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Human Embryonic Stem Cells

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Embryonic development before implantation of the embryo in the uterus includes a stage of blastocyst formation during which the initial mass of cells is segregated into an inner cell mass destined to contribute to the developing embryo and an outer layer of cells that contributes to the fetal portion of the placenta. Embryonic stem cells (ESCs) are cells derived from the inner cell mass of the developing pre-embryo that can be propagated indefinitely in culture. ESCs are characterized by the expression of a characteristic set of markers; the ability to self-renew indefinitely; a lack of contact inhibition; atypical cell cycle regulation; the ability to form teratocarcinomas in nude mice; and the ability to differentiate into ectoderm, endoderm, and mesoderm in vitro or in vivo after injection into blastocysts. This constellation of abilities distinguishes ESCs from all other cell populations, including adult stem cells.

As experience with cultures of ESCs has grown, it has become clear that although different ESC lines are similar overall, they do exhibit differences in gene expression, methylation status, X chromosome inactivation, rate of self-renewal, and ability to differentiate [1]. More importantly, the behavior of cells and their overall state change as culture conditions and the stress they are subjected to are altered. This has led to great difficulty in comparing results from one laboratory with another and even in comparing results with different passages of the same cell line. This difficulty has been compounded by the relatively limited involvement of the tools and reagent companies in developing antibodies, quantitative polymerase chain reaction (qPCR), microarray, and other reagents to accelerate the discovery process. Adding to this difficulty is the relatively high cost of maintaining multiple lines (for comparisons) in any given laboratory and a lack of incentive for the providers who initially derived the lines to provide detailed characterization of cell lines or compare them with each other. Political, legal, and ethical issues have further compounded the difficulty in using ESCs. The issues and rules governing the use of ESCs differ widely from country to country, and from state to state in the United States, making for a fractured and disorganized stem cell community.

Uses of embryonic stem cells

Perhaps the most common use of mouse ESCs has been to generate transgenic mice. In the early 1980s, soon after the successful isolation of ESC lines, Capecchi and colleagues showed that it was possible to insert foreign DNA into a specific genomic locus by means of a process termed *homologous recombination* [2]. This has allowed the replacement of defective genes with a corrected copy or insertion of reporter molecules to trace the development of cells or alter the levels of a particular product to assess the effect of changing a single protein. This remarkable ability to study directed changes throughout development has offered unprecedented insight into the biology of complex organisms and has transformed developmental biology. Although human embryonic stem cells (hESCs) cannot be used in the same manner to study in vivo development, similar strategies of

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altering gene expression can be performed with much the same efficiency and fidelity in hESCs. By recapitulating development in vitro, hESCs have provided a window into early developmental processes, such as X chromosome inactivation, regulation of methylation, segregation of maternal and paternal mitochondria, evolutionary differences, speciation and differentiation of germ cells, and other early developmental events. The ability to generate ESC lines from embryos that carry a genetic defect diagnosed at the time of blastocyst implantation (ie, preimplantation genetic diagnosis [PGD]) has important potential benefits in understanding the etiology and progression of congenital diseases. Lines carrying genetic defects that affect neuromuscular development have been isolated from human blastocysts, and more than 100 such lines are available [3]. These lines can be used in comparative gene expressions studies to understand the effect of a particular genetic abnormality on development. In addition, somatic cell nuclear transfer (SCNT) has allowed investigators [4] to transfer a nucleus from any cell in the body to a fertilized enucleated egg to generate novel patient-specific ESC lines (therapeutic cloning) that can be used for a variety of purposes, such as the treatment of diseases and replacement of human tissue or organs for transplantation.

Many other uses of hESCs can be envisaged (Fig. 1). These include modifying transplant strategies to take advantage of the unique properties of hESC behavior in vivo; using hESCs in gene and drug discovery strategies; and using hESCs to understand the basic biology of early developmental events, such mitochondrial segregation, regulation of telomerase expression, or maintenance of the pluripotent state. These additional or alternative uses of hESCs do not require fundamental scientific breakthroughs; rather, they represent an adaptation of existing technology to hESC cultures. They are an important and less often discussed aspect of the advantages of hESCs over other cell types. In subsequent sections, the authors elaborate on the uses of ESC lines as they relate to central nervous system (CNS) disorders. It is important to note that although there is much excitement about their potential use, hESCs are not currently used in any clinical or commercial application.

