In the adult mammalian central nervous system (CNS), most of the mechanisms responsible for cell proliferation and migration are shut off; thus, the capacities for self-repair and cellular replacement are greatly diminished. Observations that cell division continues in some regions of the adult brain and some resulting cells become neurons prompted attempts to identify the progenitor cells responsible for both embryonic and postnatal neural developments. The term stem cells refers to a population of cells that is capable of extended self-renewal and the ability to generate multilinage (neurons and glia) cell types (Figure). The growing interest in the isolation and propagation of stem cells and studying stem cell biology is driven by 2 goals. The first goal is to establish an in vitro system to elucidate the fate pathways of individual neural progenitors. Such a system can be used to determine signaling molecules controlling the generation of specific neural cell lineages and to dissect cellular mechanisms underlying the progressive processes of commitment, fate determination, and differentiation during neural development. The second goal is to use such cultured, well-characterized cells for therapeutic applications in the treatment of neurologic and neurodegenerative diseases.

In many neurologic diseases, specific neural cell types or circuits degenerate. These losses may be due to genetic abnormalities of the diseased cell or may be due to the absence of enzymes, neurotransmitters, trophic factors or cofactors, or the presence of toxic substances or physical injury. Treatment of CNS disease or injury could focus on one or more steps leading to cell loss. Clinical trials show that neuron replacement for neurodegenerative diseases, such as Parkinson and Huntington diseases is feasible. Grafts of freshly dissected fetal substantia nigra have shown promising long-term clinical improvement in some patients with Parkinson disease. The improvements were associated with the survival of the grafts and dopaminergic reinnervation of the striatum. Reports of fetal striatal transplantation in patients with mild to severe Huntington disease suggest that neurotransplantation may improve some of the cognitive symptoms associated with the disease and may modify the course of the disease.

Ex vivo gene therapy is an alternative approach that has been used in various experimental models of neurologic diseases. This approach consists of in vitro transfer of genes to cells and their subsequent transplantation into the brain. The transplanted cells act as carriers of therapeutic products that correct or at least compensate for the neurologic manifestation of diseases. At present, nonreplicating primary neurons are not an ideal donor candidate since they cannot be maintained for prolonged periods in cultures and are refractory to genetic manipulations. Immortalized neural cells have been explored for their use in gene transfer and therapy. Immortalized neural cell lines have been generated by retroviral transduction of oncogenes into neuroepithelial precursors derived from the developing brain. Recently, it has become possible to generate cells from the CNS that express neuronal and glial properties to varying degrees without the use of immortalizing oncogenes, by relatively simple manipulations in tissue culture. Various cytokines and/or growth factors have been used to propagate neural progenitor cells in culture. By virtue of their biological properties, progenitor and/or stem cells represent good candidates for multiple cell-based therapies for neural diseases. These properties include the

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ability to divide in culture, enabling ex vivo gene manipulation prior to grafting. In addition, being of CNS origin and having the capacity to survive and integrate in the host cytoarchitecture following grafting, stem cells can provide not only a continuous supply of therapeutic molecules throughout the CNS but also may replace dysfunctional neural cells. Both non-neural and neural cells genetically modified in vitro to produce a single neurotransmitter, enzyme or neurotrophic factor have been used successfully as donor material for grafting to prevent neu- ronal loss and/or reverse some behavioral and functional deficits associated with CNS disorders.4-6

Previous studies have shown that basic fibroblast growth factor (FGF-2) has a broad survival and growth effect on cells cultured from different regions of the embryonic brain, and large amounts of FGF-2 are present in all regions of fetal brain, suggesting an important role for this growth factor in early neurogenesis. The mitogenic property of FGF-2 has allowed the isolation and establishment of long-term cultures of progenitors from different regions of the adult CNS.7-9 A strategy was developed to first find the conditions needed to isolate and culture progenitor cells from fetal CNS, with the idea of then finding the conditions necessary for isolating precursor cells from adult CNS regions. Tissues from different regions of the brain and spinal cord of embryonic and adult rats are microdissected, mechanically dissociated and enzymatically digested, and cell suspensions were prepared. Cell suspensions are plated on uncoated plastic tissue culture plates in serum-free medium containing 20 ng/mL of FGF-2 on polyornithine and laminin-coated plates. A day after plating, only a small number of cells attach and a variety of morphologies in cultures from all regions are seen. Cells start to grow and divide slowly, as indicated by the increase in number of cells that incorporate bromodeoxyuridine, from a few cells after a 1-day incubation, to almost all cells in culture, after a 4-day incubation. These precursors proliferate in the presence of FGF-2 and withdraw from cell cycle on removal of FGF-2. The FGF-2 responsive cells are maintained through multiple passages and can be frozen down for long-term storage and then cultured again. The FGF-2 expanded progenitor cultures are characterized by immunostaining for various antigens. A number of cells cultured from all regions express nestin, an intermediate filament protein found in neuroepithelial stem cells, as well as β-tubulin isotype III that is a neuronal marker. A number of glial fibrillary acidic protein (GFAP) expressing astrocytes and cells of oligodendroglial lineage, as indicated by Rip staining, an antibody recognizing a cytoplasmic antigen specific to oligodendrocytes, are present in the cell cultures. More recently, using cloning and subcloning analyses we demonstrate that some of the FGF-2 responsive progenitors have the characteristics of multipotent stem cells in vitro, in that genetically marked clones are self-renewing and have the potential to give rise to neurons, astrocytes, and oligodendrocytes.

