The Leading Edge of Stem Cell Therapeutics

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Abstract

Stem cells, by virtue of their defining property of self-renewal, represent an unlimited source of potentially functional human cells for basic research and regenerative medicine. Having validated the feasibility of cell-based therapeutic strategies over the past decade, mostly through the use of rodent cells, the stem cell field has now embarked upon a detailed characterization of human cells. Recent progress has included improved cell culture conditions, long-term propagation, directed differentiation, and transplantation of both human embryonic and somatic stem cells. Continued progress in understanding basic human stem cell biology, combined with a better handle on the fundamental pathophysiology of human diseases one wishes to target (including the use of human stem cells in primate and other large animal models of human disease), should help to move this technology closer to clinical application.

PD: Parkinson's disease

HD: Huntington's disease

hESC: human embryonic stem cell

NSC: neural stem cell

EC: embryonal carcinoma

mESC: mouse embryonic stem cell

INTRODUCTION

Stem cell biology is currently changing our views on mammalian development, disease, and therapy. Diseases such as Parkinson's disease (PD), Huntington's disease (HD), diabetes mellitus, cardiac failure, and cancer are now discussed in the context of the progress made in stem cell research. The physiological repertoire of a stem cell includes coordinated control of growth and differentiation, as well as the induction of apoptosis, which distinguish them from malignant cancer cells. Stem cells are the most primordial cells of the organism (the embryonic stem cell) or of a given organ (the somatic stem cell). By definition, they are proliferative and self-renewing yet must also give rise to "daughter" cells that comprise the array of specialized mature cells (in the right ratio) that constitute a given organ. Importantly, in the course of development, reservoirs of residual stem cells are established within a given organ that appear to maintain the homeostasis of that organ in the face of lifelong perturbations. A stem cell restores equipoise through a number of actions—only one of which entails the replacement of dysfunctional or senescent cells. Other actions include changing the extracellular milieu or even restoring molecular balance intracellularly to cells the stem cell is designed to "chaperone."

Differentiation of a stem cell into a particular cell type is best assayed by combining morphological, immunophenotypic, and functional criteria. In this review, we discuss the fundamental characteristics of human embryonic stem cells (hESCs) and somatic (tissue-specific stem cells [using the neural stem cell (NSC) as a prototypical somatic stem cell] and highlight their potential roles in clinical therapy.

STEM CELL TYPES

Embryonic Stem Cells

The concept that a pluripotent cell can be perpetuated indefinitely and induced to differentiate rests on pioneering work with teratocarcinomas and embryonal carcinoma (EC) cells. Kleinsmith & Pierce (1) demonstrated that a single EC cell could form a tumor and also give rise to differentiated cell types. Although the developmental potential of EC cells is remarkable, they are still clearly cancer cells and their growth and differentiation is difficult to control. In 1981, two hallmark papers reported the successful derivation of pluripotent embryonic ESCs from the inner cell mass of blastocyst-stage mouse embryos (2, 3). ESCs, still capable of generating teratomas when ectopically injected into mice, were easier to control in terms of growth and differentiation compared to EC cells. Moreover, passaged mouse ESCs (mESCs) differentiated into all cell types of the body including germ cells and generated an entire mouse under appropriate conditions, paving the way for engineering genetically modified mouse models (4). The excitement over pluripotent cell lines increased further with the successful isolation of hESCs (5). Similar to their mouse counterparts, hESCs have unlimited self-renewal capacity and can be induced to differentiate into cell types of the three germ layers. Moreover, because hESCs mimic aspects of early development, these cells not only hold great promise for regenerative medicine, but also for increasing our knowledge about early stages of human embryology, which are so far not accessible for experimentation.

The transcription factors Oct-4, Sox-2, Nanog, and myc are essential for maintaining pluripotency and self-renewal of both mouse and human ESCs (6). (It is likely that other factors will be recognized as research in the field continues.) Nevertheless, fundamental differences exist between mouse and human cells regarding the activated signaling pathways that ensure the molecular signature of pluripotency. For instance, in mESCs leukemia inhibitory factor (LIF) activates Stat3 signaling and bone morphogenetic protein 4 (BMP4) inhibits neural differentiation, thereby promoting their self-renewal under feeder-free conditions (7, 8).

In contrast, LIF and Stat3 signaling are not sufficient to maintain hESCs in an undifferentiated state (9, 10). Additionally, BMP4 was found to promote differentiation of hESCs to trophectoderm (11). There is increasing evidence that high concentrations of fibroblast growth factor 2 (FGF-2) and inhibition of BMP signaling support feeder-independent growth of hESCs (12). Altogether, these fundamental species differences emphasize the point that data accumulated on animal stem cells cannot simply be extrapolated to human cells. Detailed and systematic analysis of human cell lines is of paramount importance if this technology is to be used in a clinical context.