Uses of embryonic stem cells for treating central nervous system disorders

An obvious application for hESCs is to use them as a source of cells for transplantation. Indeed, much of the current focus is on isolating cells of the appropriate phenotype for cell replacement. hESC lines can be expanded indefinitely in vitro with little or no change of properties [5,6]. Induced by appropriate exogenous signaling molecules (eg, adding retinoic acid [RA]) or by

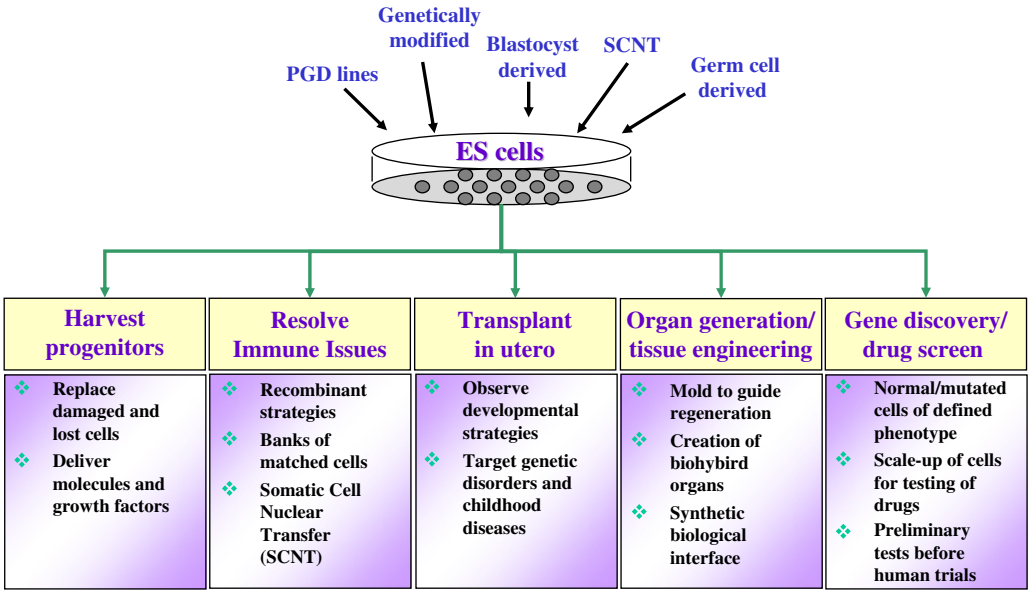

Fig. 1. Schematic shows sources of ESCs and the uses of ESCs for stem cell–based therapies.

omitting the use of basic fibroblast growth factor (bFGF), hESCs can generate differentiated progenies by way of embryonic bodies (EBs), which are three-dimensional aggregates in suspension culture. Other methods of ESC differentiation include growing them on a PA6 stromal cell line or direct differentiation by prolonged culturing without passaging [7]. A list of methods for neural differentiation is summarized in Table 1. Using such methods, investigators have successfully isolated multiple types of neurons, neural stem cell populations, astrocytes, and oligodendrocyte precursors [8]. ESC-derived differentiated populations have been transplanted in animal models; some efficacy has been noted, and problems have been identified. Perhaps the most critical problem identified so far is the persistence of undifferentiated populations that become a possible risk for tumor formation [9,10]. Studies with mouse ESCs have even suggested that a small number of undifferentiated cells can be sufficient to induce a tumor given enough time [11]. Although equivalent studies have not been performed in human beings, less extensive studies transplanting small numbers of cells into the brain have suggested that tumor formation with persistence of undifferentiated cells is not unexpected [12,13]. This suggests that rigorous purification is required to ensure that no undifferentiated cells pass through into a therapeutic product.

Other less concerning but equally important issues include the presence of xeno-material (ie, contaminated by nonhuman products) in the currently available lines. Because most hESCs are grown on mouse fibroblast feeder cells, there is a potential risk that the use of feeder layers or conditioned medium generated from feeder cells may lead to the contamination with animal molecules, which may be attacked by the human immune system (eg, Neu5Gc) [14]. Activation of long interspersed nuclear elements (LINEs) that represent integrated proviral elements is also a concern, as is the possibility of introducing karyotypically abnormal cells generated during the culture process. Other issues include the degree of maturation of the differentiated cells that are generated and the ability to manufacture cells under good manufacturing practice (GMP) conditions. Efforts to solve these problems have been initiated, and they are likely to be adequately addressed. Derivation of new lines under strict GMP conditions has been achieved, and additional markers of different stages of differentiation have been identified [15]. Multiple methods of specific cell type isolation have been described, and feeder free cell culture systems using completely defined media are now developed [16–18]. These developments, along with the documented stability of ESCs and the ability to differentiate them into neural derivatives by multiple techniques, have allowed researchers to begin considering therapeutic targets that should be evaluated for ESC-derived cell therapy. In the next section, the authors discuss the challenges of using ESC-derived cells or any other cell product in the nervous system and how initial targets are being selected.

Table 1
Methods of neural differentiation

Methods	References
Direct differentiation	[58–60]
EB formation	[61–63]
RA treatment	[59,64,65]
Growth factor withdrawal	[64,66]
Coculture with astrocytes of PA-6	[67,68]
Stochastic differentiation followed by selection using cell surface epitopes	[65,69–71]

Abbreviations: EB, embryonal body; RA, retinoic acid.

