Review

Stem cell-based cell replacement strategies for the central nervous system

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During human development, cells of the blastocyst inner cell mass proliferate and give rise to each cell in the human body. It is that potential which focuses intense interest on these stem cells as a substrate for cell-based regenerative medicine. An increased understanding of the interrelation of processes that govern the formation of various cell types will allow for the directed differentiation of stem cells into specified cells or tissues that can ameliorate the effects of disease or damage. Perhaps the most difficult cells and tissues to derive for use in cell replacement strategies are the diverse neurons, glia and complex networks of the central nervous system (CNS). Here we present emerging perspectives on the development of neuronal and glial cells from stem cells for clinical application to CNS diseases and injury.

Neurological disorders and CNS trauma, and at least some aspects of psychiatric disorders, are associated with loss or dysfunction of CNS cell types. As mature neural cells do not divide to replenish lost cells, new cells must be generated from endogenous progenitors, neural stem cells (NSCs) or transplanted from an exogenous source. Cell loss is characteristic of most debilitating CNS diseases and injuries, including Parkinson's disease (PD), Huntington's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, stroke, and spinal cord injury (SCI). The basis of cell replacement therapy is to restore those lost cells, or provide appurtenant cells to support dysfunctional cells. Thus, most severe CNS diseases and injuries are amenable to cell replacement strategies.

Despite the complexity of human CNS architecture, CRT is a clinical reality. Reports from the early 1990s [15,25] described the results of transplantations of dopaminergic neurons into PD patients. The donor neurons were derived from the mesencephalic brain region of 7–8-week-aged embryos that contain cells specified to mature into dopaminergic neurons through a cell autonomous process. In these studies, the donor nigral neurons were transplanted into the striatum of patients. Although the cells were transplanted to an ectopic location and did not receive normal afferent innervation of the basal ganglia motor circuit, the transplanted cells engrafted and released dopamine such that motor function improved in some patients. The putative lack of basal ganglia afferents to these engrafted cells suggests that the cells released dopamine at synaptic and non-synaptic sites independent of appropriate control. If this is correct, then these experiments demonstrate the potential of transplanted cells to improve outcome through delivery of extra-circuital biological molecules without veritable cell replacement. Another salient feature of these studies is that mesencephalic determined dopaminergic cells remained committed to that phenotype even when transplanted outside the substantia nigra and into a degenerating and inflammatory environment. Likewise, the transplanted cells did not form tumors. While these studies and subsequent studies [16,40] rendered proof-of-concept for CRT, the use of fetal cells for broader clinical application is dampened by limited availability, the heterogeneous nature of the transplant, insufficient cell expansion, issues with regulatory provisions, and ethical concerns. Thus, to broaden clinical applicability, CRT is dependent upon a cell source that can circumvent these issues.

The clinical application of allogeneic CRT will require a cell type that can meet health agency regulatory guidance, such as those proposed for Chemical, Manufacturing, and Control by the United States Food and Drug Administration (US FDA) Center for Biologics Evaluation and Research, Office of Cellular, Tissue and Gene Therapeutics [http://www.fda.gov/cber/gdlns/cmcsomcell.htm]. Based on FDA guidelines to ensure the safety, identity, purity, and stability of the transplant cells, it would be prudent for manufacturers to select a cell source that is committed to a particular phenotype or tractable to a specified phenotype. Based on commercial viability, it would be prudent for manufacturers to select a cell source that is highly proliferative. Manufacturers must also avoid risk factors, such as insertional mutagenesis, in the design of production methods.

While committed neural precursor cells can be expanded, these cells are considered incompetent of producing the quantities necessary for commercial and clinical viability. Until such time as neural precursor cells can be suitably expanded, it is essential to develop...
the alternative strategy, to direct the differentiation of a cell type with virtually unlimited expansion capability. Stem cells are a logical candidate cell type for CRT. Stem cells are non-determined cells that are self-regenerative and pluripotent, or multipotent for a derived tissue type. Stem cells can be banked, expanded to near limitless supply, and directed in culture to differentiate into multiple, committed cell types. Along this line, two types of stem cells have emerged with the capacity for therapeutic application in CNS diseases and injury: neural stem cells and embryonic stem cells (ESCs).