The differentiation potential of ESCs is theoretically unlimited, and the list of different cell types that have been successfully derived from hESCs is continually increasing. Formation of the three germ layers (ectoderm, mesoderm, endoderm), a process called "gastrulation" in vivo, is one of the first developmental steps that can be recapitulated during ESC differentiation in vitro. The most widely used method to induce germ layer cells is the differentiation of ESCs into three-dimensional free-floating structures, the embryoid bodies (EBs). Using adequate protocols, cells within embryoid bodies can be further differentiated into a variety of committed cell types (**Table 1**). However, EBs inevitably contain multiple lineages and cell types, each typically a low percentage of the cellular population. Hence techniques are being derived to differentiate hESCs in monolayer cultures (omitting the step of EB formation) directly, exclusively, and efficiently toward particular desired cell types (29). Such protocols will be necessary before hESCs can be safely and reliably used in clinical situations.

Table 1 Examples for human embryonic stem cell–derived cell types

Specific cell types	References
Ectoderm	
neural precursors	13, 17
dopamine neurons	20, 64, 65, 67
motor neurons	21
retinal cells	31
keratinocytes	16
melanocytes	33
Mesoderm	
fat, cartilage, skeletal muscle	14
bone	14, 25, 29
blood cells	19, 26, 28
cardiomyocytes	27, 32
Endoderm	
prostate cells	15
hepatocytes	23
lung epithelium	30
Trophoblast	11
Primitive ectoderm	18
Germ cells	24

Somatic Stem Cells

Although the field is coming to view all of stem cell biology as a continuum of development, recognition of the existence and power of the somatic stem cell unfolded initially through an entirely different route and for different reasons than the embryonic stem cell story. In the nervous system, recognition that a multipotent cell must exist emerged from an attempt to understand the underlying mechanism behind the unexpected discovery that the central nervous system (CNS) was more plastic and resilient than had ever been imagined (36). Indeed, the CNS became the first solid-organ system in which the existence of a stem cell was recognized. In the hematopoietic system, the recognition of a stem cell grew out of attempts to identify the best cell to graft in the context of bone marrow transplantation—i.e., the cell that would most efficiently and completely reconstitute an ablated bone marrow (34, 35). Single hematopoetic stem cells (HSCs) can serially reconstitute the blood system (all lineages—-erythroid, lymphoid, myeloid, and megakaryocytic) of multiple lethally irradiated organisms.

What made these two organs so informative for the somatic stem cell field was, in some

EGF: epidermal growth factor

ways, their contrasts. That a stem cell should exist in an organ system like the blood that turns over every few weeks to months was not unexpected. It simply remained for the field to identify the most primitive cell. However, that an organ system like the brain—thought to be quite immutable following embryogenesis—should also harbor stem-like cells was a revelation. And that two organ systems that seemed so different should nevertheless harbor cells with quite similar behaviors helped revolutionize the field and ultimately gave rise to the new discipline of regenerative medicine. Stimulated by work in the CNS and the blood, other investigators began to examine other organs to discover whether they, too, harbored stem-like cells, and most were found to do so. To date, tissue-specific stem cells have been reported in the adult mammalian testis, epidermis, gut, heart, pancreas, lung, retina, vasculature, and breast. And the list is likely to grow.

In contrast to ESCs, which can give rise to all cell types of the body ("pluripotence"), somatic stem cells are believed to be capable of generating only the major cell types of their tissue of origin ("multipotence"). It is believed that, in development, a pluripotent stem cell gives rise to a somatic, tissue-specific stem cell, which then participates in organogenesis and persists throughout life in specialized microenvironments ("stem cell niches") in order to support cell turnover (i.e., contributing new cells) as well creating a supportive milieu.

The Neural Stem Cell – A Prototypical Somatic Stem Cell

NSCs—or neural progenitor cells (NPCs), which are immature cells with a somewhat more restricted neurodevelopmental potential than the NSCs—can be generated from hESCs or directly isolated from the developing CNS as well as from neurogenic regions of the adult brain (36, 38, 39, 41). It is now well-established that neurogenesis occurs throughout life in the olfactory bulb and hippocampus of mammals. By definition, NSCs generate the three major cell types of the brain, which are neurons, astrocytes, and oligodendrocytes. They should also be able to give rise to all cell types of all regions of the nervous system during development as well as reconstitute those regions following their destruction.

One challenge in working with NSCs is still the lack of definitive marker proteins and the resulting difficulty in prospectively isolating bona fide stem cells. However, the efficacy of well-characterized NSCs to populate the brain widely and to restore function has been demonstrated over the years (36, 38, 45).

Neural stem/progenitor cells have been cultured as monolayers on coated substrates or as free-floating spherical aggregates, termed "neurospheres" (36, 37). Using the neurosphere assay, proliferative cells derived from the developing and adult CNS can be propagated for extended periods of time in the presence of mitogenic factors such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). However, neurospheres are composed of heterogeneous cells that change during long-term culture, and the percentage of bona fide stem cells within neurospheres has not been shown convincingly. Although the neurosphere assay has been widely used since 1992, we have defined for the first time its actual sensitivity and specificity. Clearly, the number of neurospheres grown in suspension culture does not reflect the number of NSCs. By using time-lapse video-microscopy, we have identified that spheres are highly motile structures prone to merge with each other (82). Therefore, it is highly risky to rely solely on this assay to investigate clonality, multipotentiality, and fate choice of single NSCs. On the other hand, to our knowledge, naïve NSCs that have not been genetically modified [e.g., have expression of stemness genes enhanced (36)] are difficult to perpetuate and survive only for a few passages as monolayer cultures, in part because of the enzymatic detachment of cells at each passage. Clearly, the formulation of a uniformly calibrated and standardized

protocol for NSC propagation and characterization that would be commonly accepted by different laboratories remains a challenge.

