, A Mack, HJ Wagner, S Minger, M Matzkies, M Reppel, J Hescheler, KD Sievert, A Stenzl and T Skutella: Generation of pluripotent stem cells from adult human testis. *Nature* 456, 344-9 (2008)

30. L Simon, GC Ekman, N Kostereva, Z Zhang, RA Hess, MC Hofmann and PS Cooke: Direct transdifferentiation of stem/progenitor spermatogonia into reproductive and nonreproductive tissues of all germ layers. *Stem Cells* 27, 1666-75 (2009)

31. N Golestaneh, M Kokkinaki, D Pant, J Jiang, D DeStefano, C Fernandez-Bueno, JD Rone, BR Haddad, GI Gallicano and M Dym: Pluripotent stem cells derived from adult human testes. *Stem Cells Dev* 18, 1115-26 (2009)

32. D Bryder, DJ Rossi and IL Weissman: Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am J Pathol* 169, 338-46 (2006)

33. MF Pittenger, AM Mackay, SC Beck, RK Jaiswal, R Douglas, JD Mosca, MA Moorman, DW Simonetti, S Craig and DR Marshak: Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143-7 (1999)

34. M Krampera, G Pizzolo, G Aprili and M Franchini: Mesenchymal stem cells for bone, cartilage, tendon and skeletal muscle repair. *Bone* 39, 678-83 (2006)

35. EJ Gang, D Bosnakovski, CA Figueiredo, JW Visser and RC Perlingeiro: SSEA-4 identifies mesenchymal stem cells from bone marrow. *Blood* 109, 1743-51 (2007)

36. HE Broxmeyer, GW Douglas, G Hangoc, S Cooper, J Bard, D English, M Arny, L Thomas and EA Boyse: Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci U S A* 86, 3828-32 (1989)

37. EM Horwitz, DJ Prockop, LA Fitzpatrick, WW Koo, PL Gordon, M Neel, M Sussman, P Orchard, JC Marx, RE Pyeritz and MK Brenner: Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 5, 309-13 (1999)

38. R Quarto, M Mastrogiacomo, R Cancedda, SM Kutepov, V Mukhachev, A Lavroukov, E Kon and M Marcacci: Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* 344, 385-6 (2001)

39. EM Horwitz, DJ Prockop, PL Gordon, WW Koo, LA Fitzpatrick, MD Neel, ME McCarville, PJ Orchard, RE Pyeritz and MK Brenner: Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 97, 1227-31 (2001)

40. K Le Blanc, C Gotherstrom, O Ringden, M Hassan, R McMahon, E Horwitz, G Anneren, O Axelsson, J Nunn, U Ewald, S Norden-Lindeberg, M Jansson, A Dalton, E Astrom and M Westgren: Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 79, 1607-14 (2005)

41. TR Brazelton, FM Rossi, GI Keshet and HM Blau: From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 290, 1775-9 (2000)

42. E Mezey, KJ Chandross, G Harta, RA Maki and SR McKercher: Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science* 290, 1779-82 (2000)

43. JJ Ross and CM Verfaillie: Evaluation of neural plasticity in adult stem cells. *Philos Trans R Soc Lond B Biol Sci* 363, 199-205 (2008)

44. BE Reubinoff, P Itsykson, T Turetsky, MF Pera, E Reinhartz, A Itzik and T Ben-Hur: Neural progenitors from human embryonic stem cells. *Nat Biotechnol* 19, 1134-40 (2001)

45. V Tabar, G Panagiotakos, ED Greenberg, BK Chan, M Sadelain, PH Gutin and L Studer: Migration and differentiation of neural precursors derived from human embryonic stem cells in the rat brain. *Nat Biotechnol* 23, 601-6 (2005)

46. P Jiang, V Selvaraj and W Deng: Differentiation of embryonic stem cells into oligodendrocyte precursors. *J Vis Exp* 39 (2010)

47. I Kehat, L Khimovich, O Caspi, A Gepstein, R Shofti, G Arbel, I Huber, J Satin, J Itskovitz-Eldor and L Gepstein: Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol* 22, 1282-9 (2004)

48. JQ He, Y Ma, Y Lee, JA Thomson and TJ Kamp: Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res* 93, 32-9 (2003)

49. K Chadwick, L Wang, L Li, P Menendez, B Murdoch, A Rouleau and M Bhatia: Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. *Blood* 102, 906-15 (2003)

50. AL Olsen, DL Stachura and MJ Weiss: Designer blood: creating hematopoietic lineages from embryonic stem cells. *Blood* 107, 1265-75 (2006)

51. S Sinha, BR Wamhoff, MH Hoofnagle, J Thomas, RL Neppl, T Deering, BP Helmke, DK Bowles, AV Somlyo and GK Owens: Assessment of contractility of purified smooth muscle cells derived from embryonic stem cells. Stem Cells 24, 1678-88 (2006)

52. S Assady, G Maor, M Amit, J Itskovitz-Eldor, KL Skorecki and M Tzukerman: Insulin production by human embryonic stem cells. Diabetes 50, 1691-7 (2001)

53. KA D'Amour, AG Bang, S Eliazer, OG Kelly, AD Agulnick, NG Smart, MA Moorman, E Kroon, MK Carpenter and EE Baetge: Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 24, 1392-401 (2006)

54. S Levenberg, JS Golub, M Amit, J Itskovitz-Eldor and R Langer: Endothelial cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 99, 4391-6 (2002)

55. VL Bautch: Embryonic stem cell differentiation and the vascular lineage. Methods Mol Biol 185, 117-25 (2002)

56. JL Tremoleda, NR Forsyth, NS Khan, D Wojtacha, I Christodoulou, BJ Tye, SN Racey, S Collishaw, V Sottile, AJ Thomson, AH Simpson, BS Noble and J McWhir: Bone tissue formation from human embryonic stem cells in vivo. *Cloning Stem Cells* 10, 119-32 (2008)

57. V Sottile, A Thomson and J McWhir: In vitro osteogenic differentiation of human ES cells. *Cloning Stem Cells* 5, 149-55 (2003)

58. H Shirahashi, J Wu, N Yamamoto, A Catana, H Wege, B Wager, K Okita and MA Zern: Differentiation of human and mouse embryonic stem cells along a hepatocyte lineage. *Cell Transplant* 13, 197-211 (2004)

59. M Gropp, P Itsykson, O Singer, T Ben-Hur, E Reinhartz, E Galun and BE Reubinoff: Stable genetic modification of human embryonic stem cells by lentiviral vectors. *Mol Ther* 7, 281-7 (2003)

60. AS Daar and L Sheremeta: The science of stem cells: ethical, legal and social issues. *Exp Clin Transplant* 1, 139-46 (2003)

61. M Drukker, G Katz, A Urbach, M Schuldiner, G Markel, J Itskovitz-Eldor, B Reubinoff, O Mandelboim and N Benvenisty: Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc Natl Acad Sci U S A* 99, 9864-9 (2002)

62. M Drukker and N Benvenisty: The immunogenicity of human embryonic stem-derived cells. *Trends Biotechnol* 22, 136-41 (2004)

63. T Fujikawa, SH Oh, L Pi, HM Hatch, T Shupe and BE Petersen: Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. *Am J Pathol* 166, 1781-91 (2005)