NSCs are multipotent neural stem cells that reside in distinct CNS regions, such as the subventricular zone, the subgranular layer of the dentate gyrus, and the ependymal layer of the spinal cord central canal[18]. These cells are neurogenic and gliogenic throughout development and into adulthood. Thus, NSCs can remain uncommitted to a particular neural phenotypic endpoint for some undefined period, unlike the fetal-derived dopaminergic neurons used in PD transplants to date. Endogenous NSCs proliferate and mature in the intact and injured CNS, although functional tissue repair is limited, even with added pharmacological induction [3]. The ineffectiveness of resident NSC neurogenesis or gliogenesis to compensate for or repair CNS disease or injury reflects a deficiency of progenitor cell number, impairment caused by the local injury environment, affected NSC genetic and epigenetic makeup, or some combination of these factors.

Relevant to CRT, NSCs can be isolated from embryonic and adult CNS, maintained and expanded in cell culture, and differentiated into neural and glial progeny [5,7,11,30,34]. Although there is evidence that human neural progenitors can become depleted of the potential to differentiate into particular CNS phenotypes after several expansion rounds [47], results from animal studies demonstrate that NSCs and NSC-derived precursors survive, differentiate, integrate, repair tissue, and improve neurological and behavioral function after transplant into models of CNS disease and injury [1,4,17,33].

The ability to isolate NSCs from particular locations of adult brain allows for the possibility of deriving autogenic transplants; that is, NCs that can be produced from biopsied CNS tissue, expanded and differentiated in culture, and transplanted back into the same patient. The appeal of an autogenic transplant is that it obviates the need for immunosuppression because the transplanted cells are recognized as self. However, manufacturing and regulatory considerations aside, the genetic and acquired condition of NSCs from a diseased host and the unknown effects of age and disease on NSC propagation ex vivo limit this application. Instead, NSCs from adult biopsies might be better suited to establish research lines, to elucidate mechanisms of CNS diseases, discover phenotype-specific markers, or test pharmaceuticals for CNS diseases in multiple patient-cell populations. It is possible that cell transplants derived from autogenic NSCs can be used to treat some CNS diseases and injury (for example SCI), but for broad clinical application, NSCs will likely need to be acquired from fetal tissues or differentiated from ESCs. Despite limitations, evidence from more than a decade of studies supports NSCs as a tenable substrate for CRT in neurodegeneration.

ESCs are pluripotent cells derived from the inner cell mass of pre-implantation blastocysts. In humans, these cells propagate and differentiate through cell autonomous and cell non-autonomous processes to become the trillions of ectodermal, mesodermal, and endodermal lineage cells generated over a lifetime. This propagation and differentiation can be recapitulated to some extent in culture, as ESCs can be expanded to an almost unlimited supply of cells and directed to differentiate into diverse cell types. Of particular interest to clinical applications, ESCs can be grown in defined, rodent-free media, survive repeated freeze/bank/thaw cycles, and maintain a normal karyotype and differentiation potential for years [23,35]. Of all stem cell types, ESCs currently evoke the greatest potential for large scale production of cells from an identified source and for acting as substrates to produce cells to treat the widest range of diseases or injuries amenable to cell replacement strategies. Using various techniques to maintain and direct the differentiation of stem cells, a number of studies have demonstrated the production of committed neuronal [2,28] and glial [6,26] lineage phenotypes from ESCs. Together these results indicate that ESCs are a sustainable source of neuronal- and glial-committed cells for CRT.