MULTIPLE FUNCTIONS OF STEM CELLS DURING DEVELOPMENT AND DISEASE

Although hESCs are derived from the inner cell mass (ICM) of preimplantation embryos, their exact cellular counterpart in vivo remains elusive. It is conceivable that ESCs represent a transient cell population that occurs only during a specific developmental window. As development proceeds, the ICM gives rise to the three germ layers, lineage-restricted somatic stem cells that build the organs, and ultimately to all cell types of the body. Because derivatives of the ICM can be extensively propagated in vitro as pluripotent ESC lines, this technology can be used as a powerful model to study aspects of normal development. Stem cell biologists and clinicians share a common interest in understanding how genetic and environmental factors affect embryonic and somatic stem cells that may cause birth defects and a variety of diseases, including cancer. For instance, a recent study has demonstrated that ectopic expression of the pluripotent stem cell marker Oct-4 in the gut epithelium of adult mice can lead to tumor-like dysplastic growth, suggesting that progenitor cells can be a driving force during tumorigenesis (42). In another report, using a mouse model with mid-gestation lethal cardiac defects, Fraidenraich et al. (43) could rescue these animals by intraperitoneal injection of ESCs into the mother. The authors suggested that molecules such as insulin-like growth factor 1 (IGF 1) and WNT5a provided by transplanted ESCs reversed the congenital heart defects. The concept that stem cells can efficiently deliver gene products to correct hereditary defects has been previously shown with NSCs (36, 45–47, 55).

Once the adult structure is established, a pool of somatic stem cells in various organs maintains tissue homeostasis and integrity during normal cell turnover and after injury. This function of stem cells is obvious in the blood system, gut, and skin, whereas stem cell–based self-repair of the adult brain seems to be limited. However, our fundamental view on brain development and plasticity has changed in recent years because of progress made in the NSC field. It was widely held that neuroepithelial stem cells of the neural tube generate neuroblasts, which migrate along scaffolding processes of radial glial cells to form the cortical plate (48). However, radial glial cells, we are learning, are actually NSCs, which, at the same time, can give rise to migratory neuroblasts and guide them to their final destination in the cortex (49, 50). Another fundamental stem cell-related finding, the occurrence of adult neurogenesis, has refuted an old dogma in neuroscience. It is now well-accepted that new neurons are continuously added to two distinct brain regions (at least in the rodent): the olfactory bulb and the dentate gyrus of the hippocampus (51). Numbers of correlative studies have shown that the rate of adult neurogenesis can be modulated by a number of physiological and pathological conditions in these brain regions. However, the potential causal role of adult neurogenesis for normal brain function (e.g., learning and memory) and disease (e.g., epilepsy) still needs to be defined.

THERAPEUTIC STRATEGIES EXPLOITING STEM CELLS

Transplantation

Cell replacement. It is conceivable that transplantation of appropriate cell types, either derived from ESCs or tissue-specific stem cells, is an effective method to replace cells lost due to pathology. The first demonstration that stem cells might be used for cell replacement in a solid organ actually derived from work in the adult mammalian neocortex a decade ago (58). In an adult mouse model of experimentally induced apoptosis of pyramidal neurons, it was demonstrated that

engrafted naïve NSCs responded to this environmental cue (the apoptotic death of a particular neuronal cell type) to differentiate specifically into that cell type and project axons to their proper target region (although the normal window for cortical neurogenesis had passed). The NSCs yielded neurons only within the circumscribed 300-μm diameter of pyramidal cell death. Outside that region, or in the neocortex of an intact adult, the same clone of NSCs yielded only glia.

Since that time, many studies have documented that cell replacement is effective in developing as well as in adult tissue. However, it is important not to overgeneralize. Each case, each disease, each model, and each cell sourse must be evaluated individually. For example, depending on the source of transplanted cells, their cellular differentiation state, and the disease model used, grafted cells could just as likely die during cell preparation and early after implantation (53, 54). In a rat model of PD, Nikkhah et al. (52) found that micrografting multiple dopamine cell deposits is more effective than increasing the volume of single grafts. In a reproducible manner, this micrografting approach resulted in a better cell survival and more extensive reinnervation of the host striatum than did large single grafts. Defining the appropriate cell differentiation state before grafting (naïve versus predifferentiated) and improved transplantation techniques in combination with anti-apoptotic and cell-protective drugs are key areas that need to be better understood prior to clinical translation of stem cell therapy. In addition, establishing a standardized postoperative immunosuppressive regimen is critical, since insufficient immunosuppression has been suggested as a possible reason for the poor outcome in some clinical trials with PD patients. The continous treatment with immunosuppresive drugs (e.g., cyclosporin alone or in combination with other drugs) over several months seems to be important in order to prevent acute and delayed immunologial responses to the grafted cells (60).