## RELEVANCE TO THE PRACTICE OF NEUROLOGY

Many animal model of neurologic disease are currently being investigated for potential therapeutic intervention with CNS transplantation and/or gene therapy. Therapy through cellular and/or biopharmacological replacement, compensation repair, and preventive intervention may ameliorate symptoms of neurologic disease. A number of studies5,6,8,9,10 have reported that immortalized neural progenitors and stem cells can reintegrate appropriately into mammalian brain, and can stably express foreign genes and ameliorate functional
deficits in various animal models of neurologic diseases.

Immortalized neural stemlike cells derived from the cerebellum (C17-2), and implanted in various mouse models of neurologic dysfunction were able to replace affected neural cells (neurons and/or oligodendrocytes) in many regions throughout the host brain.10 C17-2 cells injected at birth into the cerebral ventricle in shiverer mouse, mouse mutants characterized by CNS-white matter disease, engrafted throughout the myelin basic protein-deficient brain. Transplanted cells replaced the dysfunctional oligodendrocytes with myelin basic protein expressing oligodendrocytes and preliminary studies suggest that some receptive animals may show a decrease in the symptomatic tremor. In addition, in a mouse model of neuron-specific apoptotic degeneration in adult neocortex, C17-2 cells transplanted into the cortex of adult mice differentiated into pyramidal neurons replacing the degenerated neuronal population.

Since immortalized or primary neural stem cells divide in culture, they can be stably infected with viral vectors to express factors that are appropriate for neural repair, such as substrate molecules, trophic factors, and neurotransmitters.4,5 Immortalized precursor cells engineered to express trophic factors and metabolic enzymes have been used in animal models of memory impairment (similar to Alzheimer disease), Huntington disease, and genetic neurodegenerative disorders. After genetic modification, immortalized nigral cells engineered to release dihydroxyphenylalanine and dopamine have been shown to compensate some of the deficits in rodent and primate models of Parkinson disease through neural transplantation. The grafts were shown to survive for 2 months and to produce significant improvement in apomorphine-induced rotation. HiB5 cells (an immortalized hippocampal cell line) engineered to secrete nerve growth factor (NGF) have been shown to prevent cholinergic cell loss and ameliorate performance deficits in learning and memory tasks when grafted into the nucleus basalis and/or septum of adult animal models of cognitive dysfunction.5,10 In a different context, when grafted at birth into the cerebral ventricles of the murine model of genetic neurodegenerative lysosomal storage disease mucopolysaccharidosis type VII, C17-2, genetically modified to express the human enzyme α-glucuronidase, corrected the lysosomal storage throughout the brains of these mice. In another application, genetically engineered C17-2 cells were used for successful widespread expression in the neonatal and fetal mouse brain of the α-subunit of β-hexosaminidase, a mutation which leads to accumulation of GM2 gangliosides (Tays-Sachs disease).6,10 Effective delivery of gene products to multiple CNS regions offers promise for using genetically modified immortalized neural stem cells to counteract neurodegeneration due to several causes. However, the use of oncocogene immortalized cells may not be optimal for clinical use as they have the potential for neoplastic transformation. A novel alternative to oncoogene-immortalized cells is primary cells expanded in vitro. Growth factor-expanded cell populations from both neonate and adult CNS display similar biological properties in vitro and give rise to multipotent progenitors that generate neurons and glia. Recent work showing that FGF-2 responsive cells can successfully engraft and express transgene in the adult brain has suggested that growth factor-expanded primary neural cells may have therapeutic potential. Basic fibroblast growth factor-expanded progenitors isolated from the hippocampus of adult rats were grafted to adult rat hippocampus, rostral migratory pathway, and cerebellum. In neurogenic zones of the adult rat brain, such as the granular cell layer of the hippocampus and rostral migratory pathway used to replenish neurons of the olfactory bulb, these cultured cells differentiated into site-specific neurons, while in other regions they differentiated into glia.11 The results indicate that the adult-derived hippocampal progenitors can respond to persistent neuronal differentiation cues in the adult CNS. In another study,12 progenitors isolated and cultured from fetal human CNS have been grafted into the striata of rat model of Parkinson dis-
ease. Some grafted cells differentiated into dopaminergic neurons and astrocytes, and were sufficient to decrease amphetamine-induced rotation in 2 animals.12 Using an alternate paradigm, epidermal growth factor-responsive neural stem cells were isolated from the striatum of fetal transgenic mice expressing human NGF under the control of human glial fibrillary acidic protein promoter (GFAP-hNGF stem cells). These cells can differentiate in vitro into astrocytes that express and secrete bioactive NGF. Following transplantation into the striatum of adult rodent model of Huntington disease, these GFAP-hNGF stem cells could prevent the degeneration of striatal neurons.13 Collectively, these studies suggest the possibility of novel approaches to repopulating the damaged CNS. Neural stem cells may be manipulated prior to grafting, they may be differentiated in culture toward a lineage that synthesizes factors of interest, or genetically modified to express therapeutic gene products and used in grafting strategies to replace substances that are lost after injury or disease. Alternatively, precursor cells may be directed to a neuronal lineage and used to functionally repair damaged neural systems. On the other hand, the host CNS may be manipulated to recapitulate the developmental cues required for generating appropriate cells or connections from uncommitted endogenous or grafted progenitors.