Potential short-term targets for therapeutic intervention

Although ESC-derived cells solve a potential source problem, many additional hurdles that are unique to cell therapy in the CNS remain. These are universal to cell therapy in general and would affect any cell used, irrespective of whether it was derived from an adult, a fetus, or an embryo. Briefly, the number of stem cells present in the adult is small, they are located in restricted areas of the brain, and they do not participate in the repair process to a large degree [19,20]. As a result, the brain generally does not repair itself by regenerating new neurons, and as such, cues to direct appropriate differentiation of exogenously added stem cells do not exist. Furthermore, because stem cells that are endogenous to the brain do not migrate to distal injury sites, it is unclear if any homing mechanisms are present on neural stem cells and whether signals are generated by the environment to enhance or direct stem cell migration.

An additional fact that has become clearer as experiments have been performed is that different areas of the brain are structurally distinct and perform separate functions and that their neurons, and possibly their glia, differ in their properties [21–23]. Thus, one type of replacement cell may not be adequate; instead, region-specific cells may be required. It is clear that such regionalization occurs early in development, and these differences are maintained after cells are isolated. Hence, cells can be directed toward appropriate region-specific phenotypes because they are unlikely to exist in the adult intact or damaged brain.

Finally, as routes of administration are considered, one must remember that the brain is relatively insulated from the rest of the body and that the difficulty in reaching specific areas of the brain by way of the vasculature, the ventricular system, or the parenchyma without causing damage must be taken into consideration. Cells introduced by way of the ventricular system must have the ability to cross the ependymal lining and migrate large distances to integrate themselves in a site-specific manner, and cells introduced into the vasculature must traverse the capillary-glial barrier rather than obstruct small vessels and cause end-arterial obstruction. Once they traverse these barriers, cells must then differentiate into appropriate phenotypes and make appropriate neuronal connections, which may number in the thousands in the case of neurons and may be present at distances that are huge relative to the growth rates of axons and the size of a cell.

Current technical ability and state of the art do not present any simple solution; when these basic facts are considered, the method of delivery, cell choice, cell number, and type of disease that is currently treatable become quite limited. Some targets that have been considered likely are when neuronal replacement is not required, such as in disorders in which glial cell trophic support can be provided, when cells can be used as biologic pumps to provide critical missing enzymes or growth factors, or when extensive migration is not required.

Three potential initial targets are Parkinson's disease, lysosomal storage diseases, and spinal cord injury. Although it is impossible to discuss each disease model in detail, certain generalizations are possible (Figs. 2 and 3). In general, there is no "one size fits all" cell for therapy, and decisions on the type of cell to be used, the number of cells to be used, and the method of delivery that is optimum vary.

Parkinson's disease

In treating Parkinson's disease, for example, the number of cells required would range from 100,000 to 500,000. They would likely be delivered by means of a needle into the parenchyma guided by MRI stereotactic implantation. Primarily dopaminergic neurons to the exclusion of all other cells would be important if the intent was to replace the damaged or lost dopaminergic neurons. Obtaining differentiated dopaminergic neurons of the appropriate kind in sufficient numbers is feasible, and such a differentiation procedure has been shown to work by a variety of investigators [24–28]. In the case of therapy in Parkinson's disease, migration would not be a useful property and homing to a stem cell niche, for example, would be a disadvantage. Overall, investigators have shown that when they are transplanted directly in the rat brain, hESCs form fatal teratomas, and in other studies, the dopaminergic neurons derived from ESCs do not always differentiate into dopaminergic neurons of the striatum [29]. However, this is not a problem when the ESCs are first manipulated in vitro, either by directing them down a dopaminergic neuronal phenotype, or by using fluorescence-activated cell sorting (FACS) to select specific subtypes of cells for transplantation [30], this is not a problem. In addition, genetic manipulation of mouse ESCs and hESCs can enhance the dopaminergic phenotype. The dopaminergic phenotype of the appropriate kind can be enhanced in ESC cultures using a cocktail of several factors, including Nurr1, a transcription factor involved in the differentiation of dopaminergic cells [30,31].

Many of the problems of patient selection, delivery, posttransplant monitoring, and developing an animal model have been solved, and human trials with fetal tissue transplants provide a wealth of data on which to design ESC-based therapy. The clearest problem facing clinical use is the source issue or obtaining adequate number of cells for therapy. Thus, there are no insurmountable problems or technical breakthroughs required to move ESC-derived cell therapy toward the clinic. This, however, does not mean that therapy is around the corner, but it does mean that it is feasible to contemplate moving forward; indeed, several groups have initiated early animal studies (M.S. Rao, unpublished data, 2006).