64. SA Przyborski: Differentiation of human embryonic stem cells after transplantation in immune-deficient mice. *Stem Cells* 23, 1242-50 (2005)

65. NS Roy, C Cleren, SK Singh, L Yang, MF Beal and SA Goldman: Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat Med* 12, 1259-68 (2006)

66. T Sasazuki, T Juji, Y Morishima, N Kinukawa, H Kashiwabara, H Inoko, T Yoshida, A Kimura, T Akaza, N Kamikawaji, Y Kodera and F Takaku: Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. *N Engl J Med* 339, 1177-85 (1998)

67. CJ Taylor, EM Bolton, S Pocock, LD Sharples, RA Pedersen and JA Bradley: Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. *Lancet* 366, 2019-25 (2005)

68. K Takahashi and S Yamanaka: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-76 (2006)

69. K Takahashi, K Tanabe, M Ohnuki, M Narita, T Ichisaka, K Tomoda and S Yamanaka: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861-72 (2007)

70. J Yu, MA Vodyanik, K Smuga-Otto, J Antosiewicz-Bourget, JL Frane, S Tian, J Nie, GA Jonsdottir, V Ruotti, R Stewart, Slukvin, II and JA Thomson: Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917-20 (2007)

71. K Okita, M Nakagawa, H Hyenjong, T Ichisaka and S Yamanaka: Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 322, 949-53 (2008)

72. M Stadtfeld, M Nagaya, J Utikal, G Weir and K Hochedlinger: Induced pluripotent stem cells generated without viral integration. *Science* 322, 945-9 (2008)

73. K Woltjen, IP Michael, P Mohseni, R Desai, M Mileikovsky, R Hamalainen, R Cowling, W Wang, P Liu, M Gertsenstein, K Kaji, HK Sung and A Nagy: piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* 458, 766-70 (2009)

74. H Zhou, S Wu, JY Joo, S Zhu, DW Han, T Lin, S Trauger, G Bien, S Yao, Y Zhu, G Siuzdak, HR Scholer, L Duan and S Ding: Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 4, 381-4 (2009)

75. M Stadtfeld, E Apostolou, H Akutsu, A Fukuda, P Follett, S Natesan, T Kono, T Shioda and K Hochedlinger: Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* 465, 175-81 (2010)

76. S Gajovic, L St-Onge, Y Yokota and P Gruss: Retinoic acid mediates Pax6 expression during in vitro differentiation of embryonic stem cells. *Differentiation* 62, 187-92 (1997)

77. Y Okada, T Shimazaki, G Sobue and H Okano: Retinoic-acid-concentration-dependent acquisition of neural cell identity during in vitro differentiation of mouse embryonic stem cells. *Dev Biol* 275, 124-42 (2004)

78. M Kawamorita, C Suzuki, G Saito, T Sato and K Sato: In vitro differentiation of mouse embryonic stem cells after activation by retinoic acid. *Hum Cell* 15, 178-82 (2002)

79. XJ Li, ZW Du, ED Zarnowska, M Pankratz, LO Hansen, RA Pearce and SC Zhang: Specification of motoneurons from human embryonic stem cells. *Nat Biotechnol* 23, 215-21 (2005)

80. MF Pera, J Andrade, S Houssami, B Reubinoff, A Trounson, EG Stanley, D Ward-van Oostwaard and C Mummery: Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. *J Cell Sci* 117, 1269-80 (2004)

81. SM Chambers, CA Fasano, EP Papapetrou, M Tomishima, M Sadelain and L Studer: Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* 27, 275-80 (2009)

82. J Itskovitz-Eldor, M Schuldiner, D Karsenti, A Eden, O Yanuka, M Amit, H Soreq and N Benvenisty: Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol Med* 6, 88-95 (2000)

83. S Levenberg, NF Huang, E Lavik, AB Rogers, J Itskovitz-Eldor and R Langer: Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. *Proc Natl Acad Sci U S A* 100, 12741-6 (2003)

84. P Itsykson, N Ilouz, T Turetsky, RS Goldstein, MF Pera, I Fishbein, M Segal and BE Reubinoff: Derivation of neural precursors from human embryonic stem cells in the presence of noggin. *Mol Cell Neurosci* 30, 24-36 (2005)

85. H Baharvand, NZ Mehrjardi, M Hatami, S Kiani, M Rao and MM Haghighi: Neural differentiation from human embryonic stem cells in a defined adherent culture condition. *Int J Dev Biol* 51, 371-8 (2007)

86. L Gerrard, L Rodgers and W Cui: Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. *Stem Cells* 23, 1234-41 (2005)

87. H Kawasaki, H Suemori, K Mizuseki, K Watanabe, F Urano, H Ichinose, M Haruta, M Takahashi, K Yoshikawa, S Nishikawa, N Nakatsuji and Y Sasai: Generation of dopaminergic neurons and pigmented epithelia from primate ES cells by stromal cell-derived inducing activity. *Proc Natl Acad Sci U S A* 99, 1580-5 (2002)

88. H Lee, GA Shamy, Y Elkabetz, CM Schofield, NL Harrsion, G Panagiotakos, ND Socci, V Tabar and L Studer: Directed differentiation and transplantation of human embryonic stem cell-derived motoneurons. *Stem Cells* 25, 1931-9 (2007)

89. CH Park, YK Minn, JY Lee, DH Choi, MY Chang, JW Shim, JY Ko, HC Koh, MJ Kang, JS Kang, DJ Rhie, YS Lee, H Son, SY Moon, KS Kim and SH Lee: In vitro and in vivo analyses of human embryonic stem cell-derived dopamine neurons. *J Neurochem* 92, 1265-76 (2005)

90. AL Perrier, V Tabar, T Barberi, ME Rubio, J Bruses, N Topf, NL Harrison and L Studer: Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci U S A* 101, 12543-8 (2004)

91. M Ideguchi, TD Palmer, LD Recht and JM Weimann: Murine embryonic stem cell-derived pyramidal neurons integrate into the cerebral cortex and appropriately project axons to subcortical targets. *J Neurosci* 30, 894-904 (2010)

92. J Sanchez-Ramos, S Song, F Cardozo-Pelaez, C Hazzi, T Stedeford, A Willing, TB Freeman, S Saporta, W Janssen, N Patel, DR Cooper and PR Sanberg: Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol* 164, 247-56 (2000)

93. JR Sanchez-Ramos: Neural cells derived from adult bone marrow and umbilical cord blood. *J Neurosci Res* 69, 880-93 (2002)

94. D Woodbury, EJ Schwarz, DJ Prockop and IB Black: Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 61, 364-70 (2000)

95. A Joannides, P Gaughwin, C Schwiening, H Majed, J Sterling, A Compston and S Chandran: Efficient generation of neural precursors from adult human skin: astrocytes promote neurogenesis from skin-derived stem cells. *Lancet* 364, 172-8 (2004)

96. KM Safford, SD Safford, JM Gimble, AK Shetty and HE Rice: Characterization of neuronal/glial differentiation of murine adipose-derived adult stromal cells. *Exp Neurol* 187, 319-28 (2004)

