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Review

Neural stem cells in aging and disease

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Abstract

Aging in the central nervous system is associated with progressive loss of function which is exacerbated by neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. The two primary cell replacement strategies involve transplantation of exogenous tissue, and activation of proliferation of endogenous cells. Transplanted tissue is used to either directly replace lost tissue, or to implant genetically engineered cells that secrete factors which promote survival and/or proliferation. However, successful application of any cell replacement therapy requires knowledge of the complex relationships between neural stem cells and the more restricted neural and glial progenitor cells. This review focuses on recent advances in the field of stem cell biology of the central nervous system, with an emphasis on cellular and molecular approaches to replacing cells lost in neurodegenerative disorders.

Keywords: neural stem cell - Alzheimer's - Parkinson's - stroke - aging

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Introduction

The human brain has the capacity to age gracefully, retaining relatively normal function for almost a century. However, the more prevalent course is the appearance of age-related neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD) or stroke. As the mean human population age continues to increase, research has focused on potential cellular and molecular mechanisms to expand maximum lifespan and alleviate, and possibly prevent, the appearance of devastating neurodegenerative diseases. Much attention has been given to the recent advances in stem cell therapies, which offer the potential to replace lost tissue in the aging brain. In the stem cell field, the cell replacement strategies can be divided into exogenous (*i.e.*, transplantation of stem cells) and endogenous (*i.e.*, stem cells reentering the cell cycle to replace lost cells) therapies. This review will focus on recent advances in the field of stem cell biology of the central nervous system, with an emphasis on cellular and molecular approaches to replacing cells lost in neurodegenerative disorders. We will first discuss alterations in brain structure and function that are associated with aging, including diseases that commonly affect the aging brain. We will then briefly examine the biology of neural stem cells during development and during aging. Finally, we will focus on recent advances in the field of stem cell biology, which offer hope for alleviating neurodegenerative disorders associated with aging.

Changes in the brain during aging

In order to understand the role of neural progenitor cells (NPCs) and neural stem cells (NSCs) in aging, one must first understand the process of aging in the human brain. As with other organs, there is a progressive decline in functional capacity of the brain that accompanies aging. A distinction can be made between primary aging (the gradual loss of function in the non-diseased state), and secondary aging (the progressive functional decline resulting from age-related disorders, which will be discussed in the next section). In the central nervous system (CNS), primary aging is associated with altered morphology and connectivity which alters normal function. In the absence of disease, there is a moderate reduction in the number of neurons in many areas of the brain during aging [1]. Post-mortem studies indicate an approximate reduction of 5% in brain weight and volume *per* decade after 40 years of age [1]. There is also evidence of shrinkage in several brain areas, including the frontal cortex [2, 3], the striatum [4] and midbrain structures such as the locus coeruleus and substantia nigra [5, 6], as well as an increase in lateral ventricle and sulcal volume as a result of atrophy [7]. Overall, however, it is thought that most neurons remain in the brain for the lifetime of an individual, with the exception of discrete brain regions which are continually replenished with new neurons (*i.e.*, the olfactory bulb and dentate gyrus of the hippocampus). Structural changes associated with aging include loss and reorganization of synapses, retraction and expansion of dendrites, and glial cell reactivity [8, 9].

On the cellular level, aging of the CNS is accompanied by a number of changes which impair cellular function, including elevated levels of oxidative stress and associated oxidative damage to proteins and DNA; impaired cellular metabolism; and accumulation of lipid and protein by-products, such as lipofuscin and advanced glycation end products [10]. Mitochondrial function declines with age, and is associated with an increase in damage to mitochondrial DNA as a result of oxidative damage and lack of effective DNA repair [11, 12]. A variety of signal transduction pathways are altered, affecting release of neurotransmitters and growth factors which results in impaired neuronal excitability and plasticity. While these changes generally do not themselves cause neurodegeneration, they create a permissive environment in which neurons are predisposed to neurodegenerative diseases, such as AD and PD. Additionally, several alterations seen in the aging brain are often enhanced in the diseased brain. For example, protein oxidation is even greater in brains from patients with AD and PD as compared to age-matched controls [13].

