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

Vimal Selvaraj1, Peng Jiang2, Olga Chechneva2, U-Ging Lo2, Wenbin Deng1,3

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

TABLE OF CONTENTS
1. Abstract
2. Introduction
3. Types of stem cells
   3.1. Adult stem cells
   3.2. Embryonic stem cells
   3.3. Induced pluripotent stem cells
4. Differentiation of stem cells into neural lineages
   4.1. Neural progenitors derived from stem cells
   4.2. Generation of dopaminergic neurons
   4.3. Generation of motor neurons and oligodendrocytes
   4.4. Generation of astrocytes
   4.5. Generation of microglia
5. Cell based therapies for nervous system disorders
   5.1. Cell therapy for spinal cord injury
   5.2. Cell therapy for stroke
   5.3. Cell therapy for Parkinson’s disease
   5.4. Cell therapy for amyotrophic lateral sclerosis
   5.5. Cell therapy for multiple sclerosis
6. Conclusions and future perspectives
7. Acknowledgement
8. References

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

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

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 iPSCs 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 then reprogram 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 of iPSCs will become available for cells 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 improve 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 morphogenetic 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 *Forsel,* had the broadest differentiation potential (106). These cells were able to differentiate to neural cell types of anterior-posterior central and peripheral nervous system. *Forsel* 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 *Forsel*-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 rostromedial 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
Differentiating human stem cells into neurons and glial cells for neural repair

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, Nur1, 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 (110, 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 NSC’s 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 (MN) 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...
Differentiating human stem cells into neurons and glial cells for neural repair

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 regimen of growth factors, including FGF2, EGF and PDGF.

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

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 in the CNS, the recent discovery that astrocyte pathology could be a major contributor of neuronal death in amyotrophic 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 myeloid 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 amyotrophic 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 for regenerative events at the vicinity of damaged tissue. At the secondary 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 transplantsations (189). Therefore, transplantation of 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
Differentiating human stem cells into neurons and glial cells for neural repair

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

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

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
Following up, the same group recently reported beneficial activity (223, 224). In another study, intravenous PET scans showed increases in local metabolic ganglia, no significant functional outcome was observed, transplanted into infarcts that affected only the basal immortalized human teratocarcinoma cell line when (222). For example, neuronal cells derived from an stroke have already been attempted with variable outcomes (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.

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 I 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-dihydroxyphenylalanine (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 proteolipid 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 intravenously 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.
Differentiating human stem cells into neurons and glial cells for neural repair

Owing to the roadblocks in hESCs 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


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


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)


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)


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


52. SAssady, GMaor, MAmit, JItskovitz-Eldor, KL Skorecki andMTzukerman: Insulin production by human embryonic stem cells. Diabetes 50, 1691-7 (2001)


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


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


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


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


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)


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


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


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


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)


181. Y Ke, L Chi, R Xu, C Luo, D Gozal and R Liu: Early response of endogenous adult neural progenitor cells to
Differentiating human stem cells into neurons and glial cells for neural repair


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)


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)


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


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)


227. SS Rabinovich, VI Seledtsov, NV Banul, OY Poveshchenko, VV Senyukov, SV Astrakov, DM Samarin
Differentiating human stem cells into neurons and glial cells for neural repair


249. P Soundararajan, GB Miles, LL Rubin, RM Brownstone and VF Rafuse: Motoneurons derived from
Differentiating human stem cells into neurons and glial cells for neural repair


251. DH Hwang, HJ Lee, HI 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)


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


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)


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)


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: wbndeng@ucdavis.edu

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