Safety is paramount to the use of ESC-derived cells or tissues in clinical CRT. Because ESCs are highly proliferative, unrestricted in their development and sensitive to environmental cues for differentiation, tumor and mass formation are bona fide risks for transplanted stem cell-derivates. Transplants of non-differentiated ESCs form teratomas that consist of cells from endodermal, mesodermal, and endodermal lineages [43]. Even restriction of ESCs to multipotent neural progenitors can generate nestin-positive, expanding cells [36] or other unwanted, inappropriate cells. An example of this later effect is the differentiation of transplanted, multipotent neural stem cells into astrocytes [41]. Astrocytes commonly act as support cells, but are also associated with exacerbation of scar formation and potentiation of pain perception. However, directed differentiation of pluripotent and multipotent stem cells into particular, committed precursor phenotypes prior to transplantation can restrict their post-transplantation phenotypes, address defined cellular deficits, better predict outcome, and limit adverse effects [39]. Thus, pre-differentiation is a reasonable means to mitigate the imposed or implied risk of NSC- and ESC-derived transplants and promote the regulatory and widespread acceptance of stem cell-based CRT.

Inherent to the use of pre-differentiated cells for transplant is the purity of the cells in the transplant population. A number of methods have been reported for the directed differentiation of neural and glial progenitor cells from stem cells. In an asynchronous culture, cells can be discerned within particular developmental windows from the total cell population by detection of proteins, mRNAs, or reporters expressed by that population. For safety, ESC-derived cell populations must be devoid of or contain small numbers of undifferentiated cells. Identification of undifferentiated cells based on expression of a stem cell marker such as stage-specific embryonic antigen-4 can be used to separate the cells from the transplant population. However, serial negative selection of all non-target cells from a mixed population is a less practical alternative to positive selection, if the desired phenotype has known selection markers.

Purity can be determined using cell counting/sorting devices with appropriate labels or reporters. This method, fluorescence-activated cell sorting (FACS), has the advantage of isolating live, specified cells from a mixed cell population. Cell sorting requires that the cells be labeled with fluorescent antibodies to phenotype-specific cell surface proteins or engineered to express a fluorescent protein under the control of a phenotype-specific promoter. Both techniques have potential drawbacks: immunodetection necessitates further digestion of cell surface proteins to remove the antibody label, and reporter gene expression requires genetic manipulation of the starter population. Both of these sorting methods require time, which allows for amplification of contaminant populations. Regardless of the technique, the purity of the sorted population is dependent on the label or reporter used. Use of FACS to produce a pure population infers that the purity of a target cell population can be assessed from one or two markers and that those markers are not included in the non-target cell population. This presents a problem for cell identification when the literature denotes more than one potential cell type for a single marker. In practice, though, many antibodies and promoters are accepted for separation of specific CNS phenotypes. In the event that a cell-specific promoter is available, a conjunctural method...
is the knock-in of a gene for a foreign protein [49], such as green fluorescent protein [37], under the control of the promoter to highlight the cells for selection. However, the tumorigenic risk posed by insertion methods renders the use of reporter genes or any cell-specific selection genes, such as antibiotic resistance genes, undesirable for clinical CRT as per current US FDA guidelines. Lastly, the limited yield of sorting methods reduces the commercial or clinical viability of the approach for transplantation-based approaches. Nonetheless, immunolabeled sorting methods are very useful for the production of certain ESC-derived CNS phenotypes for research purposes.

Although FACS is a powerful tool for the isolation of subpopulations of cells from a mixed population, the technique is not well suited to isolation of transplant populations for clinical applications. Thus, clinical application of cell replacement strategies necessitates refined methods of directed differentiation.