The numbers and type of cell required for therapy would be altered even for Parkinson's disease if one contemplated using ESC-derived

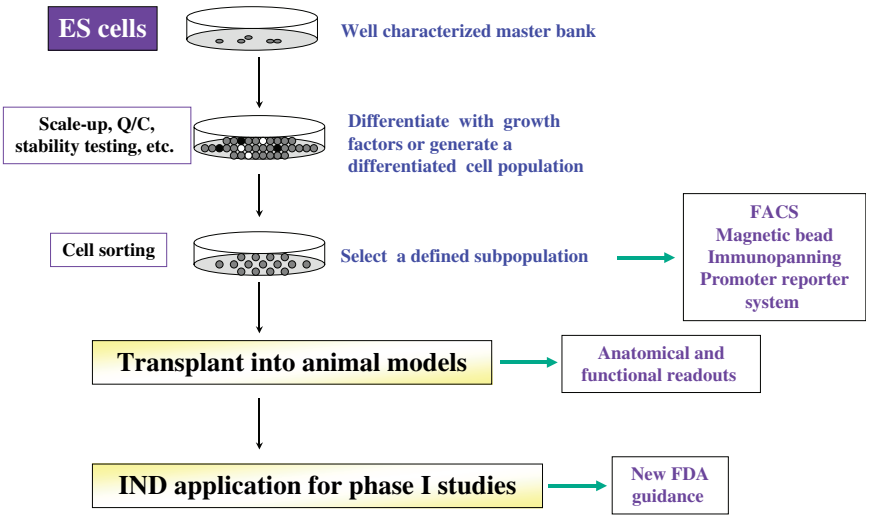

Fig. 2. Summary of strategy for ESC-derived cell transplant therapy. Q/C, quality control.

neural stem cells or their derivatives as pumps to deliver a growth factor to enhance survival of existing neurons. Here, the properties required of the cells would be that they are not electrically active, can survive in the environment, and should perhaps be delivered to the substantia nigra rather than the striatum; in addition, the numbers required would differ. The patient population selected, the animal model used, and the investigational new drug (IND) approval process would all be different. This, too, is a feasible proposition, however, and does not require any major technologic breakthrough, although some would argue that obtaining controlled persistent expression of a particular cytokine in a cell in vivo remains an unsolved problem. The authors would agree with the critics but would argue that the one cell type in which this problem could be solved with current technology is in ESCs (using mouse ESCs as an example), taking advantage of the selection process available when cells can be propagated indefinitely in culture.

Spinal cord injury

Unlike Parkinson's disease, the goal in treating spinal cord injury is not cure but mitigation, given the current technical inability to replace long

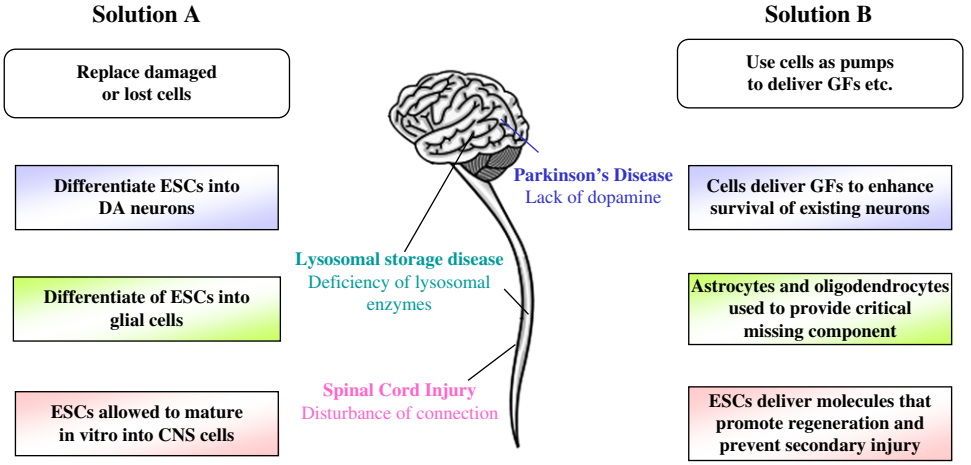

Fig. 3. Overall ESC approaches to CNS diseases; three disease models are shown, each affecting a different part of the CNS. DA, dopaminergic; GF, growth factor.

projection neurons and their connections with any fidelity or to reverse the changes that occur after prolonged denervation. The therapeutic rationale in spinal cord injury is that if one could enhance conduction in the remaining axons or mitigate the damage somewhat, the degree of incapacity after spinal cord injury would be less. A shift of two spinal segments downward after injury would alter the quality of life significantly, and if one could achieve a certain threshold of recovery, perhaps the quality of life (although not the spinal cord) would be improved. ESC-derived cells have therefore been evaluated to deliver molecules that promote regeneration, modify the host immune response, break down inhibition scars, mobilize endogenous stem cells, eliminate cellular debris, create novel bridges, limit secondary injury by protecting neurons, and replace nonneural elements that are required for optimal recovery [32–34]. Depending on the strategy elected, the cell type, mechanisms of intervention envisaged, number of cells required, mode of delivery, frequency of replacement, and timing of therapeutic intervention can vary.