97. SC Zhang, M Wernig, ID Duncan, O Brustle and JA Thomson: In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol* 19, 1129-33 (2001)

98. MT Pankratz, XJ Li, TM Lavaute, EA Lyons, X Chen and SC Zhang: Directed neural differentiation of human embryonic stem cells via an obligated primitive anterior stage. *Stem Cells* 25, 1511-20 (2007)

99. M Bibikova, E Chudin, B Wu, L Zhou, EW Garcia, Y Liu, S Shin, TW Plaia, JM Auerbach, DE Arking, R Gonzalez, J Crook, B Davidson, TC Schulz, A Robins, A Khanna, P Sartipy, J Hyllner, P Vanguri, S Savant-Bhonsale, AK Smith, A Chakravarti, A Maitra, M Rao, DL Barker, JF Loring and JB Fan: Human embryonic stem cells have a unique epigenetic signature. *Genome Res* 16, 1075-83 (2006)

100. L Iacovitti, AE Donaldson, CE Marshall, S Suon and M Yang: A protocol for the differentiation of human embryonic stem cells into dopaminergic neurons using only chemically defined human additives: Studies in vitro and in vivo. *Brain Res* 1127, 19-25 (2007)

101. G Lee, H Kim, Y Elkabetz, G Al Shamy, G Panagiotakos, T Barberi, V Tabar and L Studer: Isolation and directed differentiation of neural crest stem cells derived from human embryonic stem cells. *Nat Biotechnol* 25, 1468-75 (2007)

102. TC Schulz, SA Noggle, GM Palmarini, DA Weiler, IG Lyons, KA Pensa, AC Meedeniya, BP Davidson, NA Lambert and BG Condie: Differentiation of human embryonic stem cells to dopaminergic neurons in serum-free suspension culture. *Stem Cells* 22, 1218-38 (2004)

103. JH Kim, JM Auerbach, JA Rodriguez-Gomez, I Velasco, D Gavin, N Lumelsky, SH Lee, J Nguyen, R Sanchez-Pernaute, K Bankiewicz and R McKay: Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* 418, 50-6 (2002)

104. SH Lee, N Lumelsky, L Studer, JM Auerbach and RD McKay: Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat Biotechnol* 18, 675-9 (2000)

105. PH Schwartz, DJ Brick, AE Stover, JF Loring and FJ Muller: Differentiation of neural lineage cells from human pluripotent stem cells. *Methods* 45, 142-58 (2008)

106. Y Elkabetz, G Panagiotakos, G Al Shamy, ND Socci, V Tabar and L Studer: Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. *Genes Dev* 22, 152-65 (2008)

107. BY Hu, ZW Du, XJ Li, M Ayala and SC Zhang: Human oligodendrocytes from embryonic stem cells: conserved SHH signaling networks and divergent FGF effects. *Development* 136, 1443-52 (2009)

108. TM Jessell: Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat Rev Genet* 1, 20-9 (2000)

109. Y Elkabetz and L Studer: Human ESC-derived neural rosettes and neural stem cell progression. *Cold Spring Harb Symp Quant Biol* 73, 377-87 (2008)

110. BY Hu, ZW Du and SC Zhang: Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat Protoc* 4, 1614-22 (2009)

111. BY Hu and SC Zhang: Differentiation of spinal motor neurons from pluripotent human stem cells. *Nat Protoc* 4, 1295-304 (2009)

112. Y Yan, D Yang, ED Zarnowska, Z Du, B Werbel, C Valliere, RA Pearce, JA Thomson and SC Zhang: Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells* 23, 781-90 (2005)

113. SC Zhang: Neural subtype specification from embryonic stem cells. *Brain Pathol* 16, 132-42 (2006)

114. C Guo, HY Qiu, M Shi, Y Huang, RL Johnson, M Rubinstein, SD Chen and YQ Ding: Lmx1b-controlled isthmic organizer is essential for development of midbrain dopaminergic neurons. *J Neurosci* 28, 14097-106 (2008)

115. MP Smidt, SM Smits, H Bouwmeester, FP Hamers, AJ van der Linden, AJ Hellemons, J Graw and JP Burbach: Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene Pitx3. *Development* 131, 1145-55 (2004)

116. JR Jorgensen, B Juliusson, KF Henriksen, C Hansen, S Knudsen, TN Petersen, N Blom, A Seiger and LU Wahlberg: Identification of novel genes regulated in the developing human ventral mesencephalon. *Exp Neurol* 198, 427-37 (2006)

117. AL Ferri, W Lin, YE Mavromatakis, JC Wang, H Sasaki, JA Whitsett and SL Ang: Foxa1 and Foxa2 regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. *Development* 134, 2761-9 (2007)

118. SL Ang: Transcriptional control of midbrain dopaminergic neuron development. *Development* 133, 3499-506 (2006)

119. HH Simon, H Saueressig, W Wurst, MD Goulding and DD O'Leary: Fate of midbrain dopaminergic neurons controlled by the engrailed genes. *J Neurosci* 21, 3126-34 (2001)

120. S Kaufman: Tyrosine hydroxylase. *Adv Enzymol Relat Areas Mol Biol* 70, 103-220 (1995)

121. W Tao and E Lai: Telencephalon-restricted expression of BF-1, a new member of the HNF-3/fork head gene family, in the developing rat brain. *Neuron* 8, 957-66 (1992)

122. W Wurst and L Bally-Cuif: Neural plate patterning: upstream and downstream of the isthmic organizer. *Nat Rev Neurosci* 2, 99-108 (2001)

123. CM Gall, SH Hendry, KB Seroogy, EG Jones and JW Haycock: Evidence for coexistence of GABA and dopamine in neurons of the rat olfactory bulb. *J Comp Neurol* 266, 307-18 (1987)

124. T Kosaka, K Kosaka, Y Hataguchi, I Nagatsu, JY Wu, OP Ottersen, J Storm-Mathisen and K Hama: Catecholaminergic neurons containing GABA-like and/or glutamic acid decarboxylase-like immunoreactivities in various brain regions of the rat. *Exp Brain Res* 66, 191-210 (1987)

125. M Denham and M Dottori: Signals involved in neural differentiation of human embryonic stem cells. *Neurosignals* 17, 234-41 (2009)

126. MS Cho, DY Hwang and DW Kim: Efficient derivation of functional dopaminergic neurons from human embryonic stem cells on a large scale. *Nat Protoc* 3, 1888-94 (2008)

127. E Roussa and K Krieglstein: Induction and specification of midbrain dopaminergic cells: focus on SHH, FGF8, and TGF-beta. *Cell Tissue Res* 318, 23-33 (2004)

128. CE Chamberlain, J Jeong, C Guo, BL Allen and AP McMahon: Notochord-derived Shh concentrates in close association with the apically positioned basal body in neural target cells and forms a dynamic gradient during neural patterning. *Development* 135, 1097-106 (2008)

129. H Nakamura, T Sato and A Suzuki-Hirano: Isthmus organizer for mesencephalon and metencephalon. *Dev Growth Differ* 50 Suppl 1, S113-8 (2008)

130. L Lillien and H Raphael: BMP and FGF regulate the development of EGF-responsive neural progenitor cells. *Development* 127, 4993-5005 (2000)