"Chaperone" effects. We have demonstrated that NSCs, besides their potential for cell replacement, have important additional biological properties that could be harnessed therapeutically. There are several lines of evidence now that grafted NSCs naturally deliver trophic and cytoprotective molecules such as glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin-3 (NT-3) to the diseased brain. Other NSC-derived factors, not entirely identified and characterized, exert anti-inflammatory actions, decrease scarring, and promote angiogenesis. Experiments in aged rodents showed that NSCs releasing GDNF can rescue dopamine neurons of the mesostriatal system that were lesioned with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (45). These findings led to the concept that NSCs are endowed with inherent mechanisms for rescuing dysfunctional neurons, now called the "chaperone effect." This effect is likely part of the stem cell's repertoire of "homeostasis-promoting" actions. Although first discovered in the brain (45), this effect has now been found to be key to the action of stem cells from many organ systems in many diseases (e.g., bone marrow-derived mesenchymal stem cells for cardiac disease, umbilical cord cells in stroke).

Stem cell–based gene therapy. Depending on the specific disease, pathology in the brain can be restricted to specific sites or widely distributed. In an ideal therapeutic scenario it would be possible to target the pathological lesions while avoiding healthy tissue. Efficient delivery of therapeutic molecules to specific brain regions is still a major challenge in gene therapy. Because NSCs (endogenous and grafted) exhibit a remarkable ability to migrate with a proclivity to home to pathology and are able to integrate seamlessly into the host brain while continuing to stably express a foreign transgene, it was reasoned that these cells may be ideal vehicles for the delivery

of therapeutic molecules for CNS disorders. In fact, inflammation and molecules released during acute or chronic injuries were found to be chemo-attractants for NSCs. For instance, SDF1-α secreted during the inflammatory response (in part by macrophages, activated microglia, reactive astrocytes, and inflamed endothelium) strongly attracts migratory human NSCs even over long distances (55). Therefore, NSCs manipulated ex vivo (e.g., by viral transduction) and transplanted into the brain may be well suited for long-range delivery of therapeutically relevant molecules and drugs to CNS lesions. The efficacy of this powerful concept has been demonstrated in mouse models of lysosomal storage diseases and brain tumors (see below).

Ex Vivo Applications

Standardized cell assays. Stem cell technology offers the opportunity to develop in vitro assays based on well-defined specific human cells. Existing assays for drug screening/testing and toxicology studies have several shortcomings because they are of animal origin, immortalized cell lines, or derived from cadavers. Because these alternatives often poorly reflect the physiology of normal human cells, stem-cell derived assays (e.g., homogeneous populations of heart and liver cells) could be established in the future and may play an important role for these purposes.

Disease modeling. The flurry of new information now available on the molecular and cellular level related to human diseases (e.g., microarray data) makes it crucial to develop and test hypotheses about pathogenetic interrelations. The experimental access to hESC-derived specific cell types from all developmental stages and even from blastocysts deemed to harbor pathology based on pre-implantation genetic diagnosis may be useful in modeling and understanding aspects of human disease (22). Such cell lines would also be valuable for the testing of drugs.

Recruiting Endogenous Stem Cells

The existence of adult stem cells in various organs, including the brain, offers the opportunity to recruit these cells for tissue repair. Although it appears that adult neurogenesis is restricted to the olfactory bulb and dentate gyrus of the hippocampus, it may well be that NSCs exist along the entire adult neuraxis (40, 41), but most CNS regions are not permissive for neurogenesis under normal in vivo conditions. It has been demonstrated that cells derived from non-neurogenic regions can generate new neurons in vitro or after transplantation into a neurogenic region such as the hippocampal dentate gyrus (56, 57), suggesting that most brain areas inhibit neurogenesis. Under pathological conditions both neurogenic and non-neurogenic regions can promote the recruitment of new neurons (58). The limited number of adult NSCs, their restricted location, and the limitations of non-permissive microenvironments are major hurdles and it remains unclear if recruitment of endogenous NSCs may be a realistic clinical prospect for brain repair. Also, there is no guarantee that sufficient numbers of endogenous neural progenitors can be recruited without causing deformation of the region, or that they can be induced to generate neurons that will make proper neural connections without making wrong neural connections. Finally, the use of endogenous NPCs will likely have therapeutic potential only in diseases that are not genetically based. In diseases that are likely to result from a pre-existing genetic abnormality—e.g., such neurodegenerative conditions as HD, amyotrophic lateral sclerosis (ALS), Alzheimer's, PD, and lysosomal storage diseases (LSDs)—the endogenous NPCs will also carry a genetic defect.