The most remarkable changes in the brain may be the alterations in cognition and plasticity which occur with aging. As compared to younger individuals, older adults show different patterns of

brain area activation when performing cognitive tasks with some tasks reorganized in a manner which suggests rearrangement to optimize performance [14]. However, there is also substantial evidence for loss of brain function as individuals age, most notably resulting in impairment of functions such short-term memory. There is also evidence of age-related impairment of repair capacity following injury or disease. In the normal adult brain, most CNS repair strategies involve reorganization of existing circuitry (by mechanisms such as compensation and redundancy). In select regions, such as the olfactory bulb and hippocampus, there is the possibility of neuronal replacement through the generation of new neurons. However, these cases are the exception to the normal reaction to physical injury, which generally involves proliferation of astrocytes and formation of a glial scar. Thus, under normal circumstances, neuronal replacement is not a normal response to injury or disease. In the aging brain, there is evidence that the limited repair capacity is inhibited even more than in the normal adult brain, especially when neurodegenerative disease is evident.

Even in the absence of disease, the decline in brain function with aging may have important implications for the process of aging itself. Several lines of evidence suggest that the CNS may control lifespan [15]. The hypothalamic-pituitary axis may be involved, as mice with mutations resulting in abnormally small pituitary glands [16] or decreased levels of growth hormone [17] experience longer lifespan than normal mice. In the *Caenorhabditis elegans*, worms with mutations in insulin-like signaling pathways (notably the insulin receptor and phosphatidylinositol-3-kinase homologs *daf-2* and *age-1*) expand lifespan [18, 19]. The effect of these mutations is cell-specific, such that expression of wild-type *daf-2* or *age-1* in mutant *C. elegans* reverses the elongated lifespan effect when the proteins are expressed in neurons, but not in muscle or intestinal cells [20]. If the brain does regulate lifespan in humans, then loss of brain function during aging may be a determinant factor in the observed decline in function associated with secondary aging. The decline in function is worsened in the presence of neurodegenerative disease, thus therapy which alleviates primary and/or secondary aging is a primary goal in aging research. In the next section, we will briefly describe the primary neurodegenerative disorders associated with aging which may benefit from stem cell based therapy.

Diseases of the aging brain

Three of the most prevalent age-related neurodegenerative disorders are AD, PD and stroke. Although there are other neurodegenerative disorders which are associated with aging, these tend to have a genetic basis; for example, the trinucleotide disorder Huntington's disease is an inherited disorder. While these diseases are associated with aging, they do not result from the aging process itself and will not be addressed in this review. AD, PD and stroke share the common feature that specific populations of neurons are affected in each disorder. In AD, a disorder which affects memory and cognition, the primary regions affected are the hippocampus, cerebral cortex and amygdala. In PD, a disease characterized by motor dysfunction, there is selective loss of dopaminergic neurons in the substantia nigra. In stroke, which occurs with occlusion or rupture of a cerebral blood vessel, there is selective loss of neurons from the region(s) supplied by that blood vessel. Thus, each disease offers a relatively discrete target for potential therapies. Additionally, each disease can be mimicked both *in vitro* and *in vivo* through a variety of models. Animal models of AD include presenilin-1 knock-in mice, as well as various mutants of presenilin-1, tau and amyloid processing protein (APP). To mimic PD, selective loss of dopaminergic neurons can be produced by administration of 6-hydroxydopamine (6-OHDA) or 1-methyl- 4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP). Additionally, transgenic mice expressing mutant human α-synuclein exhibit loss of dopaminergic neurons and behavioral abnormalities resembling those seen in PD. Stroke can be induced through transient or permanent occlusion of the middle cerebral artery in rodents. Thus, the animal models needed to test stem cell-based therapies already exist and, in most cases, have been well characterized.

Traditional therapy for each of these diseases has focused on pharmacological therapeutics, with generally poor results. During AD, there is marked