131. A Liu and AL Joyner: Early anterior/posterior patterning of the midbrain and cerebellum. *Annu Rev Neurosci* 24, 869-96 (2001)

132. TC Schulz, GM Palmarini, SA Noggle, DA Weiler, MM Mitalipova and BG Condie: Directed neuronal

differentiation of human embryonic stem cells. *BMC Neurosci* 4, 27 (2003)

133. KC Sonntag, J Pruszak, T Yoshizaki, J van Arensbergen, R Sanchez-Pernaute and O Isacson: Enhanced yield of neuroepithelial precursors and midbrain-like dopaminergic neurons from human embryonic stem cells using the bone morphogenic protein antagonist noggin. *Stem Cells* 25, 411-8 (2007)

134. M Dezawa, H Kanno, M Hoshino, H Cho, N Matsumoto, Y Itokazu, N Tajima, H Yamada, H Sawada, H Ishikawa, T Mimura, M Kitada, Y Suzuki and C Ide: Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. *J Clin Invest* 113, 1701-10 (2004)

135. YS Fu, YC Cheng, MY Lin, H Cheng, PM Chu, SC Chou, YH Shih, MH Ko and MS Sung: Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: potential therapeutic application for Parkinsonism. *Stem Cells* 24, 115-24 (2006)

136. WD Richardson, HK Smith, T Sun, NP Pringle, A Hall and R Woodruff: Oligodendrocyte lineage and the motor neuron connection. *Glia* 29, 136-42 (2000)

137. KL Ligon, SP Fancy, RJ Franklin and DH Rowitch: Olig gene function in CNS development and disease. *Glia* 54, 1-10 (2006)

138. NP Pringle, WP Yu, S Guthrie, H Roelink, A Lumsden, AC Peterson and WD Richardson: Determination of neuroepithelial cell fate: induction of the oligodendrocyte lineage by ventral midline cells and sonic hedgehog. *Dev Biol* 177, 30-42 (1996)

139. UM Lim, KS Sidhu and BE Tuch: Derivation of Motor Neurons from three Clonal Human Embryonic Stem Cell Lines. *Curr Neurovasc Res* 3, 281-8 (2006)

140. XJ Li, BY Hu, SA Jones, YS Zhang, T Lavaute, ZW Du and SC Zhang: Directed differentiation of ventral spinal progenitors and motor neurons from human embryonic stem cells by small molecules. *Stem Cells* 26, 886-93 (2008)

141. S Karumbayaram, BG Novitch, M Patterson, JA Umbach, L Richter, A Lindgren, AE Conway, AT Clark, SA Goldman, K Plath, M Wiedau-Pazos, HI Kornblum and WE Lowry: Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem Cells* 27, 806-11 (2009)

142. N Baumann and D Pham-Dinh: Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev* 81, 871-927 (2001)

143. H Xue, S Wu, ST Papadeas, S Spusta, AM Swistowska, CC MacArthur, MP Mattson, NJ Maragakis, MR Capecchi, MS Rao, X Zeng and Y Liu: A targeted neuroglial reporter line generated by homologous recombination in human embryonic stem cells. *Stem Cells* 27, 1836-46 (2009)

144. GI Nistor, MO Totoiu, N Haque, MK Carpenter and HS Keirstead: Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia* 49, 385-96 (2005)

145. SM Kang, MS Cho, H Seo, CJ Yoon, SK Oh, YM Choi and DW Kim: Efficient induction of oligodendrocytes from human embryonic stem cells. *Stem Cells* 25, 419-24 (2007)

146. M Izrael, P Zhang, R Kaufman, V Shinder, R Ella, M Amit, J Itskovitz-Eldor, J Chebath and M Revel: Human oligodendrocytes derived from embryonic stem cells: Effect of noggin on phenotypic differentiation in vitro and on myelination in vivo. *Mol Cell Neurosci* 34, 310-23 (2007)

147. JE Gil, DH Woo, JH Shim, SE Kim, HJ You, SH Park, SH Paek, SK Kim and JH Kim: Vitronectin promotes oligodendrocyte differentiation during neurogenesis of human embryonic stem cells. *FEBS Lett* 583, 561-7 (2009)

148. L Buzanska, EK Machaj, B Zablocka, Z Pojda and K Domanska-Janik: Human cord blood-derived cells attain neuronal and glial features in vitro. *J Cell Sci* 115, 2131-8 (2002)

149. VR Dasari, DG Spomar, CS Gondi, CA Sloffer, KL Saving, M Gujrati, JS Rao and DH Dinh: Axonal remyelination by cord blood stem cells after spinal cord injury. *J Neurotrauma* 24, 391-410 (2007)

150. MS Rao and M Jacobson: Astrocyte development. In: Developmental Neurobiology. Kluwer Academic/Plenum Publishers, New York (2005)

151. RP Skoff: Gliogenesis in rat optic nerve: astrocytes are generated in a single wave before oligodendrocytes. *Dev Biol* 139, 149-68 (1990)

152. SW Levison and JE Goldman: Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. *Neuron* 10, 201-12 (1993)

153. NP Pringle, S Guthrie, A Lumsden and WD Richardson: Dorsal spinal cord neuroepithelium generates astrocytes but not oligodendrocytes. *Neuron* 20, 883-93 (1998)

154. GE Gray and JR Sanes: Migratory paths and phenotypic choices of clonally related cells in the avian optic tectum. *Neuron* 6, 211-25 (1991)

155. P Malatesta, E Hartfuss and M Gotz: Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* 127, 5253-63 (2000)

156. SC Noctor, AC Flint, TA Weissman, RS Dammerman and AR Kriegstein: Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 409, 714-20 (2001)

157. F Doetsch, I Caille, DA Lim, JM Garcia-Verdugo and A Alvarez-Buylla: Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97, 703-16 (1999)

158. ED Laywell, P Rakic, VG Kukekov, EC Holland and DA Steindler: Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci U S A* 97, 13883-8 (2000)

159. F Doetsch: The glial identity of neural stem cells. *Nat Neurosci* 6, 1127-34 (2003)

160. A Buffo, I Rite, P Tripathi, A Lepier, D Colak, AP Horn, T Mori and M Gotz: Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. *Proc Natl Acad Sci U S A* 105, 3581-6 (2008)

161. AM Clement, MD Nguyen, EA Roberts, ML Garcia, S Boillee, M Rule, AP McMahon, W Doucette, D Siwek, RJ Ferrante, RH Brown, Jr., JP Julien, LS Goldstein and DW Cleveland: Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302, 113-7 (2003)

162. A Trounson: The production and directed differentiation of human embryonic stem cells. *Endocr Rev* 27, 208-19 (2006)

163. DS Lee, K Yu, JY Rho, E Lee, JS Han, DB Koo, YS Cho, J Kim, KK Lee and YM Han: Cyclopamine treatment of human embryonic stem cells followed by culture in human astrocyte medium promotes differentiation into nestin- and GFAP-expressing astrocytic lineage. *Life Sci* 80, 154-9 (2006)

164. S Wislet-Gendebien, P Leprince, G Moonen and B Rogister: Regulation of neural markers nestin and GFAP expression by cultivated bone marrow stromal cells. *J Cell Sci* 116, 3295-302 (2003)