LSD: lysosomal storage disease

Nuclear Reprogramming or Somatic Cell Nuclear Transfer

Nuclear reprogramming or somatic cell nuclear transfer (SCNT), also referred to as "therapeutic cloning" in the lay press, is a

new technology that may have potential to create patient-specific pluripotent cell lines. Conceptually, the nucleus of a fully differentiated cell (e.g., skin cell) is transferred into a denucleated egg and developed into blastocysts that then can be used to establish stem cell lines. By using SCNT, Rideout et al. (59) derived an ESC line from immune-deficient Rag2 mice. The authors then repaired the gene defect in these ESCs, generated ESC-derived hematopoetic precursors, and successfully reconstituted the blood system of the same immune-deficient Rag2 mice. Although promising experience has been accumulated on animal cells, it is currently not known if similar strategies can be applied to human cells. Results obtained so far indicate that nuclear reprogramming of human cells is more challenging and intricate than reprogramming of animal cells. Future experimentation will be required to determine whether the limitations of nuclear reprogramming of human cells can be controlled and the efficiency of this technology increased. To date, it has not yet been achieved.

STEM CELLS AND NEUROLOGICAL DISEASES

Various brain disorders have been suggested as potential targets for stem cell therapy. We focus here on four CNS diseases that could benefit from the different therapeutic capabilities of stem cells. For further reading on stroke and multiple sclerosis, see the articles by Suwanwela & Koroshetz and de Jager & Hafler, respectively, in this volume.

Parkinson's Disease (PD)

PD is a progressive neurodegenerative disorder characterized mainly by the loss of nigrostriatal dopaminergic neurons, which leads to characteristic clinical symptoms such as tremor, rigidity, and bradykinesia. Promising proof-of-principle experience was acquired with clinical transplantation of fetal mesencephalic grafts into patients in the early 1990s (60). It has been demonstrated that transplantation of dopamine-producing neurons into the dopamine-deficient striatum, the axonal target region of the substantia nigra, is an effective therapy. Because of problems with fetal tissue availability, difficulties in defining the best treatment conditions, and variation in functional outcome (a subset of patients who appeared to benefit from the fetal tissue also went on to develop refractory dyskinesias for unclear reasons), this approach has not been developed into a standardized clinical therapy. Therefore, the reliable generation of standardized dopamine neurons, preferably the A9 neuron subtype of the midbrain, in unlimited numbers is critical for future cell therapy in PD. Derivation of unlimited numbers of dopamine neurons from immortalized NSC lines (61) and ESCs is currently the most promising paradigm in animal experiments. Using differentiation protocols that recapitulate some aspects of development, midbrain-type dopamine neurons expressing specific transcription factors have been derived from animal (mouse, monkey) and human ESCs (62–67). In some studies, transplantation of these cells resulted in functional improvement in rat and monkey models of PD. However, the in vivo functionality of hESC-derived dopamine neurons remains to be shown.

Although considerable progress has been made recently towards a stem cell-based therapy of PD, several issues need to be addressed before clinical application. First, the currently available protocols for hESCs yield considerable proportions of dopamine neurons, yet a highly purified and homogenous population of midbrain-type human dopamine neurons has not been generated. Second, it is still unclear if these hESC-derived dopamine cells can survive, reinnervate the striatum, and restore function in animal models of PD. Third, because contamination with undifferentiated cells may be a potential risk for teratoma formation, the safety of hESC-derived grafts has to be established. Fourth, additional strategies such as postoperative rehabilitation after

stem cell therapy may be necessary to maximize therapeutic efficacy for individual patients (68). The clinical experience with fetal grafts suggests that the patient's disease history is an important parameter and that cell therapy most likely will not be the method of choice for every parkinsonian patient. Patient selection seems to be as crucial as graft placement and post-operative immunosuppression. Finally, the adverse side effects such as dyskinesias observed in some patients after transplantation of fetal grafts need careful consideration (60).

Huntington's Disease

Cell replacement strategies for HD are based on a wealth of experimental evidence and a small number of clinical pilot studies. HD is a hereditary disorder (autosomal dominant) caused by a CAG-repeat expansion mutation on chromosome 4. The neuropathological hallmark is the nuclear and cytoplasmatic deposition of huntingtin fragments, resulting in a progressive and selective neuronal loss of GABAergic medium spiny striatal neurons. Clinically, HD presents with a triad of motor, cognitive, and psychiatric symptoms and signs and progresses relentlessly over 15 to 20 years, leading inevitably to the death of the affected individuals. At present, there is no disease-modifying treatment or cure for HD patients. Transplantation of NSCs may offer a novel treatment option that may slow, halt, or even reverse the progression of this devastating illness (69). Experimental studies in animal models of HD have provided convincing evidence that fetal neural tissue can survive transplantation, grow, and establish functional afferent and efferent connections with the host brain. These observations correlated with an amelioration of lesion-induced behavioral deficits including abnormal locomotion, chorea, dystonia, and dementia (69, 70). Furthermore, several independent open-labeled clinical phase 1 trials on HD patients have demonstrated clearly that the neural transplantation approach is feasible, and can be established ethically and safely with a minimum risk to the patient (71–73). Three of five grafted patients demonstrated long-term stability of clinical performance and even clinical improvements on some symptoms up to six years following fetal neural tissue grafting (74). These clinical changes were paralleled by results reported from electrophysiological tests and fluorodeoxyglucose-positron emission tomography (FDG-PET) scans. A postmortem analysis in a transplanted HD patient who died 18 months after grafting, from causes unrelated to the procedure, clearly demonstrated that human fetal striatal neural tissue can survive successfully, form synaptic contacts, and resist the underlying disease process (72). **Figures 1** and **2** give examples of graft analyses of an HD patient who was treated with fetal striatal tissue. While not proven, conventional wisdom in the field is that the cellular component within primary fetal tissue that accounts for its efficacy is the population of NPCs. Therefore, the further assumption is that purified populations of NPCs would be even more effective–by virtue of being able to administer larger numbers of homogenous NPCs in their optimal state of differentiation more reliably.