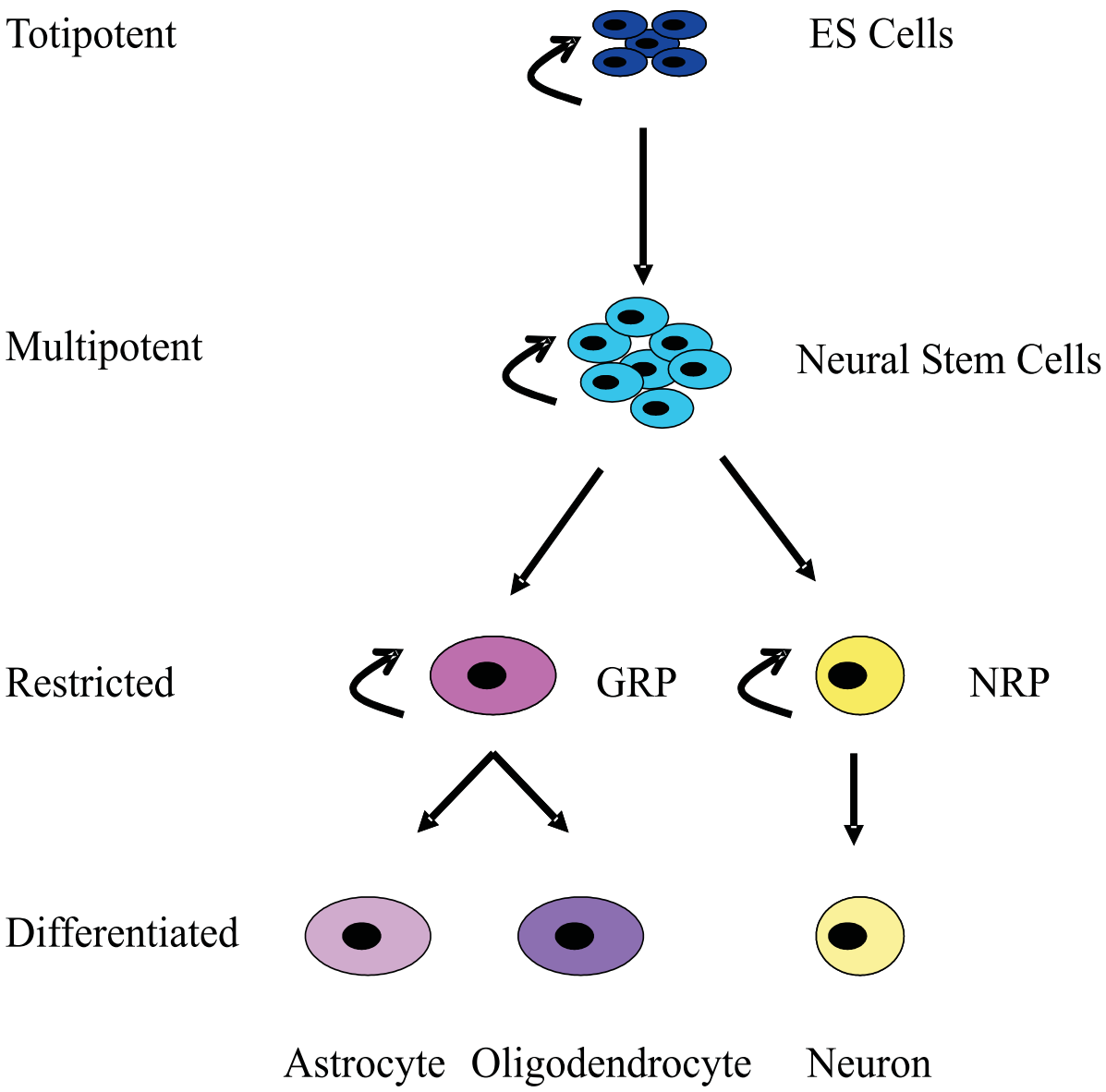

Fig. 1 Generalized outline of the lineage relationship between stem cells and differentiated cells of the central nervous system. As cells become more specialized, the different types of progeny they can produce becomes more restricted, such that multipotent stem cells are capable of producing all cell types, while neuron restricted precursors (NRPs) and glial restricted precursors (GRPs) produce only neurons and glia, respectively. Note that expansion of cellular pools can occur at several stages of development, allowing for division of multiple types of "stem cells" simultaneously.

atrophy of the cerebral cortex with a disproportionate loss of cholinergic neurons. Traditional therapy has sought to enhance cholinergic function with cholinesterase inhibitors, such as tacrine. However, clinical trials revealed little to no improvement in cognitive function with this therapy. In PD, therapy has focused on drugs which enhance dopaminergic function. The primary therapy is L-dopa, which is converted to dopamine in the striatum. Although L-dopa is the most widely used therapeutic agent for PD, its effectiveness diminishes after 3-5 years of continuous treatment. In stroke, treatment usually focuses on minimizing the secondary damage following reperfu-