RELEVANCE TO THE STUDY OF NEUROSCIENCE

A basic aim in developmental neuroscience is to define factors regulating neurogenesis and gliogenesis within the CNS. In vivo fate mapping procedures support the existence of multipotent cells that give rise to both neurons and glia. Confirming the results of fate mapping, both epidermal growth factor and FGF-2 have been used as mitogens to expand CNS stem cells in vitro.5,14 Lineage experiments show that growth factor-expanded cells from both fetal and adult CNS are multipotent, differentiate into neurons, astrocytes and oligodendrocytes, and possess self-renewal ca-
capacity, thus fulfilling the criteria of stem cells. Isolation and characterization of pluripotent stem cells provide an in vitro system to examine cellular and molecular mechanisms underlying lineage diversification, fate choice, progressive restriction of fate choice, and to learn how terminal differentiation and specification of various phenotypes within a particular lineage are achieved. Several in vitro studies demonstrate the role of epigenetic factors in regulating the differentiation fate of neural precursor cells. Ciliary neurotrophic factor and thyroid hormone have been shown to act in an instructive manner on the multipotential stem cell, directing it to a committed astrocytic and oligodendrocytic fate, respectively. Also, bone morphogenetic proteins promoted astrocytic differentiation from epidermal growth factor–derived stem cells. In contrast platelet-derived growth factor), insulinlike growth factor 1, and brain-derived growth factor have been reported to support neuronal differentiation.

However, the in vitro system for neural development may not reflect the actual in vivo events, since growing cells in culture and exposing them to nonphysiological levels of growth factors may alter the endogenous properties and the developmental capacity of these cells and thus may not reflect the tree characteristics of their in vivo counterparts. It is also possible that a cell-intrinsic developmental program is important in regulating lineage, and growth factors may act mainly as survival factors or mitogens for committed cells. The factors that control stem cell behavior in vivo to maintain them in a quiescent, uncommitted state and mechanisms that can trigger their proliferation and differentiation remain to be clarified. The interplay between extracellular and cell-autonomous regulatory factors in lineage determination, as well as the signaling pathways involved, remains to be clarified. Recent grafting studies of growth factor–expanded progenitors show that these cells can engraft into structures of origin as well as into heterotopic regions of the adult rat, and can acquire characteristics appropriate for neurons in their new environment. The data suggest that progenitors do not show regional specificity and cells grafted to new sites generate region-specific neurons in response to local cues. Thus, regional heterogeneity of the brain may result from extracellular signals acting on uncommitted progenitors during migration and differentiation.

APPLICATIONS

It has been established that growth factor expandable neural stem cells with self-renewal and multilineage potential are present in the adult mammalian CNS. However, the field of stem cell technology is fairly recent and many questions have not been answered. For example, what is the role, function, and location of the stem cells within the adult CNS? Do stem cells exist as dormant or proliferating cells? Whether the proliferating cells observed in vivo are the same cells as those isolated in vitro? Whether stem cells can be induced to proliferate in vivo in response to growth factors, then migrate and differentiate into specific phenotypes when provided with the appropriate environmental signals? The possibility that growth factors may influence neural progenitors in vivo have been supported by finding that intracerebroventricular administration of growth factors increased proliferative progenitors in the subventricular zone and some of them differentiated into neurons.

In considering applications of stem cell technology to human disease, reports suggest the existence of neural precursors in the human CNS. Studies are currently underway to establish whether the human precursors have properties similar to rodent stem cells and thus the extent to which studies of rat CNS be extended to human CNS. Novel strategies in the treatment of neurologic diseases may involve a combination of gene therapy, progenitor isolation and propagation, and grafting procedures to generate cells ideally suited for delivery of therapeutic substances to prevent cell loss and promote regeneration and functional recovery. Continuing studies to understand the endogenous in vivo properties of stem cells and to identify the factors that direct the generation of specific lineages will serve to guide the development of strategies to manipulate stem cells present in situ to repopulate either injured or degenerating regions of the adult CNS.

REFERENCES


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