Taken together, the extensive experimental data in animal models as well as the more limited clinical data clearly support the further development of stem cell-based restorative therapies for HD.

Several other reasons support the notion that stem cells may be ideal candidates for HD treatment. First, NSCs preferentially differentiate into GABAergic cells, which is the prevailing neuron cell type to be replaced in HD. Second, they have already been shown to survive, reinnervate the striatum, and restore function in animal models. Third, they can be produced in large numbers necessary to develop a standardized clinical therapy that could widely be used. Compared to PD, HD may offer an advantage because stem cells can be grafted organotypically into the striatum. Finally, grafting of genetically modified stem cells may be considered to target recently

identified key events in the pathogenesis of HD (75).

Most stem cell work in HD to date has involved NPCs. Anatomical and functional reconstruction of the striatal synaptic circuitry by hESCs has yet to be demonstrated in HD animal models.

Lysosomal Storage Diseases

As stated above, NSCs display an impressive migratory potential after transplantation and disseminate widely in the brain parenchyma. The concept that migratory NSCs may be exploited for the delivery of important molecules was first demonstrated in a mouse model of mucopolysaccharidosis type VII (MPS VII), which shares many biochemical, pathological, and clinical features with human MPS VII (Sly disease). This incurable lysosomal storage disease (LSD) is caused by an inherited single gene deficiency of β-glucuronidase (GUSB), which is a secreted enzyme involved in the degradation of glycosaminoglycans. Mice and humans deficient in GUSB show neurodegeneration throughout the brain (e.g. cerebral neocortex, hippocampus, cerebellum), and progressive mental retardation is a typical clinical symptom in humans. Human and murine GUSB-expressing NSCs were grafted into the ventricles of newborn mice. Remarkably, NSCs not only disseminated widely throughout the neuraxis, but also cross-corrected lysosomal function in mutant cells. Increased GUSB concentrations were found up to 8 months after transplantation (44). Following a similar approach, Lacorazza et al. (76) transplanted retrovirally transduced NSCs into the brains of fetal and newborn mice that were deficient for β-hexosaminidase A (Hex A). This enzyme deficiency characterizes the neurodegenerative Tay-Sachs disease. The authors reported enzyme expression for up to 8 weeks and increased levels of Hex A activity (76). Another genetic disease that has been studied with a similar stem cell-based gene therapy is Niemann-Pick disease (77). Pathogenetically, in Niemann-Pick disease, the lack of acid sphingomyelinase (ASM) results in accumulation of sphingomyelin and cholesterol in lysosomes. Grafting of genetically modified adult neural precursor cells reversed lysosomal storage pathology in mice up to 10 weeks.

It is important to note that wild-type, normal NSCs, because they constitutively produce a normal complement of lysosomal enzymes, may not require genetic engineering to be useful. Of course, if one desires an overproduction of this or other enzymes, they can be readily engineered via viral gene transfer techniques.

Although at the time of this writing, there have been no clinical trials employing NSCs for actual patients with LSDs, it is likely that such patients will be the subjects in the first legitimate clinical trials using NSCs. The delay in launching clinical trials has been due to the reluctance of regulatory agencies to approve experimental therapies in children, the age group of patients that typically present with these lethal neurodegenerative diseases. Also, there has been insufficient capital invested in stem cell therapies from the private sector, particularly for such rare diseases as the LSDs.

Brain Tumors

Despite progress made over the past decades in medicine, the poor prognosis of malignant brain tumors, particularly of glioblastoma multiforme (GBM), has not changed significantly. The median survival of GBM patients is only 12–14 months, and surgical tumor resection or combined radio-chemotherapy offers very modest increase in survival. One difficulty in targeting and eliminating GBM is the fact that these malignant cells are highly invasive, with diffuse and widespread distribution throughout the brain parenchyma. A second reason why brain tumors have been difficult to target therapeutically may be explained by our poor knowledge about the first steps of carcinogenesis and the identity of the tumor-initiating cell(s). Recent experimental data

suggest that the presence of stem-like cells that have lost the ability to control growth and differentiation ("cancer stem cells") correlate with the malignancy grade and poor prognosis of brain neoplasias (78). Brain tumor-forming cancer stem cells may well be the product of deregulated and transformed NSCs, though this remains to be proven. Based on the findings that stem cells and neoplastic cells exhibit similar properties (e.g., migratory potential, self-renewal, molecular signature), we suggested that these cells indeed may represent two sides of the same coin (79).