In general, two modes of delivery have been envisaged: parenchymal, within or on either side of the lesion, or lumbar puncture (outpatient procedure). Both are relatively practical because surgery is often performed to stabilize the spine and lumbar puncture is relatively safe, even in a spinal injury. Lumbar puncture offers the ability to deliver a much larger number of cells and allows for repeated injections. It is likely, however, that because the blood-brain barrier is compromised, the immune response and chance of rejection are much higher than in Parkinson's disease or lysosomal storage diseases. Most investigators have assumed that neuronal replacement would not be as important as glial replacement; the cell selected should preferably have limited neuronal differentiation and limited migration potential, and this migratory ability, should it exist, should preferably be directed toward a lesion.

Currently, researchers have shown that transplanted ESCs are capable of giving rise to the three primary types of nervous system cells, which can then integrate into the spinal cord to promote functional recovery [35]. Other studies have shown that similar transplantation into areas of damaged spinal cord with demyelination induced selective differentiation into oligodendrocytes that myelinate host axons [36,37]. Recent results from Bakshi and colleagues [38] have shown that cells delivered by means of lumbar puncture can survive and integrate at the site of damage (as long as the damage includes the pia and arachnoid). They developed a rat model to mimic lumbar puncture delivery and used it to show therapeutic improvement.

Thus, the strategy that has begun to evolve in the treatment of spinal cord injury is to treat with cells differentiated from ESCs that are biased toward the glial fate and to deliver the cells by means of direct injection or by means of lumbar puncture with immune suppression. Rat models are used to mimic the injury, and early and late intervention is being attempted.

Lysosomal storage disorders

In lysosomal storage disorders that involve multiple systems, enzyme replacement therapy and bone marrow transplantation (BMT) have been used [39]. In these attempts, primary graft rejection in lysosomal storage disorders is a significant problem, which means that 50% of all BMTs have to be repeated. In addition, BMT after the onset of significant neurologic signs does not lead to an improvement in neurologic function. In cases of enzyme replacement, delivery to the CNS is a problem and deterioration of neurologic function may continue. Several researchers have used animal models of lysosomal storage disorders and have shown that cell replacement of a small percentage of cells (as long as they are widely dispersed) can lead to anatomic and functional improvement [40]. Although scientists have used stem cells, the assumption has been that it is the glial cells that are of importance, given the fact that glial cells migrate and release enzyme that is taken up by adjacent cells, whereas neuronal differentiation is limited in these models. Astrocytes and oligodendrocytes are likely important for replacement, because demyelination after oligodendrocyte degeneration is an important component of many of these disorders. Thus, cell therapy is not geared to replacing damaged or lost neurons but is directed to using cells as delivery pumps to provide a critical missing component.

The requirements of cells here are their ability to secrete the missing protein, their ability to survive in a potentially toxic environment, and their ability to migrate extensively and to respond to appropriate cues to cease migration and proliferation. Stem cells, which need a niche for integration or survival, are perhaps not the

optimal choice; rather, glial progenitors that migrate during development and after injury and can remyelinate axons are perhaps the more logical choice. Investigators have shown that such strategies work in animal models [41] and are evaluating ESCs as a source for such glial progenitors.

Overall, these examples show that the cell replacement therapies that have been contemplated are disease specific and that the number of cells used, the properties required, the mode of intervention, and even the cell type(s) used can vary widely.

Problems facing investigators

Although much progress in recent research on neural cell transplantation has been reported, there are still many challenges for the wide application of this kind of therapy in clinical practice. These are summarized in Table 2. Among the potential obstacles in the application of ESCs to cell therapy, immunologic rejection is one problem that still has to be solved for the clinical application of hESCs. This is because on differentiation, hESCs express the major histocompatibility complex (MHC) molecules according to the information carried by their own genome [42]. Therefore, the derivation of immunocompatible cloned ESCs through SCNT, or "therapeutic cloning," might be one of the alternatives to circumvent immune problems [4,43,44]. The machinery involved in activating the human oocyte is not completely understood, however, and trials with nonhuman primates were unsuccessful because of technical limitations. Another problem faced is the possibility of teratoma formation when hESCs are used for transplantation. These precursors, which can potentially form teratomas, are present as a result of a contaminating population of less differentiated cells because of the inability to direct ESC differentiation efficiently and, hence, the transplantation of highly heterogeneous cell populations. One solution is cell sorting of the differentiated cell population through specific cell-surface markers or by engineering a lineage selection marker into the ESCs by genetic manipulation before implantation to generate a defined population of ESCs for use in vivo [45,46].

Table 2
Problems facing investigators

Problems facing investigators	Comments
CMC issues	Xeno-issues, lot-to-lot variability, release criteria, measures of potency and efficacy
Animal model issues	Is the model appropriate? Are primate models critical? Can human-to-human transplants be modeled in human-to-mouse experiments
Tracking and tracing and follow-up issues	Biodistribution, tumor formation studies, labeling
Patient selection criteria	Allelic variability, long-term immune suppression, methods of delivery
Tracking and tracing and follow-up issues	Noninvasive monitoring, engineering suicide genes

Abbreviation: CMC, chemistry, manufacturing, and controls.