165. BH Choi: Hematogenous cells in the central nervous system of developing human embryos and fetuses. *J Comp Neurol* 196, 683-94 (1981)

166. P Rezaie and D Male: Colonisation of the developing human brain and spinal cord by microglia: a review. *Microsc Res Tech* 45, 359-82 (1999)

167. MA Eglitis and E Mezey: Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci U S A* 94, 4080-5 (1997)

168. AR Simard and S Rivest: Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J* 18, 998-1000 (2004)

169. WJ Streit and QS Xue: Life and death of microglia. *J Neuroimmune Pharmacol* 4, 371-9 (2009)

170. M Schwartz, O Butovsky, W Bruck and UK Hanisch: Microglial phenotype: is the commitment reversible? *Trends Neurosci* 29, 68-74 (2006)

171. TB Yu, YS Cheng, P Zhao, DW Kou, K Sun, BH Chen and AM Wang: Immune therapy with cultured microglia grafting into the injured spinal cord promoting the recovery of rat's hind limb motor function. *Chin J Traumatol* 12, 291-5 (2009)

172. K Takata, Y Kitamura, D Yanagisawa, S Morikawa, M Morita, T Inubushi, D Tsuchiya, S Chishiro, M Saeki, T Taniguchi, S Shimohama and I Tooyama: Microglial transplantation increases amyloid-beta clearance in Alzheimer model rats. *FEBS Lett* 581, 475-8 (2007)

173. D Narantuya, A Nagai, AM Sheikh, K Wakabayashi, Y Shiota, T Watanabe, J Masuda, S Kobayashi, SU Kim and S Yamaguchi: Microglia transplantation attenuates white matter injury in rat chronic ischemia model via matrix metalloproteinase-2 inhibition. *Brain Res* 1316, 145-52 (2010)

174. T Tsuchiya, KC Park, S Toyonaga, SM Yamada, H Nakabayashi, E Nakai, N Ikawa, M Furuya, A Tominaga and K Shimizu: Characterization of microglia induced from mouse embryonic stem cells and their migration into the brain parenchyma. *J Neuroimmunol* 160, 210-8 (2005)

175. DN Angelov, S Arnhold, C Andressen, H Grabsch, M Puschmann, J Hescheler and K Addicks: Temporospatial relationships between macroglia and microglia during in vitro differentiation of murine stem cells. *Dev Neurosci* 20, 42-51 (1998)

176. I Napoli, K Kierdorf and H Neumann: Microglial precursors derived from mouse embryonic stem cells. *Glia* 57, 1660-71 (2009)

177. SL Rossi and HS Keirstead: Stem cells and spinal cord regeneration. *Curr Opin Biotechnol* 20, 552-62 (2009)

178. M Ronaghi, S Erceg, V Moreno-Manzano and M Stojkovic: Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? *Stem Cells* 28, 93-9 (2009)

179. Q Chen, Y Long, X Yuan, L Zou, J Sun, S Chen, JR Perez-Polo and K Yang: Protective effects of bone marrow stromal cell transplantation in injured rodent brain: synthesis of neurotrophic factors. *J Neurosci Res* 80, 611-9 (2005)

180. Y Li and G Raisman: Sprouts from cut corticospinal axons persist in the presence of astrocytic scarring in long-term lesions of the adult rat spinal cord. *Exp Neurol* 134, 102-11 (1995)

181. Y Ke, L Chi, R Xu, C Luo, D Gozal and R Liu: Early response of endogenous adult neural progenitor cells to

acute spinal cord injury in mice. *Stem Cells* 24, 1011-9 (2006)

182. AJ Mothe and CH Tator: Proliferation, migration, and differentiation of endogenous ependymal region stem/progenitor cells following minimal spinal cord injury in the adult rat. *Neuroscience* 131, 177-87 (2005)

183. J Silver and JH Miller: Regeneration beyond the glial scar. *Nat Rev Neurosci* 5, 146-56 (2004)

184. QL Cao, RM Howard, JB Dennison and SR Whittemore: Differentiation of engrafted neuronal-restricted precursor cells is inhibited in the traumatically injured spinal cord. *Exp Neurol* 177, 349-59 (2002)

185. BJ Cummings, N Uchida, SJ Tamaki and AJ Anderson: Human neural stem cell differentiation following transplantation into spinal cord injured mice: association with recovery of locomotor function. *Neurol Res* 28, 474-81 (2006)

186. BJ Cummings, N Uchida, SJ Tamaki, DL Salazar, M Hooshmand, R Summers, FH Gage and AJ Anderson: Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. *Proc Natl Acad Sci U S A* 102, 14069-74 (2005)

187. M Vroemen, L Aigner, J Winkler and N Weidner: Adult neural progenitor cell grafts survive after acute spinal cord injury and integrate along axonal pathways. *Eur J Neurosci* 18, 743-51 (2003)

188. DJ Webber, EJ Bradbury, SB McMahon and SL Minger: Transplanted neural progenitor cells survive and differentiate but achieve limited functional recovery in the lesioned adult rat spinal cord. *Regen Med* 2, 929-45 (2007)

189. CP Hofstetter, NA Holmstrom, JA Lilja, P Schweinhardt, J Hao, C Spenger, Z Wiesenfeld-Hallin, SN Kurpad, J Frisen and L Olson: Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. *Nat Neurosci* 8, 346-53 (2005)

190. T Mitsui, JS Shumsky, AC Lepore, M Murray and I Fischer: Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and motor functions, decreases thermal hypersensitivity, and modifies intraspinal circuitry. *J Neurosci* 25, 9624-36 (2005)

191. RP Bunge, MB Bunge and Rish: Electron microscopic study of demyelination in an experimentally induced lesion in adult cat spinal cord. *J Biophys Biochem Cytol* 7, 685-96 (1960)

192. FM Bareyre: Neuronal repair and replacement in spinal cord injury. *J Neurol Sci* 265, 63-72 (2008)

193. HS Keirstead, G Nistor, G Bernal, M Totoiu, F Cloutier, K Sharp and O Steward: Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. *J Neurosci* 25, 4694-705 (2005)

194. YW Zhang, J Denham and RS Thies: Oligodendrocyte progenitor cells derived from human embryonic stem cells express neurotrophic factors. *Stem Cells Dev* 15, 943-52 (2006)

195. DP Ankeny, DM McTigue and LB Jakeman: Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. *Exp Neurol* 190, 17-31 (2004)

196. D Cizkova, J Rosocha, I Vanicky, S Jergova and M Cizek: Transplants of human mesenchymal stem cells improve functional recovery after spinal cord injury in the rat. *Cell Mol Neurobiol* 26, 1167-80 (2006)

197. S Wu, Y Suzuki, Y Ejiri, T Noda, H Bai, M Kitada, K Kataoka, M Ohta, H Chou and C Ide: Bone marrow stromal cells enhance differentiation of cocultured neurosphere cells and promote regeneration of injured spinal cord. *J Neurosci Res* 72, 343-51 (2003)

198. SH Yoon, YS Shim, YH Park, JK Chung, JH Nam, MO Kim, HC Park, SR Park, BH Min, EY Kim, BH Choi, H Park and Y Ha: Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. Stem Cells 25, 2066-73 (2007)