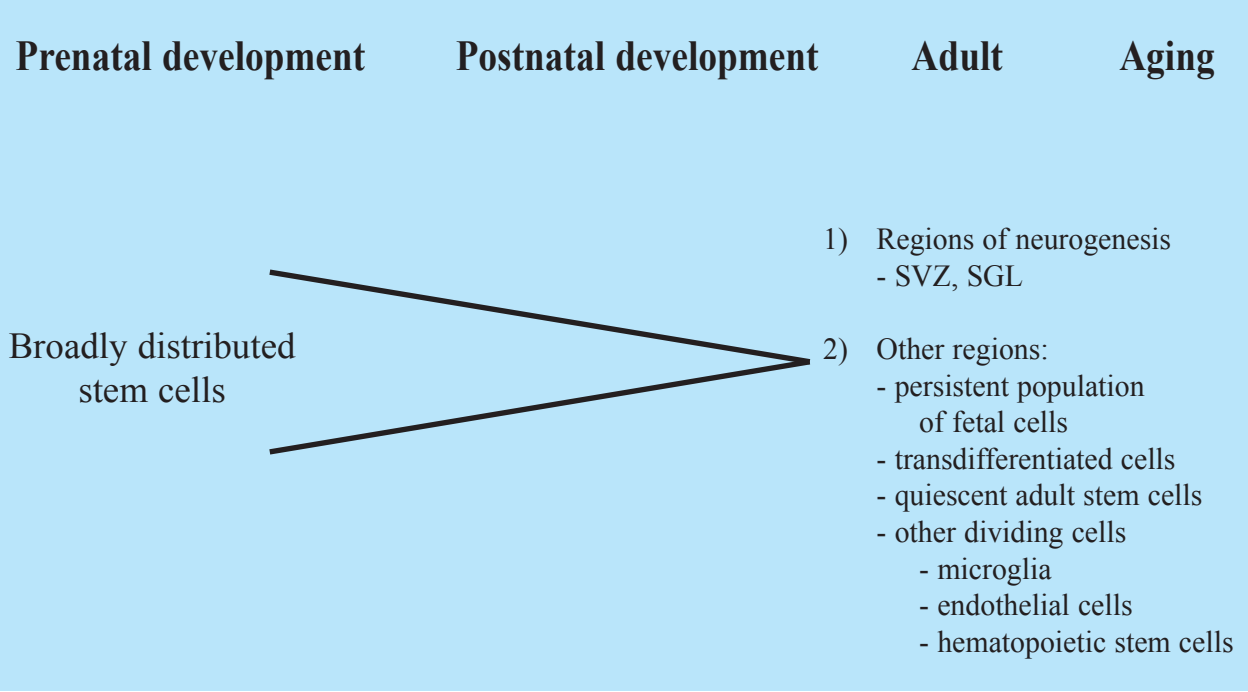

Fig. 2 As brain development progresses from prenatal to adult, there is a progressive restriction in the localization of NSCs which is accompanied by a general decrease in the proliferative capacity of these cells. In the adult and aging brain, there is also an increase in the potential types of cells which can divide following appropriate stimulation, ranging from quiescent adult stem cells to non-neural cells such as microglia and hematopoietic stem cells.

sion of the injured area using agents such as creatine and antioxidants; however, treatments which are successful in experimental animals are generally not effective in humans, with the possible exception of controlled hypothermia [21].

In the early stages of each of these diseases- AD, PD and stroke- there is a relatively discrete population(s) of neurons which is affected, making these diseases ideal targets for cell replacement therapy using stem cells. The question facing modern medicine is how best to use stem cells to produce functional recovery in neurodegenerative disorders. In recent years it has been noted that the adult brain has the capacity to replace lost neurons in several select regions of the CNS, such as the olfactory bulb and the hippocampus. Additionally, stem cells have been described in the fetal brain and the adult human subependymal zone, hippocampus and cortex. Estimations of the number and proliferative capacity of these cells suggests the possibility that therapy through replacement of lost cells by mobilizing endogenous stem cells or transplantation of exogenous cells may be a realistic therapeutic approach. In order to best use these cells, however, we must first understand their basic biological properties.

Stem cells and the brain

Biology of neural stem cells

For decades, scientific dogma maintained that the adult mammalian nervous system was incapable of creating new neurons. However, the demonstration of neurogenic regions in the adult human brain led to an explosion of research in the area of adult neurogenesis. It is becoming evident, however, that neurogenesis involves not only multipotent stem cells but also a variety of other cell precursors, as observed during normal development in the CNS. During the course of neural development, there is a progressive restriction of developmental potential, from the multipotent stem cell to the final differen-

