Application of Neural Stem Cells

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Running Title: Neural stem cells are uniquely suited for cell replacement and gene therapy in neurological disorders

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Abstract

Investigations into the biology of neural stem cells (NSCs) informs not only our understanding of development, plasticity, degeneration, aging, an repair in the nervous system, but also serves as a bellwether for new insights into these processes in other organ systems, as well. Stem cells, it i believed, play a pivotal role in the earlie development of the nervous system. Their potential therapeutic role is predicated in part on the hypothesis that these developmental processes may be reinvoked at later stages in life in order t re-create damaged portions of the nervous system. Indeed, properties of stem cells which can be isolated, grown in abundance *ex vivo*, and reimplanted seamlessly into recipient organs efficiently -- suggest that they, indeed, display predisposition towards repopulating damaged central nervous system (CNS) regions. This area of investigation has come to be an interface between developmental biology and regenerative medicine (including cellular and molecular approaches to organ reconstruction). Because NSCs were the first solid organ stem cell to be recognized and characterized, they have served as a prototype for understanding the developmental role and therapeutic potential of stem cells in other systems as well.

Key words: neural stem cells, cell replacement, gene therapy, neurological disorders

초 록

신경줄기세포는 미성숙, 미분화된 세포로 계속하여 증식하여 자가갱신 (self-renew)하고, 전체 신경계를 이루는 다양한 신경세포로 분화하는 분화의 다능성 (multipotency)을 보이며, 발달단계의 신경계 및 손상된 신경계에서 신경세포를 재구성 및 재생하는 세포를 일반적으로 의미한다. 이러한 신경줄기세포의 생물학적 특성에 관한 연구는 신경계의 발달, 가소성, 변성, 노화 및 재생기전을 밝히기 위하여 필수적일 뿐만 아니라, 손상된 신경계에 있어서 신경줄기세포를 이용한 세포 및 유전자치료에 적용하여 난치성 신경계질환에 대한 새로운 재생의학적 치료를 가능하게 할 것이다. 현재 인간을 포함한 포유동물의 신경줄기세포를 생체 외에서 대량으로 증식 배양할 수 있고, 질환동물의 신경계에 이식 시 공여세포는 생착, 이주, 분화 및 외부 유전자의 확고한 발현을 보이며, 숙주동물의 신경계에 구조적 및 기능적으로 통합됨이 확인되어 향후 기전규명을 통한 임상시도 적용과 다른 장기에서도 줄기세포를 이용한 재생치료에 적용될 수 있을 것이다.

중심단어: 신경줄기세포, 신경계질환, 세포치료, 유전자치료

Since neurobiologists identified cells with surprising plasticity, multipotency, and a propensity for dynamically shifting their fates within cultures obtained from the developing and mature CNS over 10 years ago[1-4], the existence of such cells

challenged the prevailing dogma that the nervous system was rigidly and immutably constructed. NSCs, as these plastic cells came to be termed, began to acquire the interest of not just the developmental community but also that of the neural repair, gene therapy, and transplant communities when it was recognized that they could be expanded in culture and reimplanted into the mammalian brain where they would reintegrate appropriately and stably express foreign genes[5-9]. Their abundance, multipotency, ease of manipulation, and engraftability made this strategy an attractive alternative for CNS gene therapy and repair.

NSCs are the most primordial and least committed cells that exist in the developing and even adult nervous system[10, 11]. Multipotent NSCs are operationally defined by their ability to self-renew, to differentiate into cells of all glial and neuronal lineages throughout the neuraxis, and to populate developing or degenerating CNS regions[12-16]. Thus their use as graft material can be considered analogous to hematopoietic stem cell-mediated reconstitution and gene transfer[17].