In addition, the expansion methods for hESCs have to be optimized not only to generate a xeno-free culture of ESCs by the replacement of animal feeder layers or serum with those of human origin but to prevent chromosomal abnormalities as the result of the culture methods used [47]. Hence, routine procedures for measuring the stability of cells should be implemented, in addition to the monitoring of heterogeneity and differentiation capabilities of these hESCs [48].

At the present time, cell transplantation therapy has been performed with human cells that are transplanted into rodents. By itself, this has its limitations, because marker expression seems to be similar on the superficial level but several differences have been noted, as summarized in Table 3. Differences between equivalent stem cell populations have also been observed, which is why it is necessary to develop standardized characterization tests and to compare isogenic and xenogenic transplants using well-characterized cells to determine whether the results can be extrapolated to human studies. In a review by Ginis and Rao [49], the differences between human and rodent signaling, species differences that affect stem cell transplantation, the effect of different transplant surgical differences, differences between animal models and human disease, and the issue of allelic variability in human beings are among several important points that were discussed.

In summary, although certain therapeutic targets are considered near-term possibilities, there

Table 3
Differences between human and rodent stem cells

Features	Rodents	Human beings
Neural stem cells		
Timing of appearance and spatial distribution of NSCs and neural precursors	E105, astrocytes appear late	5 weeks; astrocytes appear early
Cell cycle and number of cell divisions, telomerase biology	Short, telomerase high, LIF promotes differentiation	Long, telomerase low, LIF promotes growth in some reports
Effects of NGF, PDGF, CNTF, and LIF on NSC differentiation	Differences reported	Differences reported
Expression of MHC class 1	Not detected	High levels detected early in development
CD95 (Fas) expression	Not present	Present
Expression of glial marker A2B5 on neuronal precursors	Not seen in rat cortical or spinal cord cultures	Seen in a substantial proportion of cortical neurons
Expression of NG2 on oligodendrocyte precursors	Expressed by rodent precursors	May not be expressed
Expression of GFAP in neural stem cells	Not seen in fetal NSCs	Seen in culture in neuronal cells and NSCs
Embryonic stem cells		
Colonies	Piled, no distinct borders	Flat, with distinct borders
Population doubling time	12 hours	36 hours
LIF dependence	Yes	No
LIF receptor/gp 130	Yes	No
Inhibition of LIF signaling by SOCS1	No	Yes
FGF dependence	No	Yes
Cloning efficiency	Good	Difficult
Differentiation with RA	Low RA levels	10-fold higher levels
SSEA-1	Present	Absent
SSEA-3	Absent	Present
SSEA-4	Absent	Present
TRA-1, TRA-1-81	Not detected with available antibody	Readily detected
GCTM-2	Not detected	Present
β-III tubulin	Not seen	Low-level expression reported
Integrin expression	α5, α6, and β1	α6, α1, β1, β2, β3, and β4

Abbreviations: CNTF, ciliary neutrophic factor; FGF, fibroblast growth factor; GFAP, glial fibrillary acidic protein; LIF, leukemia inhibitory factor; MHC, major histocompatibility complex; NGF, nerve growth factor; NSC, neural stem cell; PDGF, platelet-derived growth factor; RA, retinoic acid, SOCSI, suppressor-of-cytokine-signalling-1.

Modified from Ginis I, Rao MS. Toward cell replacement therapy: promises and caveats. Exp Neurol 2003;184:64.

remain several hurdles before one can assume that cell therapy is likely to become a routine part of the therapeutic armamentarium. Protocols to generate large numbers of cells under GMP conditions have to be developed, the US Food and Drug Administration (FDA) and other regulatory agencies need to be satisfied that the risk/benefit ratios are appropriate, methods of isolation and purification need to be in place, GMP-grade cells need to be available, and appropriate animal models need to be available. These processes, although feasible, have not yet been completed for any single therapeutic intervention, although many companies are racing to complete the appropriate studies.

Longer term potential uses

Other near- or medium-term targets are the treatment of stroke to mitigate the duration and

extent of injury rather than completely repairing the injury; cell replacement in cerebral palsy; and localized repair of demyelinated plaques in multiple sclerosis, Devic's disease, and transverse myelitis. Diseases that require extensive neuronal loss, large-scale migration of cells, or extensive axonal regrowth with appropriate synaptic connections are beyond current technology and would require additional breakthroughs, although such a time frame is difficult to estimate. For example, if cell fusion allows survival of damaged neurons, thereby maintaining accurate connectivity and eliminating the requirement of extensive neuronal outgrowth, perhaps a large number of additional diseases can be treated. This is not possible with current technology, however, although the authors remain optimistic that breakthroughs are going to occur and expand the utility of cell therapy. Although breakthroughs, by definition, are unpredictable, some potential targets that investigators are working toward are discussed here.

Resolving immune issues

In all cell therapy, preventing graft rejection is a problem much like in organ transplantation. Perhaps the most relevant data come from pancreatic islet transplants in human patients, in which immune suppression is required and careful attention to the type of agent used is critical, because stem and progenitor cell populations tend to be more susceptible to the toxic effects of these agents. Preliminary data from several investigators have suggested that the brain is not immune privileged, particularly in damaged brains, in which the blood-brain barrier is often disrupted [22,23].