199. F Callera and RX do Nascimento: Delivery of autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique in patients with spinal cord injury: a preliminary safety study. Exp Hematol 34, 130-1 (2006)

200. KK Jain: Cell therapy for CNS trauma. Mol Biotechnol 42, 367-76 (2009)

201. HC Park, YS Shim, Y Ha, SH Yoon, SR Park, BH Choi and HS Park: Treatment of complete spinal cord injury patients by autologous bone marrow cell transplantation and administration of granulocyte-macrophage colony stimulating factor. Tissue Eng 11, 913-22 (2005)

202. J Goolsby, MC Marty, D Heletz, J Chiappelli, G Tashko, D Yarnell, PS Fishman, S Dhib-Jalbut, CT Bever, Jr., B Pessac and D Trisler: Hematopoietic progenitors express neural genes. Proc Natl Acad Sci U S A 100, 14926-31 (2003)

203. GJ del Zoppo, K Poeck, MS Pessin, SM Wolpert, AJ Furlan, A Ferbert, MJ Alberts, JA Zivin, L Wechsler, O Busse and et al.: Recombinant tissue plasminogen activator in acute thrombotic and embolic stroke. Ann Neurol 32, 78-86 (1992)

204. R Dubinsky and SM Lai: Mortality of stroke patients treated with thrombolysis: analysis of nationwide inpatient sample. *Neurology* 66, 1742-4 (2006)

205. CT Ekdahl, Z Kokaia and O Lindvall: Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience* 158, 1021-9 (2009)

206. C Buhnemann, A Scholz, C Bernreuther, CY Malik, H Braun, M Schachner, KG Reymann and M Dihne: Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain* 129, 3238-48 (2006)

207. T England, P Martin and PM Bath: Stem cells for enhancing recovery after stroke: a review. *Int J Stroke* 4, 101-10 (2009)

208. O Lindvall and Z Kokaia: Stem cells in human neurodegenerative disorders--time for clinical translation? *J Clin Invest* 120, 29-40 (2010)

209. V Darsalia, SJ Allison, C Cusulin, E Monni, D Kuzdas, T Kallur, O Lindvall and Z Kokaia: Cell number and timing of transplantation determine survival of human neural stem cell grafts in stroke-damaged rat brain. *J Cereb Blood Flow Metab* (2010)

210. MM Daadi, AL Maag and GK Steinberg: Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. *PLoS One* 3, e1644 (2008)

211. MM Daadi, Z Li, A Arac, BA Grueter, M Sofilos, RC Malenka, JC Wu and GK Steinberg: Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. *Mol Ther* 17, 1282-91 (2009)

212. S Kelly, TM Bliss, AK Shah, GH Sun, M Ma, WC Foo, J Masel, MA Yenari, IL Weissman, N Uchida, T Palmer and GK Steinberg: Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *Proc Natl Acad Sci U S A* 101, 11839-44 (2004)

213. T Kallur, V Darsalia, O Lindvall and Z Kokaia: Human fetal cortical and striatal neural stem cells generate region-specific neurons in vitro and differentiate extensively to neurons after intrastriatal transplantation in neonatal rats. *J Neurosci Res* 84, 1630-44 (2006)

214. M Bacigaluppi, S Pluchino, L Peruzzotti Jametti, E Kilic, U Kilic, G Salani, E Brambilla, MJ West, G Comi, G Martino and DM Hermann: Delayed post-ischaemic neuroprotection following systemic neural stem cell transplantation involves multiple mechanisms. *Brain* 132, 2239-51 (2009)

215. AL Ho, S Keshavarzi and ML Levy: Exploitation of genetically modified neural stem cells for neurological disease. *Adv Exp Med Biol* 671, 74-92 (2010)

216. KM Harms, L Li and LA Cunningham: Murine neural stem/progenitor cells protect neurons against ischemia by HIF-1alpha-regulated VEGF signaling. *PLoS One* 5, e9767 (2010)

217. K Toyama, O Honmou, K Harada, J Suzuki, K Houkin, H Hamada and JD Kocsis: Therapeutic benefits of angiogenetic gene-modified human mesenchymal stem cells after cerebral ischemia. *Exp Neurol* 216, 47-55 (2009)

218. H Ohtaki, JH Ylostalo, JE Foraker, AP Robinson, RL Reger, S Shioda and DJ Prockop: Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. *Proc Natl Acad Sci U S A* 105, 14638-43 (2008)

219. YP Liu, H Seckin, Y Izci, ZW Du, YP Yan and MK Baskaya: Neuroprotective effects of mesenchymal stem cells derived from human embryonic stem cells in transient focal cerebral ischemia in rats. *J Cereb Blood Flow Metab* 29, 780-91 (2009)

220. T Onda, O Honmou, K Harada, K Houkin, H Hamada and JD Kocsis: Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. *J Cereb Blood Flow Metab* 28, 329-40 (2008)

221. RH Miller, L Bai, DP Lennon and AI Caplan: The potential of mesenchymal stem cells for neural repair. *Discov Med* 9, 236-42 (2010)

222. F Locatelli, A Bersano, E Ballabio, S Lanfranconi, D Papadimitriou, S Strazzer, N Bresolin, GP Comi and S Corti: Stem cell therapy in stroke. *Cell Mol Life Sci* 66, 757-72 (2009)

223. D Kondziolka, L Wechsler, S Goldstein, C Meltzer, KR Thulborn, J Gebel, P Jannetta, S DeCesare, EM Elder, M McGrogan, MA Reitman and L Bynum: Transplantation of cultured human neuronal cells for patients with stroke. *Neurology* 55, 565-9 (2000)

224. D Kondziolka, GK Steinberg, L Wechsler, CC Meltzer, E Elder, J Gebel, S Decesare, T Jovin, R Zafonte, J Lebowitz, JC Flickinger, D Tong, MP Marks, C Jamieson, D Luu, T Bell-Stephens and J Teraoka: Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. *J Neurosurg* 103, 38-45 (2005)

225. OY Bang, JS Lee, PH Lee and G Lee: Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol* 57, 874-82 (2005)

226. JS Lee, JM Hong, GJ Moon, PH Lee, YH Ahn and OY Bang: A Long-Term Follow-Up Study of Intravenous Autologous Mesenchymal Stem Cell Transplantation in Patients With Ischemic Stroke. *Stem Cells* 28, 1099-1106 (2010)

227. SS Rabinovich, VI Seledtsov, NV Banul, OV Poveshchenko, VV Senyukov, SV Astrakov, DM Samarin

and VY Taraban: Cell therapy of brain stroke. *Bull Exp Biol Med* 139, 126-8 (2005)

228. SI Savitz, J Dinsmore, J Wu, GV Henderson, P Stieg and LR Caplan: Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. *Cerebrovasc Dis* 20, 101-7 (2005)

229. K Pollock, P Stroemer, S Patel, L Stevanato, A Hope, E Miljan, Z Dong, H Hodges, J Price and JD Sinden: A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. *Exp Neurol* 199, 143-55 (2006)