CHARACTERISTICS OF CNS DISEASES

The pathologic lesions of many neurological disorders are often globally dispersed throughout the brain. Such diseases include the inherited neurodegenerative diseases of the pediatric age group (e.g., inborn errors of metabolism such as the lysosomal storage diseases; leukodystrophies; hypoxic-ischemic encephalopathy) and some adult CNS diseases (e.g., multiple sclerosis and Alzheimer's disease). Widespread enzyme and/or neural cell replacement throughout the cerebrum may be beneficial for some of these global diseases. Other disorders are manifested by a loss of more discrete neural populations affecting a more limited area of the CNS, such as Parkinson's disease, some aspects of Huntington's disease, and perhaps aspects of spinal cord contusion. Engraftment of specific types of neurons or glia into circumscribed areas may be therapeutic in some of these cases. For example, one might envision engraftment of NSC-derived GABA-ergic neurons to replace the spiny striatal neurons lost in Huntington's disease or NSC-derived dopaminergic neurons to replace those lost in Parkinson's disease. However, as our knowledges about disease processes grow, we are beginning to learn that one probably needs to replace more than just one neural cell type in a disease. For example, in a disease such as amyotrophic lateral sclerosis (ALS), a disorder characterized by progressive motor neuron degeneration, we are beginning to learn that astrocyte replacement may be just as critical as replacing motor neurons. Conversely, in multiple sclerosis, a white matter disease characterized by oligodendrocyte degeneration, replacing neurons and their axonal connections may be critical for the restoration of function. These same caveats may apply to a number of diseases in which replacement of multiple cell types may be the key to neurological reconstitution and to reconstructing a damaged milieu[18].

BIOLOGICAL AND THERAPEUTIC POTENTIALS OF NEURAL STEM CELLS

Although the degree to which the mammalian CNS supports the birth of neurons and other cell types outside of their classical spatial or temporal developmental windows has become an area of intense investigation and debate[19-22], most agree that one of the repositories of whatever plasticity exists is the NSC, residing lifelong within various secondary germinal zones of the brain. Indeed, it was the observation that exogenous multipotent NSCs could respond to the prevailing cues of normal and abnormal microenvironments that first suggested the existence of spontaneous compensatory mechanisms for genetic or acquired deficiencies[21, 23-25], including neurogenesis beyond its normal confines. It is acknowledged, however, that these compensations alone are not sufficient to redress neurological deficits in the most devastating of injuries. This poor regenerative ability may be due to the restricted location and limited number of NSCs and/or to limitations imposed by the surrounding microenvironment, which may not be supportive or instructive for neuronal differentiation. Therefore, NSCs expanded ex vivo in culture and

then implanted intracerebrally to regions in need of repair may overcome those limitations that are related to inadequate numbers of NSCs in proximity to the defective region. Whether the environment itself may also inhibit exogenous NSCs from surviving or differentiating towards replacement cells is a possibility. However, a number of transplantation experiments have suggested that neurogenic cues are transiently elaborated during degenerative processes (perhaps recapitulating developmental cues) and that exogenous NSCs are able to sense and respond appropriately to those. In other words, NSCs appear to respond in vivo to neurogenic signals not only when they occur appropriately during development, but even when induced at later stages by certain neurodegenerative processes, for example, during apoptosis. Thus, the degree to which these natural processes might be augmented by supplying exogenous NSCs with or without exogenous stimulants and/or molecular prompting and priming is the primary focus today of regenerative neurobiology[23, 26-31].

NSCs possess other inherent biologic properties that make them attractive for the treatment of metabolic, degenerative, traumatic, or other lesions in the brain, particularly those that are widely distributed. They are easy to administer (often directly into the cerebral ventricles), they are readily engraftable, and they circumvent the blood-brain barrier. A preconditioning regime is not required prior to administration (e.g., total body irradiation) as is necessary for bone marrow transplantation. NSCs appear to accommodate to the region of engraftment, perhaps obviating the necessity for obtaining donor cells from many specific CNS regions or the imperative for precise targeting during reimplantation. The NSCs and their progeny intermingle nondisruptively with endogenous neural progenitor/stem cells, responding to the same spatial and temporal cues in a similar manner. Because of their ability to develop into integral cytoarchitectural components of many regions throughout the host brain as neurons, astrocytes, oligodendrocytes, and even incompletely differentiated but quiescent progenitors, NSCs may be able to replace a range of missing or dysfunctional neural cell types within the same region. This is important in the likely situation in which return of function may require the reconstitution of the entire milieu of a given regionfor example, not just the neurons but also the glia and support cells required to nurture, detoxify, guide, and/or myelinate the neurons. The NSCs, particularly in certain differentiation states, might express various genes of interest intrinsically (e.g., many neurotrophic factors, lysosomal enzymes, angiogenic factors, anti-inflammatory molecules, anti-oxidants), or they can be engineered ex vivo to do so since they are readily transduced by gene transfer vectors. These gene products can be delivered to the host CNS in a direct, immediate, and stable manner. While NSCs can migrate and integrate widely throughout the brain, particularly when implanted into germinal zone—sallowing reconstitution of enzyme or cellular deficiencies in a global manner—this extensive migratory ability is present even in the parenchyma of the diseased adult and aged brain. NSCs may be attracted, even across long distances, to regions of neurodegeneration in brains of all ages, including old age. Despite their extensive plasticity, NSCs never give rise to cell types inappropriate to the brain (e.g., muscle, bone, teeth) or yield neoplasms. The use of NSCs as graft material in the CNS may be considered almost analogous to hematopoeitic stem cellmediated reconstitution of the bone marrow[26, 28].