Although several strategies have been proposed to address this immune issue, ESCs offer three possible unique solutions that cannot be contemplated in other cell populations to date. One possibility is to generate a universal donor line by engineering the cells to eliminate the human leukocyte antigen (HLA) locus. Such recombination strategies can be performed in ESCs but not in other cell populations. Another strategy is to generate matched cells by SCNT. This is possible and has been demonstrated in multiple species, although recent claims that this was successful in human patients were shown to be fraudulent [50]. Nevertheless, the technology exists and, in principle, can be optimized. A third more mundane strategy is to develop banks of matched cells much like organ transplants are matched. The efficiency of generating ESCs has steadily increased, and the number of cell lines derived is increasing exponentially. The idea of a stem cell bank with HLA records for matching is feasible. Indeed, Taylor and colleagues [51] estimated that approximately 150 hESC lines would be needed for most of the population in the United Kingdom and that as few as 10 might suffice for the identification of cell lines that could serve a larger number of patients, such as lines homozygous for common HLA types [52]. Although one could theoretically do the same with other stem cell populations, it currently seems to be difficult, given the limited propagation possible with most such populations.

In utero transplantation

Complexities and developmental limitations of adult hosts for cell transplants need not preclude the utility of stem cell–based therapies. A special opportunity for stem cell therapy may exist early in development when migration and connectivity cues are present and appropriate feedback loops exist to regulate appropriate site-specific development. It is also worth pointing out that identification of antigens as self versus nonself occurs late in development and that cells transplanted before this stage are likely to be recognized as self, and thus do not provoke an immune response. Because the uterus can be readily accessed throughout embryonic development, specific tissues can be targeted with relative ease, and a diagnosis can be made early in development, many congenital disorders could, in principle, be targeted without the problems associated with later stage intervention. For example, Ourednik and colleagues [53] have shown integration of stem cells into the ventricular zone after intrauterine transplantation in monkeys.

Although any cell that has the capability of integrating into the tissue targeted for therapy and responding appropriately to local signals is useful for intrauterine replacement, the authors suggest that fetal- or ESC-derived cells are the most appropriate. In the opinion of these authors, it is unlikely that appropriate cues to direct ESCs to differentiate are going to be available at all stages of embryonic development. It is also clear that in utero transplantation cannot be an option for diseases that manifest late in development or cannot be unambiguously diagnosed early. For genetic disorders and childhood diseases, however, in utero stem cell therapy may be a possibility

that offers a high potential for success, because it co-opts a normal developmental strategy. Possible disease targets are glycogen storage disorders, cerebral palsy, and other disorders in which candidate cells are available and the diagnosis can be made early.

Organ replacement

A more involved version of stem cell transplantation is organ replacement. It is, of course, impossible to consider replacing the entire brain, but nerves, ganglia, peripheral sensory structures, and perhaps segments of the spinal cord can be considered. In this application of stem cell biology to therapy, a mold of some sort is typically used (or at least contemplated). This mold guides the proper alignment and physical disposition of the cells or combinations of cell types and the extracellular matrix to produce a reasonable facsimile of an organ. Advances in biomaterial engineering and stem cell technology have made the creation of biohybrid organs feasible [54], and the problems of obtaining oriented growth of axons or myelinating oriented axons have been solved to a large extent [55]. Advances have also been made in providing hybrid biologic or synthetic structures for organs and tissue for which it is still impossible to generate synthetic organs. This approach of mechanical or electronic devices that signal to biologic tissues should, in turn, process the information [56,57]. This synthetic biologic interface is one in which stem cells and progenitor cells derived from ESCs may play a useful role as well (Fig. 4).

Organ generation in vivo

An even more radical possibility is to develop nerves, ganglia, and sensory tissue in swine by transplantation of human stem cells or progenitor cells during development. The in vivo development of transplanted cells would be shaped by cues that cannot be replicated as yet, and the chimeric tissue, along with the transplanted stem and progenitor cells, can be harvested when it has reached an appropriate size. This tissue could then be transplanted as and when the need arises. Over time, the host immune system would destroy the xenobiotic cells while sparing the matched human cells, which would gradually expand to replace the destroyed cells, leaving fully formed human tissue that maintains the complex morphology and contours of the normal tissue. With their innate ability to differentiate into multiple phenotypes present in a particular tissue, their large replicative capacity, and their ability to obtain large numbers of undifferentiated or partially differentiated cells, ESCs represent the ideal cell source.