An ideal procedure for directed differentiation produces a pure population of cells at a single developmental time point. Despite advancements in rodent and human developmental biology over the past 20 years, a 100% pure population has yet to be produced. A number of laboratories, including ours, have been able to produce high purity populations of oligodendrocyte progenitor cells from human ESCs (hESCs) [20,29,48]. OPCs are precursor cells that are developmentally restricted to produce oligodendrocytes that myelineate axons and support neuronal function. Each of the approaches in the literature resulted in a high-purity and high-yield population of hESC-derived OPCs. Our approach produced a population with approximately 95% GalC, RIP, and O4-positive cells with oligodendrocyte morphology, with less than 5% of the cells positive for non-oligodendrocyte markers GFAP and Tuj1, or markers of undifferentiated ESCs. The Izrael et al. protocol produced a population with greater than 90% O4- and Olig1-positive cells with oligodendrocyte morphology. These successes reflect the uniquely advanced state of our understanding of the development of this cell population, and the characterization of the lineage intermediates.

A cell replacement therapy should result in graft integration, attenuation of clinical decline, and/or enhancement of functional outcome. When hESC-derived OPCs were transplanted into the shiverer mouse model of dysmyelination, the cells integrated into the white matter, differentiated into oligodendrocytes, formed multilayered compact myelin, and produced MBP-positive areas within the spinal cord [29] and brain [20]. Thus, these progenitors demonstrated the capacity to engraft and differentiate into functional, mature cells in animal models. However, many CNS diseases and injuries are multifactorial disorders that present an environment detrimental to the survival and integration of transplanted cells, containing pathological features such as inflammation and excitotoxicity. SCI is a congruous model for the study of hESC-derived OPC transplants in complex environments, as the injury leads to secondary degeneration, a loss of oligodendrocytes, and chronic and progressive demyelination [44]. To examine the capacity of hESC-derived OPCs to integrate and improve functional recovery in the injured CNS, these cells were transplanted into an animal model of SCI [22]. In this model, transplanted hESC-derived OPCs localized to white matter, differentiate into myelinating oligodendrocytes, and improve recovery of locomotor function without harmful effects [9]. Although the contribution of denervation to functional loss is difficult to ascertain in SCI due to temporally overlapping pathological processes, the results indicate that hESC-derived OPCs can improve functional outcome. Taken together, these data support the use of hESCs for generating donor cells for clinical treatment of SCI.

It is notable that these studies did not directly examine the role of hESC-derived OPCs on axonal degeneration, neuron loss, cavitative, and inflammation, all of which are characteristic of secondary degeneration following SCI. Oligodendrocytes can affect neurofilament phosphorylation and enhance cortical neuron survival [45] through secretion of soluble factors such as glial-derived neurotrophic factor (GDNF) and insulin-like growth factor-1 (IGF-1). These data suggest that hESC-derived OPCs might attenuate SCI secondary damage and/or benefit functional outcome through support of endogenous neuronal survival and axon outgrowth. To determine if transplanted OPCs can exert pleiotropic effects on injured CNS, we treated neuron cultures with conditioned media from hESC-derived OPCs or control media and examined survival, proliferation, and neurite outgrowth. Cortical neuronal cultures treated with OPC-conditioned media showed increased viability and expanded at a higher rate relative to cultures treated with control media [14]. In addition, treatment with hESC-derived OPC conditioned media led to increased neurite length of cortical [14] and sensory [48] neurons. These results suggest that hESC-derived OPCs produce one or more soluble, neurotrophic factors that promote neuronal function, and concur with a growing literature that suggests that oligodendrocytes provide trophic support for neurons and axons. Specific neuronal effects linked to oligodendrocyte-soluble factor interactions include induction of neurofilament accumulation within axons, clustering of sodium channels along axons, and maturation and stabilization of axons [10,21,38].