ISOLATION AND PROPAGATION OF MURINE AND HUMAN NSCs

Neural cells with stem cell properties have been isolated/derived from the embryonic, neonatal, and adult rodent and human CNS using several different in vitro expansion methods. The recognition of marker proteins consistent with an immature phenotype has contributed to the characterization of such cells. Commonly used NSC markers have included Nestin and Vimentin, intermediate filament proteins; Musashi 1, an RNA-binding protein; and Sox1, a transcription factor. These markers are selective rather than specific for NSCs because they are also expressed at lower levels in neural progenitor cells and even in nonneural cells (e.g., muscle and endothelium). Therefore, better than

simply looking for the presence of markers are functional assays for stem-like behavior. The operational definition of a neural stem cell is a single cell that can give rise to clonally related progeny of all neural cell types (neuronal and glial) that can populate the developing nervous system and repopulate ablated regions of the nervous system as well as yield daughter cells with identical potential. Such cells have been found to proliferate in serum-free culture medium in response to either basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF), suggesting the presence of both receptors on their cell surface as a defining characteristic, although cells in the very early rodent embryo are responsive to only bFGF and subsequently develop EGF-responsiveness. Whereas bFGF can substitute for serum in maintaining the mitosis of NSCs, EGF maintains cell division only half as well as serum[1, 6, 9, 10-13, 26].

A stem cell is a cell with mitotic potential by definition. While mitotic, a stem cell retains its greatest degree of multipotency. In other words, its exit from the cell cycle is accompanied by a rapid cascade of commitment steps and a progressive narrowing of potential, often under the instruction of pertinent external environmental cues. The extent to which an NSC's commitment is preprogrammed within the cell (i.e., cell autonomous) versus the extent to which it is determined by external signals from the milieu (i.e., cell nonautonomous) remains to be determined and controversial. It is possible that the number of cell divisionsand hence the degree to which multipotency and environmental responsiveness can be maintainedchanges with the age of the stem cell or the age of the organism within which the stem cell resides. Certain genes including those that help mediate mitotic action can also be transduced into a stem cell to maintain its ability to self-renew, blunt senescence, hold commitment in abeyance, and/or preserve multipotency. One such gene is myc, the overexpression of which will maintain stem-like behavior in an NSC. The gene does not preclude responsiveness of the NSC to normal environmental and growth control cues and is downregulated following transplantation in vivo. Other genes that have been used include telomerase and SV40 large T antigen[1-4, 26].

Like any type of dividing cell maintained in serum-free medium without an adherent substrate, NSCswhen maintained in those culture conditionscan form floating clusters (cells of a similar nature typically have great attraction for each other). The presence of such floating clusters (called neurospheres) is not synonymous with a defining characteristic of a stem cell, or of a cell of neural identity, or even of a bona fide clone. The ability to form a sphere can suggest that the cells are actively proliferating, one of the requirements of a stem cell; however, that is the extent to which sphere-forming ability is useful. The remaining aspects of the operational definition of a stem cell as outlined above must be fulfilled. The presence of a panel of markers (e.g., vimentin, nestin, musashi1), rather than just one marker, will support but not cinch this assessment.

Maintaining an NSC in a proliferative state in vitro—whether by epigenetic or genetic manipulation—does not appear to subvert its ability to respond to appropriate environmental cues in vivo following transplantation into the developing brain: to migrate throughout the brain and to differentiate into neurons, astrocytes, and oligodendrocytes. It remains to be determined, however, whether prolonged passaging of cells in vitro produces fundamental changes in these cells that begin to change them from their in vivo predecessors and counterparts[26, 28, 29].