tiated cell (Figure 1). For the purposes of this review, a stem cell is defined as any cell capable of both self-renewal through symmetric divisions and production of non-stem cells through asymmetric divisions. As cells develop from embryonic stem (ES) cell to differentiated cell, there are a number of intermediate cells which are restricted in their developmental potential. These cells are distinct from cells whose developmental fate has already been determined, which are labeled as precursor cells. Neural stem cells (NSCs) are defined as cells which can generate all three major types of cells in the CNS: neurons, astrocytes and oligodendrocytes. In general, NSCs are more restricted in their differentiation potential as compared to the multipotent ES cells. It is important to note that while NSCs have been identified in both fetal and adult tissue, there are notable differences between the properties and localization of stem cells derived from these tissues (Figure 2). For example, FGFR4 is a marker for stem cells in fetal tissue [22] but is not detectable on adult stem cells. Additionally, within the CNS there are specific subclasses of neural stem cells which exhibit self-renewal and the capacity to differentiate into different types of neurons, but are relatively restricted in the types of differentiated cells they produce. For example, pluripotent neuroepithelial cells derived from human fetal spinal cord are limited in their lineage potential, generating neurons and astrocytes but not oligodendrocytes [23]. Cells can also be limited in their differentiation capacity depending on the region from which they are originally isolated. For example, stem cells derived from the mesencephalon generate dopaminergic neurons more readily than other precursor populations [24]. Similarly, retinal stem cells produce retinal cells more easily than stem cells derived from the forebrain or spinal cord. Thus, these "stem cells" are not capable of easily producing any cell type, but are included in the definition of "stem cell" because of their ability to self-renew and generate several types of differentiated progeny.

Cells with a more restricted potential than NSCs, termed precursor cells, have also been described in the human CNS. Cells that are committed to becoming glial cells or neuronal cells are labeled as glial restricted precursors (GRPs) or neuronal restricted precursors (NRPs), respectively. Using these definitions, GRPs and NRPs have been identified in fetal and adult tissue. Neuronal precursors have been described in the adult subventricular zone [25] and the hippocampus [26, 27]. These cells generally express polysialated NCAM (PSA-NCAM), which is expressed by NRPs and immature neurons, and can be used to isolate NRPs from human fetal tissue [28]. Like stem cells, cells expressing PSA-NCAM appear to have different properties, including expression pattern and capacity for self-renewal, in the fetus as compared to the adult, although the physiological basis for this observation is not yet understood [29]. Similarly, cells which are restricted to oligodendrocyte lineage have been described in adult human white matter [30, 31]. These cells express the cell-surface protein A2B5 which can be used to select for these cells in culture [32], yielding cells that are similar to the rodent O2A cell. Astrocyte restricted precursor cells have also been isolated through sequential passaging of multipotent stem cells derived from cultured human spinal cord tissue [23]. Astrocyte precursors have not yet been directly isolated from human tissue, however at least two different types are evident in rodents [33, 34]. Astrocytes are themselves interesting in terms of self-renewal and differentiation capacity. Astrocytes are normally quiescent *in vivo*; however, they are capable of reentering the cell cycle and dividing indefinitely. Even more interesting is recent data suggesting that astrocytes are capable of dedifferentiating then re-differentiating into neurons in culture [35, 36].

Thus, neural tissue in both the fetal and adult human CNS is comprised of multiple classes of dividing cells, including multipotent NSCs, restricted precursors and astrocytes which are capable of dedifferentiating and reentering the cell cycle. Theoretically, these cells are an endogenous source of new cells to replace cells that are lost during injury or disease. In reality, however, the potential of these cells is not realized in the adult CNS under most conditions. A major question currently facing the stem cell field is how to utilize these cells to replace lost cells either by promoting the proliferation, differentiation and survival of endogenous cells, or by transplanting new cells into damaged areas. For either approach to be successful, however, requires an understanding of the relationship between cell

Table 1. Mechanisms underlying aging in the central nervous system. Although there is little information regarding the effect of stressors on neural stem cells, it can be hypothesized that age-associated changes which affect somatic cells may potentially affect NSCs. Age-related changes in NSCs, such as decreased proliferative capacity in neurogenic regions, may also occur independently of these processes.

Processes underlying aging in the central nervous system: somatic and stem cells
DNA damage and instability
Altered protein synthesis and degradation
Mitochondrial instability and mutations
Decreased telomerase expression and activity
Elevated free radical production and decreased free radical scavenging
Accumulation of somatic mutations

lineages and the factors that influence developmental fate.