230. P Limousin, P Krack, P Pollak, A Benazzouz, C Ardouin, D Hoffmann and AL Benabid: Electrical stimulation of the subthalamic nucleus in advanced Parkinson's disease. *N Engl J Med* 339, 1105-11 (1998)

231. H Yuan, ZW Zhang, LW Liang, Q Shen, XD Wang, SM Ren, HJ Ma, SJ Jiao and P Liu: Treatment strategies for Parkinson's disease. *Neurosci Bull* 26, 66-76 (2010)

232. O Lindvall and A Bjorklund: Cell therapy in Parkinson's disease. *NeuroRx* 1, 382-93 (2004)

233. O Lindvall, S Rehncrona, P Brundin, B Gustavii, B Astedt, H Widner, T Lindholm, A Bjorklund, KL Leenders, JC Rothwell, R Frackowiak, D Marsden, B Johnels, G Steg, R Freedman, BJ Hoffer, A Seiger, M Bygdeman, I Stromberg and L Olson: Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch Neurol* 46, 615-31 (1989)

234. O Lindvall, G Sawle, H Widner, JC Rothwell, A Bjorklund, D Brooks, P Brundin, R Frackowiak, CD Marsden, P Odin and et al.: Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. *Ann Neurol* 35, 172-80 (1994)

235. O Lindvall, H Widner, S Rehncrona, P Brundin, P Odin, B Gustavii, R Frackowiak, KL Leenders, G Sawle, JC Rothwell and et al.: Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putaminal implants. *Ann Neurol* 31, 155-65 (1992)

236. CR Freed, RE Breeze, NL Rosenberg, SA Schneck, E Kriek, JX Qi, T Lone, YB Zhang, JA Snyder, TH Wells and et al.: Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *N Engl J Med* 327, 1549-55 (1992)

237. CR Freed, NL Rosenberg, SA Schneck and RE Breeze: Improved drug responsiveness following fetal tissue implant for Parkinson's disease. *Neurochem Int* 20 Suppl, 321S-327S (1992)

238. TB Freeman, CW Olanow, RA Hauser, GM Nauert, DA Smith, CV Borlongan, PR Sanberg, DA Holt, JH Kordower, FJ Vingerhoets and et al.: Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. *Ann Neurol* 38, 379-88 (1995)

239. JH Kordower, Y Chu, RA Hauser, TB Freeman and CW Olanow: Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* 14, 504-6 (2008)

240. JY Li, E Englund, JL Holton, D Soulet, P Hagell, AJ Lees, T Lashley, NP Quinn, S Rehncrona, A Bjorklund, H Widner, T Revesz, O Lindvall and P Brundin: Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* 14, 501-3 (2008)

241. CR Freed, PE Greene, RE Breeze, WY Tsai, W DuMouchel, R Kao, S Dillon, H Winfield, S Culver, JQ Trojanowski, D Eidelberg and S Fahn: Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 344, 710-9 (2001)

242. CW Olanow, CG Goetz, JH Kordower, AJ Stoessl, V Sossi, MF Brin, KM Shannon, GM Nauert, DP Perl, J Godbold and TB Freeman: A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol* 54, 403-14 (2003)

243. CW Olanow, K Kieburtz and AH Schapira: Why have we failed to achieve neuroprotection in Parkinson's disease? *Ann Neurol* 64 Suppl 2, S101-10 (2008)

244. DR Rosen, T Siddique, D Patterson, DA Figlewicz, P Sapp, A Hentati, D Donaldson, J Goto, JP O'Regan, HX Deng and et al.: Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362, 59-62 (1993)

245. K Yamanaka, SJ Chun, S Boillee, N Fujimori-Tonou, H Yamashita, DH Gutmann, R Takahashi, H Misawa and DW Cleveland: Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nat Neurosci* 11, 251-3 (2008)

246. JM Harper, C Krishnan, JS Darman, DM Deshpande, S Peck, I Shats, S Backovic, JD Rothstein and DA Kerr: Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron-injured adult rats. *Proc Natl Acad Sci U S A* 101, 7123-8 (2004)

247. DM Deshpande, YS Kim, T Martinez, J Carmen, S Dike, I Shats, LL Rubin, J Drummond, C Krishnan, A Hoke, N Maragakis, J Shefner, JD Rothstein and DA Kerr: Recovery from paralysis in adult rats using embryonic stem cells. *Ann Neurol* 60, 32-44 (2006)

248. H Wichterle, I Lieberam, JA Porter and TM Jessell: Directed differentiation of embryonic stem cells into motor neurons. *Cell* 110, 385-97 (2002)

249. P Soundararajan, GB Miles, LL Rubin, RM Brownstone and VF Rafuse: Motoneurons derived from

embryonic stem cells express transcription factors and develop phenotypes characteristic of medial motor column neurons. *J Neurosci* 26, 3256-68 (2006)

250. AC Lepore, B Rauck, C Dejea, AC Pardo, MS Rao, JD Rothstein and NJ Maragakis: Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. *Nat Neurosci* 11, 1294-301 (2008)

251. DH Hwang, HJ Lee, IH Park, JI Seok, BG Kim, IS Joo and SU Kim: Intrathecal transplantation of human neural stem cells overexpressing VEGF provide behavioral improvement, disease onset delay and survival extension in transgenic ALS mice. *Gene Ther* 16, 1234-44 (2009)

252. DA Kerr, J Llado, MJ Shamblott, NJ Maragakis, DN Irani, TO Crawford, C Krishnan, S Dike, JD Gearhart and JD Rothstein: Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury. *J Neurosci* 23, 5131-40 (2003)

253. SH Appel, JI Engelhardt, JS Henkel, L Siklos, DR Beers, AA Yen, EP Simpson, Y Luo, G Carrum, HE Heslop, MK Brenner and U Popat: Hematopoietic stem cell transplantation in patients with sporadic amyotrophic lateral sclerosis. *Neurology* 71, 1326-34 (2008)

254. L Mazzini, K Mareschi, I Ferrero, E Vassallo, G Oliveri, N Nasuelli, GD Oggioni, L Testa and F Fagioli: Stem cell treatment in Amyotrophic Lateral Sclerosis. *J Neurol Sci* 265, 78-83 (2008)

255. I Ferrero, L Mazzini, D Rustichelli, M Gunetti, K Mareschi, L Testa, N Nasuelli, GD Oggioni and F Fagioli: Bone marrow mesenchymal stem cells from healthy donors and sporadic amyotrophic lateral sclerosis patients. *Cell Transplant* 17, 255-66 (2008)

256. A Vercelli, OM Mereuta, D Garbossa, G Muraca, K Mareschi, D Rustichelli, I Ferrero, L Mazzini, E Madon and F Fagioli: Human mesenchymal stem cell transplantation extends survival, improves motor performance and decreases neuroinflammation in mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 31, 395-405 (2008)

257. HR Martinez, MT Gonzalez-Garza, JE Moreno-Cuevas, E Caro, E Gutierrez-Jimenez and JJ Segura: Stem-cell transplantation into the frontal motor cortex in amyotrophic lateral sclerosis patients. *Cytotherapy* 11, 26-34 (2009)