Defining the similarities and differences between cancer cells and stem cells may help not only to understand the early steps of tumor formation, but also to design new therapeutics. In fact, the first studies to demonstrate that grafted NSCs can migrate over very long distances, home to the tumor mass, or even track single highly invasive cancer cells have opened exciting new avenues for the treatment of all cancers. Aboody et al. (80) demonstrated that murine and human NSCs, grafted to a number of different locations (intratumoral, contralateral hemisphere, intraventricular, intravenously injected into the tail vein), show a remarkable tropism to pathology in the adult brain, the first example of which was a brain neoplasm. The authors then grafted NSCs that were manipulated to express the pro-drug-converting enzyme cytosine deaminase. Because cytosine deaminase converts the systemically administered prodrug to 5-flurouracil, a chemotherapeutically active compound, tumor cells could be specifically targeted and the tumor mass dramatically reduced. Similar experiments by other groups confirmed that migratory NSCs modified to express a wide variety of therapeutic genes are powerful weapons against brain cancer. Different genetic and nongenetic strategies in NSC-mediated brain tumor therapeutics have been recently suggested (see table 1 in Reference 79).

Several factors released by different cells (e.g., neoplastic cells, tumor stroma, endothelial cells of tumor angiogenesis) are likely to contribute to this pronounced tumor-tropism of NSCs. The chemokine receptor CXCR4 and its ligand SDF-1α seem to play an important role in chemo-attracting NSCs to neoplastic lesions. Other molecules reported to date that attract migratory NSCs include stem cell factor (SCF), monocyte chemoattractant protein-1 (MCP1), and vascular endothelial growth factor (VEGF) (79). Other factors that promote the homing of stem cells are likely those that emanate from the damage to normal tissue rendered by the tumors.

The use of stem cells against cancer represents "low-hanging fruit" in the translation of stem cells to clinical practice.

PERSPECTIVES

Stem cells form organs, maintain tissue homeostasis and integrity in the adult, and represent a powerful source for cell and gene therapy. We have described two prototypical stem cells—pluripotent hESCs and multipotent somatic stem cells as modeled by the NSC—and highlighted their potentials for clinical use. Currently, it seems prudent to pursue the systematic characterization of both pluripotent and multipotent stem cells in parallel and not to study one type to the exclusion of the other. We expect that the stem cell field, and ultimately the patient, will benefit from a synergistic and integrative approach that may require the use of different stem cell types—even within the same individual at different stages of his disease. For stem cells to be developed as a therapeutic tool, problems such as standardized protocols, reproducibility, quality control, and safety need to be addressed (81). But in the meantime, stem cells may contribute to the treatment of human diseases in other ways. First, because stem cells can produce a great number of human cells, they could be used for large-scale drug screening and testing. Second, stem cells offer the opportunity to develop cellular models of human disease and to study aspects of complex pathogenetic interrelations. Stem cell technology

will likely play an equally important role in disease modeling and cell therapy. Third, stem cell therapies must be compared to conventional therapies, and be guided by the medical commitment to first do no harm. Eventually, clinical trials with small numbers of selected patients could be initiated for candidate diseases such as the lethal hereditary neurodegenerative diseases of childhood (e.g., LSDs), brain tumors, and possibly HD and ALS. In each case, one would be taking advantage primarily of stem cells as vehicles for molecular therapies rather than for cell replacement (with the possible exception of striatal manifestations of HD). Effective large-scale cell replacement awaits more sophisticated research into both the behavior of the stem cell and the needs of the diseases.

Stem cell research is a multidisciplinary and multilayered challenge, and the current pace of progress in the field is taking this technology closer to clinical application.