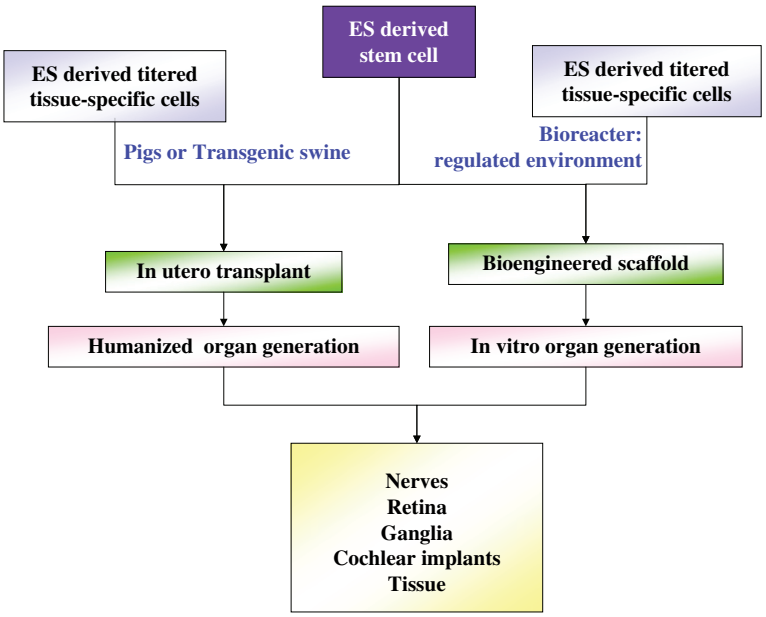

Fig. 4. Schematic shows how ESCs can be used for organ regeneration.

Drug discovery and gene discovery strategies for the central nervous system

It is important to keep in mind that there are few models of human CNS cells. Most tumor lines derived from CNS tumors have not propagated well. Glial cell lines do not retain their differentiation ability, and neuronal tumors of the CNS are rare. Stem cells from neuroepithelial tumors have not been well characterized, and it has been difficult to immortalize human stem and progenitor cells without introducing significant functional changes. ESCs thus offer the potential of obtaining functionally and genetically normal or mutated cells of a defined phenotype in sufficiently large numbers so that large-scale analytic methodology could be used for drug screening or gene discovery.

ESCs also provide an invaluable window into development and, coupled with the ability to monitor the effect of a single mutation or a particular drug at any stage of the development process, provide a way to examine the etiology or progression of a disease, model the effect of perturbation, and identify candidate therapeutic measures. ESCs could also improve the overall process of drug discovery by supplying the materials required for cell-based screens and assays. With their characteristics of immortality, self-renewal, and pluripotency, ESCs possess ideal qualities for a cell-based drug screening tool. They can be cultured indefinitely and triggered to create any particular cell type desired, and isogenic lines of any cell type can be obtained. Indeed, those with specific genotypes (allele combinations) can, in principle, be generated with ease. A panel of appropriate cell types provides a test bed to identify and characterize functional properties of protein factors and chemicals Antibodies also can be validated in the context of such an assay. Pharmaceutic researchers might, for example, seek out molecules that control cell growth and differentiation. Alternatively, they might simply use the panel to examine potential toxicity in vitro as a surrogate for and preliminary to expensive and time-consuming human trials. These considerations suggest that ESCs may bridge the large gap between assays that involve purified protein and those that use animal models.

Summary

To date, some stem cell– or progenitor cell–based treatments are already in use, and some cell treatments are in early clinical studies. Most of the reported benefits have come from hematopoietic stem cells (HSCs) and HSC-related products. The next cell type that may prove useful is the mesenchymal stem cell (MSC), although MSCs may not prove to be effective in treating most neurologic disorders. ESCs and fetal-derived neural stem cells and progenitor cells are being explored for use in lysosomal storage disorders and treatment of Parkinson's disease, spinal cord injury, and stroke. StemCells (Palo Alto, California) and Geron (Menlo Park, California) are two companies with submitted IND applications. The numbers of new IND filings have soared, and the FDA has provided much better guidance on the use of allogeneic cells for therapy.

Unfortunately, the promise of these preliminary results and the relative ease with which one can perform some of these experiments have led to a proliferation of offshore clinics that purport to use stem cells to treat any or all diseases. Nevertheless, the authors believe that success in this field is likely to come in measured paces based on accepted scientific protocols and the performance of rigorous peer-reviewed experiments with no unseemly haste to rush to the clinic.

In the near future, solving the immune rejection issue should expand the potential use of these cells. Large banks to provide matched ESC-derived products (analogous to blood banks), gene targeting to remove the immune locus, or SCNT to develop matched lines or better immune therapy should allow much wider use of cells than is currently possible. In the longer term, perhaps the transdifferentiation issue or migration and homing issues can be solved so as to increase the number of "druggable" targets. In parallel, it can be projected that novel delivery methods and better tools and reagents to sort, select, isolate, and grow appropriate populations are likely to refine treatment modalities and make cell therapy a more reliable and routine procedure.

It is important to remember that hESCs were identified less than a decade ago and that progress, despite the many hurdles in place, has been rapid. Although it is difficult to predict the future, another decade of concerted effort should bring about dramatic changes.

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