258. H Deda, MC Inci, AE Kurekci, A Sav, K Kayihan, E Ozgun, GE Ustunsoy and S Kocabay: Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: a 1-year follow-up. *Cytotherapy* 11, 18-25 (2009)

259. HF McFarland and R Martin: Multiple sclerosis: a complicated picture of autoimmunity. *Nat Immunol* 8, 913-9 (2007)

260. DM Wingerchuk: Current evidence and therapeutic strategies for multiple sclerosis. *Semin Neurol* 28, 56-68 (2008)

261. L Bai, DP Lennon, V Eaton, K Maier, AI Caplan, SD Miller and RH Miller: Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia* 57, 1192-203 (2009)

262. E Zappia, S Casazza, E Pedemonte, F Benvenuto, I Bonanni, E Gerdoni, D Giunti, A Ceravolo, F Cazzanti, F Frassoni, G Mancardi and A Uccelli: Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood* 106, 1755-61 (2005)

263. RK Burt, Y Loh, B Cohen, D Stefoski, R Balabanov, G Katsamakis, Y Oyama, EJ Russell, J Stern, P Muraro, J Rose, A Testori, J Bucha, B Jovanovic, F Milanetti, J Storek, JC Voltarelli and WH Burns: Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. *Lancet Neurol* 8, 244-53 (2009)

264. E Capello, L Vuolo, F Gualandi, MT Van Lint, L Roccatagliata, L Bonzano, M Pardini, A Uccelli and G Mancardi: Autologous haematopoietic stem-cell transplantation in multiple sclerosis: benefits and risks. *Neurol Sci* 30 Suppl 2, S175-7 (2009)

265. A Uccelli and G Mancardi: Stem cell transplantation in multiple sclerosis. *Curr Opin Neurol* 23, 218-25 (2010)

266. A Nasef, N Ashammakhi and L Fouillard: Immunomodulatory effect of mesenchymal stromal cells: possible mechanisms. *Regen Med* 3, 531-46 (2008)

267. AJ Nauta, AB Kruisselbrink, E Lurvink, R Willemze and WE Fibbe: Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. *J Immunol* 177, 2080-7 (2006)

268. M Rafei, PM Campeau, A Aguilar-Mahecha, M Buchanan, P Williams, E Birman, S Yuan, YK Young, MN Boivin, K Forner, M Basik and J Galipeau: Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner. *J Immunol* 182, 5994-6002 (2009)

269. G Constantin, S Marconi, B Rossi, S Angiari, L Calderan, E Anghileri, B Gini, SD Bach, M Martinello, F Bifari, M Galie, E Turano, S Budui, A Sbarbati, M Krampera and B Bonetti: Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. *Stem Cells* 27, 2624-35 (2009)

270. S Slavin, BG Kurkalli and D Karussis: The potential use of adult stem cells for the treatment of multiple sclerosis and other neurodegenerative disorders. *Clin Neurol Neurosurg* 110, 943-6 (2008)

271. S Pluchino, A Quattrini, E Brambilla, A Gritti, G Salani, G Dina, R Galli, U Del Carro, S Amadio, A Bergami, R Furlan, G Comi, AL Vescovi and G Martino: Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* 422, 688-94 (2003)

272. S Pluchino, A Gritti, E Blezer, S Amadio, E Brambilla, G Borsellino, C Cossetti, U Del Carro, G Comi, B t Hart, A Vescovi and G Martino: Human neural stem cells ameliorate autoimmune encephalomyelitis in non-human primates. *Ann Neurol* 66, 343-54 (2009)

273. M Aharonowiz, O Einstein, N Fainstein, H Lassmann, B Reubinoff and T Ben-Hur: Neuroprotective effect of transplanted human embryonic stem cell-derived neural precursors in an animal model of multiple sclerosis. *PLoS One* 3, e3145 (2008)

274. CC Shih, SJ Forman, P Chu and M Slovak: Human embryonic stem cells are prone to generate primitive, undifferentiated tumors in engrafted human fetal tissues in severe combined immunodeficient mice. *Stem Cells Dev* 16, 893-902 (2007)

275. PS Knoepfler: Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. *Stem Cells* 27, 1050-6 (2009)

276. A Vats, RC Bielby, NS Tolley, R Nerem and JM Polak: Stem cells. *Lancet* 366, 592-602 (2005)

277. C Foroni, R Galli, B Cipelletti, A Caumo, S Alberti, R Fiocco and A Vescovi: Resilience to transformation and inherent genetic and functional stability of adult neural stem cells ex vivo. *Cancer Res* 67, 3725-33 (2007)

278. E Gerdoni, B Gallo, S Casazza, S Musio, I Bonanni, E Pedemonte, R Mantegazza, F Frassoni, G Mancardi, R Pedotti and A Uccelli: Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. *Ann Neurol* 61, 219-27 (2007)

279. T Schinkothe, W Bloch and A Schmidt: In vitro secreting profile of human mesenchymal stem cells. *Stem Cells Dev* 17, 199-206 (2008)

280. CP Hofstetter, EJ Schwarz, D Hess, J Widenfalk, A El Manira, DJ Prockop and L Olson: Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci U S A* 99, 2199-204 (2002)

281. F Soldner, D Hockemeyer, C Beard, Q Gao, GW Bell, EG Cook, G Hargus, A Blak, O Cooper, M Mitalipova, O Isacson and R Jaenisch: Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* 136, 964-77 (2009)

282. IH Park, N Arora, H Huo, N Maherali, T Ahfeldt, A Shimamura, MW Lensch, C Cowan, K Hochedlinger and GQ Daley: Disease-specific induced pluripotent stem cells. *Cell* 134, 877-86 (2008)

283. JT Dimos, KT Rodolfa, KK Niakan, LM Weisenthal, H Mitsumoto, W Chung, GF Croft, G Saphier, R Leibel, R Goland, H Wichterle, CE Henderson and K Eggan: Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321, 1218-21 (2008)

284. AD Ebert, J Yu, FF Rose, Jr., VB Mattis, CL Lorson, JA Thomson and CN Svendsen: Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 457, 277-80 (2009)

285. F Jia, KD Wilson, N Sun, DM Gupta, M Huang, Z Li, NJ Panetta, ZY Chen, RC Robbins, MA Kay, MT Longaker and JC Wu: A nonviral minicircle vector for deriving human iPS cells. *Nat Methods* 7, 197-9 (2010)

286. L Warren, PD Manos, T Ahfeldt, YH Loh, H Li, F Lau, W Ebina, PK Mandal, ZD Smith, A Meissner, GQ Daley, AS Brack, JJ Collins, C Cowan, TM Schlaeger and DJ Rossi: Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 7, 618-30 (2010)

Key Words: Stem cells, iPS cells, Differentiation, Neural Progenitors, Cell Therapy, Neurons, Oligodendrocytes, Astrocytes, Microglia, Spinal Cord Injury, Stroke, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Review

Send correspondence to: Wenbin Deng, Department of Cell Biology and Human Anatomy, School of Medicine, University of California at Davis, Institute of Pediatric Regenerative Medicine, Shriners Hospitals for Children, Sacramento, CA 95817, Tel: 916-453-2287, Fax: 916-453-2288, E-mail: wbdeng@ucdavis.edu

http://www.bioscience.org/current/vol17.htm