Adapting similar strategies, researchers have isolated stable populations of human NSCs or progenitors from the respective regions (e.g., telencephalon, diencephalons, midbrain, cerebellum, spinal cord, etc.) of the whole developing human CNS and cultured them in vitro as neurospheres with epigenetic tools for a long-term. Preliminary experiments show that cells within neurospheres have specific regional or temporal characteristics with regard to growth, differentiation, and gene expression patterns according to the anatomical sites of CNS from which they are isolated. But, their regional and /or temporal specificity of human NSCs or progenitors are not irreversible, and can be altered by local microenvironmental inductive cues[32]. The principal differences between human NSCs and mouse NSCs so far seem to be the issues of cell cycle (protracted in human cells with

a strong predilection to exit the cell cycle and differentiate, or to cease cycling entirely after about 100 doublings), but many of the important biological principles gleaned from examining rodent cells have been conserved in the human CNS[9, 16-18, 32]. Lines of engraftable human NSCs (hNSCs) have been isolated from fetal and adult specimens that, in many ways emulate their rodent counterparts[12, 13]. For example, hNSCs can participate in CNS development (including of subhuman primates), respond to local cues, migrate to widely disseminated CNS regions including homing to areas of intracranial pathology, express transgenes, replace missing neural cells, and, in some cases, help promote functional improvement in some disease models[6, 9, 33-35]. In pilot studies on the contused adult rat spinal cord, hNSCs yield neurons that make long distance connections both rostral and caudal to the lesion that appear to facilitate the conduction of cortico-spinal impulses and concomitant behavioral improvement[36]. In other pilot experiments in which hNSCs were implanted in key regions along the spinal cord of the SOD1 transgenic mouse model of amyotrophic lateral sclerosis (ALS), a significant proportion of the recipients experienced a remarkable preservation of motor function and, in some animals, nearly a doubling of lifespan[18].

Transplantation into lesioned monkeys not only assays the hNSCs' response to a neuro-degenerative milieu that closely mimics that of humans, but also begins to lay the groundwork for clinical translation by requiring practical protocols for the administration of cells to large recipients (e.g., number of cells to inject, placement and number of injections, rate of delivery) while establishing safety and efficacy. One recent set of experiments has analyzed the fate and impact of hNSCs in the MPTP-induced model of dopamine (DA) depletion in African Green Monkeys, an authentic animal model of Parkinson's disease (PD). In pilot studies, hNSCs appeared to colonize the mesostriatum, with some spontaneously converting to tyrosine hydroxylase (TH)-expressing cells. Given that hNSCs, like murine NSCs, intrinsically produce many neurotrophic and neuroprotective factors, the improvement in DA activity observed in some recipients is likely the combined effect of not only DA cell replacement but also the provision by hNSCs of factors promoting the survival and enhanced function of host DA neurons and their nigrostriatal connections[17]. These dual mechanisms will likely be therapeutically significant.

THERAPEUTIC USES OF NEURAL STEM CELLS IN ANIMAL MODELS

Many CNS diseases are characterized by global degeneration or dysfunction. Treatment for these disorders requires widespread gene and/or cell replacement, as well as the regeneration or protection of broad networks of neural circuitry. The ability of NSCs to integrate into germinal zones from which inherently migratory progenitors can be launched, combined with the fact that NSCs travel long distances to home in on pathologic regions, makes these cells ideallyperhaps uniquelysuited for this task[5, 8, 16, 17, 23, 24, 26, 28,29,31, 37-39].

This ability was first observed in a mouse model of one of the genetically based neurodegenerative diseases, a lysosomal storage disease known as mucopolysaccaridosis type VII (MPS VII) (β-glucuronidase deficiency). This mouse, however, served as model for neurologic diseases in which a single gene was defective throughout the brain leading to cell death and demise of the animal. Treatment of global metabolic lesions required broad and diffuse enzyme replacement. Implanting wild-type, diffusely engrafting, enzyme-producing NSCs into the telencephalic ventricles of these mutant mice as newborns—creating chimeric fore-brains—essentially reversed the cerebral manifestations of the disease through a process known as cross-correction. This process was essentially like a bone marrow transplant to the brain[5]. Similar approaches have proven promising for other enzyme deficiencies, including the gangliosidoses, where -hexosaminidase requires repletion to thwart the pathological accumulation of GM_2 ganglioside in the brain. Other types of molecules can be used in such a paradigm—including neurotrophic, neuroprotective, anti-inflammatory, and antineoplastic agents. Although NSCs express baseline amounts of a particular enzyme or neuroprotective factor, NSCs can be

genetically modified to enhance the production of these molecules or complement their presence by the production of additional molecules to increase their therapeutic potential. Indeed, such approaches have proven useful for delivering nerve growth factor (NGF) to cholinergic systems of the septum and nucleus basalis magnocellularis to induce sprouting and to reverse cognitive deficits in rodent models of Alzheimer's disease and aging, and for delivering NGF and brain-derived neurotrophic factor (BDNF) for neuroprotection against excitotoxic lesions in the striatum mimicking Huntington's disease. One can even use NSCs spewing forth disease-causing molecules to quickly and inexpensively create a transgenic mouse brain model of a disease for subsequent study and cure.