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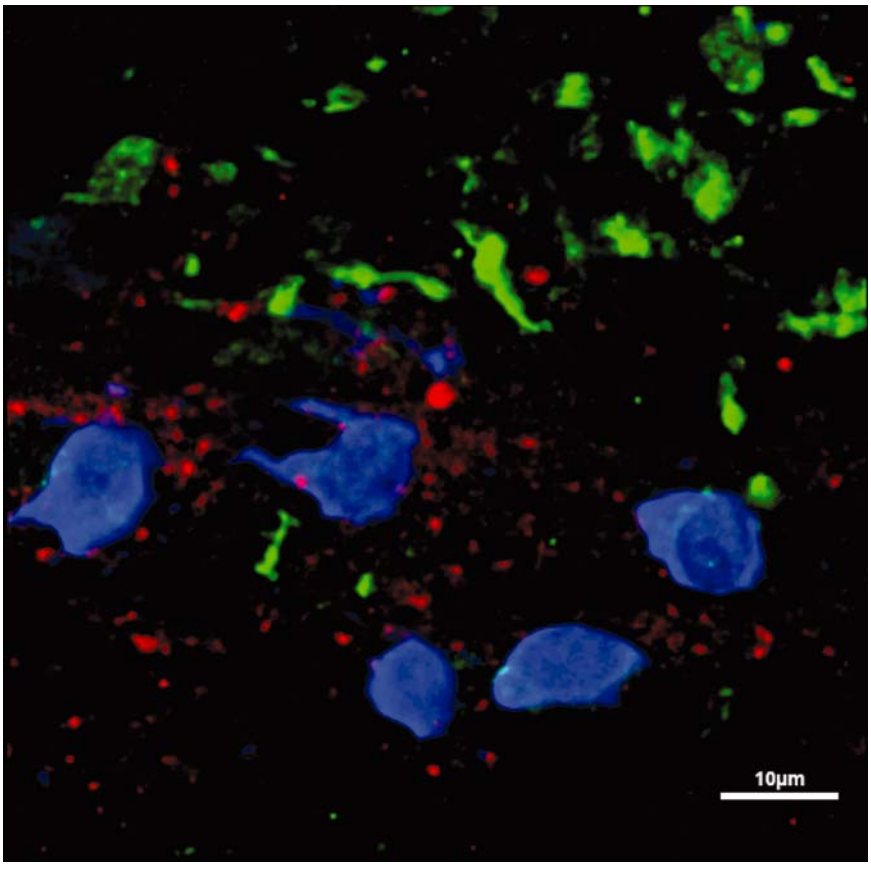

Figure 1

Confocal image showing immunostaining for doublecortin (*green*; a marker for immature migratory neuroblasts), synaptophysin (*red*; a presynaptic marker), and DARPP-32 (*blue*; a marker for striatal neurons) in the caudate and putamen of a Huntington's disease (HD) patient who died of an unrelated cause six months after grafting of fetal striatal progenitor cells (P. Capetian, R. Knoth & G. Nikkhah, manuscript in preparation).

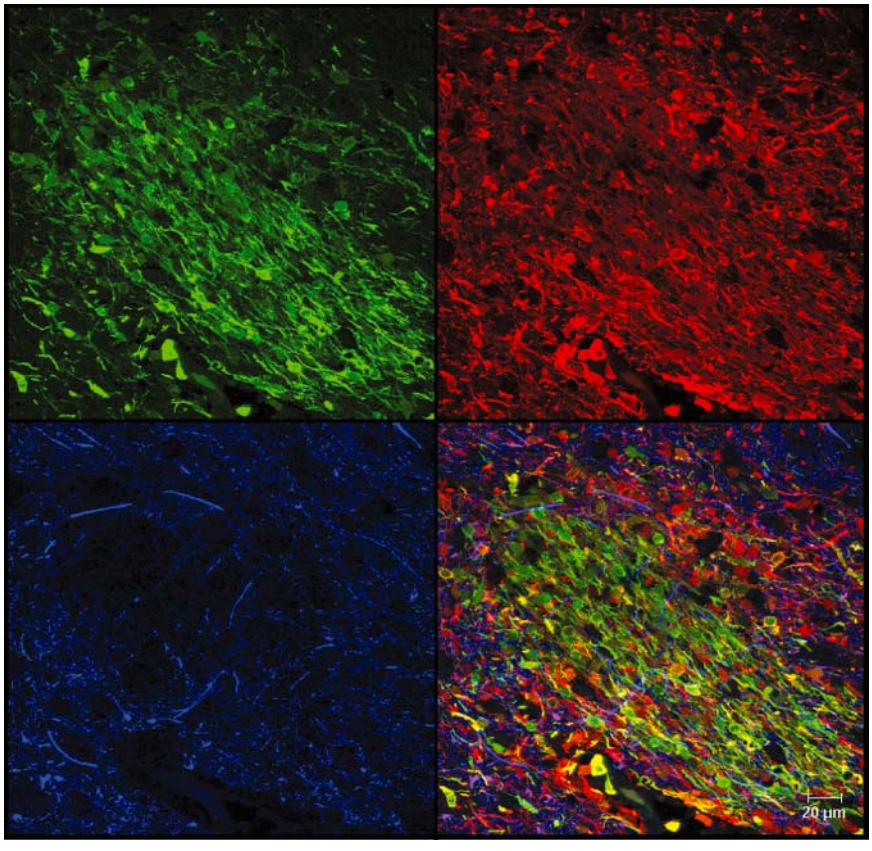

Figure 2

Confocal analysis of the graft of the same HD patient as in **Figure 1**. Immunolabeling for tyrosine hyxdroylase (*blue*; a marker for striatal dopaminergic afferents) suggests a moderate innervation of the graft zone still expressing the immature neuronal markers β-III-tubulin (*red*) and doublecortin (*green*) six months after transplantation. The fourth panel represents the merged channel (P. Capetian, R. Knoth & G. Nikkhah, manuscript in preparation).