Addressing the identity of soluble factors expressed by hESC-derived OPCs, a recent study by Zhang et al. [48]. Analysis of gene microarrays determined that 49 growth factor mRNAs were highly expressed by hESC-derived OPCs. Secreted proteins were detected from conditioned media using antibody arrays and ELISAs. The hESC-derived OPC expressed and secreted factors included neurotrophic factors IGF-1, brain-derived neurotrophic factor (BDNF), NT-3, and nerve growth factor (NGF). Additional secreted growth factors of interest were hepatocyte growth factor (HGF), transforming growth factor-β1 (TGFB1), transforming growth factor-β2 (TGFB2), stem cell factor (SCF), vascular endothelial growth factor (VEGF), activin A, and midkine, all of which are reported to act as neurotrophins. The detected concentrations of these three proteins, namely midkine, HGF, and activin A, were each within the 1–10 ng/ml range described for each protein’s in vitro neurotrophic effects [13,19,27]. However, the synergistic effect produced by the summation of these proteins is more pertinent and effective than each individual concentration. Of particular significance to expression and secretion of these neurotrophic proteins is the absence of neuronal or other glial cells within the culture. The concentrations of these proteins in the conditioned media, then, represent hESC-derived OPC unstimulated, basal secretion. It is reasonable to expect that neuronal or glial co-cultured cells could alter OPC protein expression, as might the transplant environment of diseased or injured CNS tissue. Nonetheless, an extrapolation of these neurotrophic data to SCI models would suggest that OPCs might produce multiple, beneficial effects on host tissue, such as suppression of inflammation, promotion of axonal regeneration, and homeostatic maintenance. It is also possible that host cytokines, such as IFNγ, might play a reciprocal role to favor engraftment of hESC-derived OPCs [31,32]. As the role of these pleiotropic effects are elucidated, it is possible that hESC-derived OPCs will be useful as substrates for broader CNS applications, for example protein-drug delivery [39], apart from remyelination.

Despite evidence that hESC-derived OPCs can comply with most of the proposed standards for use in clinical CRT, the concern about rejection posits the use of immunosuppression. The primary concern that necessitates immune suppression is the presentation of xenogeneic or allogeneic antigens by the transplanted cells. The potential for presentation of xenogeneic antigens, such as Neu5Gc, on the surface of hESC-derived cells is a result of propagation on feeder layers. However, several studies now show that passage of hESCs in feeder-free conditions eliminates any issue of serum cytotoxicity [8,31]. While this practice reduces the risk of immunogenicity due to incorporated non-human antigens, the constitutive
presence of allogeneic antigens presents a more persistent problem. It is important to note in this regard that undifferentiated hESCs appear immunologically privileged [24], and that some other hESC-derivatives display similar immunological characteristics [12,31]. Although, as hESC-derived OPCs mature and engraf to the recipient, it is possible that the cells will express surface antigens that can evoke an immune response. This unknown is an appropriate cause to use an immunosuppressive regimen for hESC-derived CRT.

A number of approaches have been proposed to circumvent the need for life-long immunosuppression after cell transplants. These methods include genetic modifications of cells to replace MHC genes, express Fas ligand, and/or modifications of other antigenic epitopes. Also included are somatic cell nuclear transfer (SCNT) and genetic modifications to create induced pluripotent stem cells (iPSCs) from recipient fibroblasts [42,46]. The benefit of SCNT and induced pluripotency is that they can be generated from the patient, so the genetic make-up is identical. A current major setback to the use of genetic modification is that the alterations can make the cells susceptible to cancerous growth. Unless the current induction technique or regulatory guidelines change, it is unlikely that we will see genetically modified cells replace ESCs as a cell source for CRT in the short term. What may be a more workable option is to use the advances in hESC culture developed over the past 10 years to create banks of new hESC lines. These lines could better match to patients and perhaps reduce the immunosuppressive regimen.

For patients treated with CRT, patients thus far show that the strategy is effective and safe provided that the transplanted cells are functional and stable. The generation of high-purity hESC-derived OPCs provides a precedent for the use of a sustainable cell source for CRT. Although several regulatory and manufacturing hurdles still exist for the commercial and clinical viability of hESC-derived OPCs, their translation to the clinical setting will provide a lasting infrastructure and regulatory pathway for the use of other hESC-derivates in CRT.

References