In addition to widespread molecular therapy, one can imagine widespread neural cell replacement therapy as well. Although a number of diseases are characterized by such a deficiency, mouse mutants characterized by CNS-wide white matter pathology have provided some of the clear models for these because a single cell type (the oligodendrocyte) is deficient throughout the brain with a relatively straightforward readout (demyelination or dysmyelination). For example, the oligodendroglia of the dysmyelinated shiverer (shi) mouse are dysfunctional because they lack myelin basic protein (MBP) essential for effective myelination. Therapy, therefore, requires widespread replacement with normal MBP-expressing oligodendrocytes. When undifferentiated murine NSCs were transplanted at birth into the ventricles of newborn shi mice (allowing NSCs access to the SVZ), NSCs became integrated throughout the shi brain with repletion of significant amounts of MBP. Accordingly, of the many donor cells which differentiated into oligodendroglia, a subgroup myelinated about 40% of host neuronal processes. In some recipient animals, the symptomatic tremor decreased. In similar experiments, NSCs have been harvested from adult rat brains, expanded with mitogens in culture, differentiated into oligodendrocyte precursors ex vivo, and implanted into the myelin-deficient (md) rat. These NSCs, too, produced robust amounts of new myelin. Finally, it has recently been demonstrated that neural progenitor/stem cells of human origin have a similar remyelinating capacity in the shi mouse brain. Therefore, global cell replacement seems feasible for some pathologies if cells with stem-like features are employed. The ability of NSCs to remyelinate is of particular importance because dysmyelination/demyelination plays an important role in many genetic (e.g., leukodystrophies, inborn metabolic errors) and acquired (traumatic, infectious, asphyxial, ischemic, inflammatory) neurodegenerative processes.

In the experiments described previously, in which a particular neural cell type was impaired, the multipotent NSCs shifted their fate to yield more of the particular type of needed cell (in those cases, oligodendrocytes). Such observations began to highlight another important characteristic of the NSC: that it might be capable of "sensing" the deficiency of a particular cell type—or a particular niche may be created by the absence of a particular cell type—such that the NSC can alter its fate to compensate. This phenomenon was observed for neurons in two other circumstances. The meander tail (mea) mouse mutant is characterized by the failure of a sufficient number of granule cell (GC) neurons to develop in certain regions of the cerebellum. NSCs (both of mouse and human origin) implanted at birth into the EGL were capable of repopulating large GC-deficient regions with GCs—at the expense of progeny of other cells type—ssuggesting that environmental signals (in this case, the absence of a requisite number of GCs) may have pushed undifferentiated, multipotent cells towards repletion of this inadequately developed cell type. That this phenomenon can also occur outside the classical developmental timeframe was illustrated by the fact that NSCs, when implanted into an adult rodent neocortex in which a narrow region was depleted of projection pyramidal neurons, differentiated into precisely that deficient cell type, but only within the abnormal region. Outside that region the NSCs became nonneurons in the cortex, their anticipated normal developmental fate. In other words, again, there was a differentiation shift.

The examples cited above highlight the fact that an abnormal environment can direct the behavior of the NSCs. However, they leave the impression that the exogenous NSCs alone are responsible for

conferring the needed reparative or regenerative element or molecule. The situation is actually more complex and ultimately richer. We are beginning to learn that the response between an NSC and the injured host is one of ongoing reciprocal interactions. In response to implanted NSCs in the context of an injured CNS, the host nervous system also contributes to its own repair. This has been illustrated in a few examples: Hypoxia-ischemia (HI), a common cause of neurologic disability in adults and children, causes much of its damage from extensive loss of cerebral parenchyma and of the cells and connections that reside there. When NSCs were implanted into these regions of extensive degeneration (particularly when transiently supported by biodegradable scaffolds), robust reciprocal interactions ensued spontaneously between the exogenous implant and the injured host brain, which resulted in substantial reconstitution of parenchyma and anatomic connections as well as reduction of parenchymal loss, secondary cell loss, inflammation, and scarring. Similar results were observed in the hemisectioned adult rodent spinal cord in which evidence of an upregulated host neuronal regenerative response was noted.

The impact of NSCs in rescuing endangered host neurons was particularly evident in a series of experiments in aged rodents in which the nigrostriatal system was impaired. In most neurodegenerative disorders (e.g., Parkinson's disease), neurons do not die abruptly but undergo a gradual albeit inexorable deterioration in function with death as a terminal event. Often the neurons that die comprise a discrete subtype, although their malfunction has recently been postulated to result from an impairment in cross-talk from their nonneuronal neighbors. To explore the effect of NSCs on this type of pathological process, researchers generated a mouse model of Parkinson's disease by sequential systemic administration of high doses of the selective dopaminergic (DA) toxin MPTP (1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine) to aged mice. The MPTP permanently impaired (but did not kill outright) the DA neurons of the mesostriatal nuclei. When such mice received implants of murine NSCs unilaterally into the right substantia nigra (SN) and ventral tegmental area (VTA), the donor NSCs migrated to the MPTP-lesioned sites. The DA cell population and their functional nigrostriatal projections were reconstituted as demonstrated by renewed expression of such DA-specific molecules as the DA transporter (DAT) and, importantly, by behavior. Although this restoration of DA function was graft-dependent, it was not predominantly due to differentiation of the donor NSCs into DA neurons. Although such differentiation was noted, the majority of the now functional DA neurons were, surprisingly, rescued host DA neurons. The beneficial effect of NSCs on preserving dysfunctional neurons might be explained by the fact that, although some NSCs do differentiate into the absent neuronal type, many of the NSCs differentiate into astroglia or remain as undifferentiated NSCs. These chaperone cells constitutively produce substantial amounts of neurosupportive agents. One such prominent example in this case, which may be among such mediating molecules, is glial cell linederived neurotropic factor (GDNF), a factor known to be neuroprotective of ventrally located neurons such DA neurons and spinal ventral horn cells (motor neurons). A similar observation, in fact, is beginning to emerge from the implantation of human NSCs into the MPTP-lesioned subhuman primate model of Parkinson's disease, as well as the implantation of murine and human NSCs into the spinal cords of the superoxide dismutase 1 (SOD1) transgenic mouse model of amyotrophic lateral sclerosis (ALS), a disease characterized by progressive, virulent motor neuron degeneration. These observations suggest that exogenous NSCs may not only replenish inadequate pools of endogenous NSCs for the purposes of compensating for missing neural cells, but may also serve to reactivate or enhance endogenous regenerative capacities.

FUTURE STUDIES

NSCs hold great promise for regenerative medicine. However, successful translation of the NSC's therapeutic potential in animal models to actual human diseases will rely on further thorough characterization of many biological and clinical issues. In the developing and adult brain, further

detailed characterization of human NSCs, including identification of their markers, mapping of the neural cell lineages involved in brain development, and their functional roles in adult brain is required. We still don't know the exact mechanisms on self-renewal and differentiation of NSCs in vitro and in vivo, so a better understanding of the methods for controlling the propagation and phenotype-specification of NSCs should be made. In addition, identification of the genetic and epigenetic processes that confer NSC identity to an immature cell and ultimately mature neural cell-type specification has to be performed. In case of the transplantation of NSCs into animal models, the functional integration of engrafted NSCs, for example, neurons' ability to form synaptic connections that are electrophysiogically active and appropriate, and the behavioral recovery should be examined in details. Also required is a better understanding of the pathophysiology of the diseases to be targeted, that is, knowing what aspects actually require repair and which cell types really require replacement or rescue. Also, it is important to learn what the best source of NSCs will be—(a) isolated directly from the neuroectoderm of fetuses or adults; (b) derived from embryonic stem (ES) cells that have been directed towards a neuroectodermal and NSC lineage; (c) isolating NSCs from nonneural but more accessible structures (e.g., bone marrow, skin, retina, blood). Genomic methods (such as DNA microarrays) and proteomic approaches may provide valuable tools for identifying these genes and their products. Better genetic and epigenetic markers of NSC identity may be useful in mapping the precise location of adult NSCs as potential targets for new pharmaceuticals aimed at specific and selective activation, mobilization, and recruitment. The neural differentiation genes may be useful targets for agents aimed at producing specific cell types from these NSCs. Finally, it is clear that complex diseases, such as those affecting the nervous system, will require complex and multifaceted solutions—including pharmacologic, genetic and molecular, cell replacement, tissue engineering, angiogenic, anti-inflammatory, antiapoptotic, pro-regenerative, proneurite-outgrowth-promoting therapies. The NSC, as a key player in a set of fundamental developmental mechanisms, may serve as the glue that holds many of these strategies together. However, determining how these strategies may be intelligently, effectively, and safely orchestrated in practical manner in approaching actual patients will require a good deal of careful investigation.

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