Regulation of stem cell proliferation, differentiation and survival

Under normal conditions, there are several possible outcomes for a neural stem cell. Stem cells may remain quiescent and not undergo division, or may undergo programmed cell death (apoptosis) and cease to exist. Alternatively, stem cells may reenter the cell cycle and proliferate to generate new stem cells (through symmetric divisions) or differentiated progeny and undifferentiated stem cells (through asymmetric divisions). Further, a stem cell may "mature" into a different type of stem cell (*i.e.*, a fetal stem cell may become an adult stem cell), creating a diverse set of possible outcomes for initiation of stem cell division. The final outcome depends on the information received by the stem cell in the form of hormones, growth factors, *etc*., and is influenced by factors such as cell density, energy metabolism, receptor expression, and cross-talk between signaling pathways [37].

A number of factors have been identified which affect NSC and NPC proliferation, differentiation and survival both *in vitro* and *in vivo*. During development, several factors regulate cell proliferation and fate. For example, bone morphogenic proteins (BMPs) suppress neural differentiation in the embryo, which can be antagonized by noggin, thereby promoting neurogenesis [38]. A similar function of BMPs and noggin has been identified in the adult subventricular zone (SVZ) [39]. Growth factors, most notably epidermal growth factor (EGF) and fibroblast growth factor (FGF), promote proliferation of NSCs and precursor cells. In the cortical SVZ, EGF promotes gliogenesis while FGF promotes neuronal differentiation [40, 41]. Brain-derived neurotrophic factor (BDNF) may promote neural differentiation and survival in the hippocampal dentate gyrus and cerebral cortex [42, 43]. Cytokines also play a role in determining cell proliferation and fate. Leukemia inhibiting factor (LIF) and ciliary neurotrophic factor (CNTF), which activate a heterodimer comprised of a gp130 receptor and a partner receptor (the LIF receptor or CNTF receptor, respectively), have different actions depending on the developmental stage of the NSC. In mouse ES cells, LIF or CNTF is essential for maintenance of neural stem cells in an undifferentiated state [44-47]. However, in both the developing and adult mouse CNS, activation of the gp130 receptor promotes gliogenesis through differentiation and/or survival of astrocytes [48-50]. The same factors can also promote the generation and survival of oligodendrocytes *in vitro* [51-53]. Thus, the effect of factors which affect NSCs and NPCs regulation must be considered in the context of the stage of development of the cell (ES cell *vs.* lineage restricted precursor).

Stem cells and the aging brain

In the adult mammalian brain, stem cells are localized in two areas: the subventricular zone (SVZ) of the lateral ventricle and the subgranular layer (SGL) of the hippocampal denate gyrus. Cells in the SVZ follow the rostral migratory stream (RMS) to become interneurons in the olfactory bulb, while cells in the SGL migrate a short distance to differentiate into hippocampal granule neurons [54, 55]. Stem cells of the SVZ spread across most of the lateral wall of the lateral ventricle, while stem cells of the SGL are limited to the thin SGL. As a result of the greater number of stem cells in the SVZ, this region has been characterized to a greater extent than the SGL. However, NSCs comprise such a small percent of the cells in both adult neurogenic regions that deriving a pure population for studies *in vitro* is difficult. It can be predicted that stem cells will be affected by many of the same processes which affect somatic cells during aging (Table 1). Preliminary evidence suggests that stressors which accompany aging, such as increased oxidative stress and DNA damage, may be more damaging to stem cells than somatic cells, and that damage which occurs to NSCs early in development may affect NSCs present in the adult brain. For example, prenatal stress inhibits neurogenesis and affects learning and memory in the adult hippocampus [56], suggesting a heightened vulnerability of NSCs to damage caused by stress. However, there is little information regarding NSC vulnerability to age-related changes in the adult brain.

What is known is that NSCs can be induced to reenter the cell cycle following exercise [57-59], exposure to an enriched living environment [60, 61], or following injury caused by cerebral ischemia [62] or severe seizures [63]. However, the capacity for neurogenesis diminishes with age, even in the absence of disease. In the aging rat hippocampus, bromodeoxyuridine (BrdU) incorporation by neuronal progenitor cells is decreased compared to younger adult rats as a result of decreased proliferation [64]. The loss of proliferative capacity and thus replenishment of hippocampal granule neurons may contribute to age-related deficits in memory and learning, which are mediated by the hippocampus. The loss of proliferation in the hippocampus was not found in other neurogenic regions, such as the lateral ventricle wall, suggesting a specific age-related impairment in the hippocampus [64]. Hippocampal neural progenitors retain the ability to proliferate, as demonstrated by the restoration of hippocampal neurogenesis following adrenalectomy in aged rats [65]. Thus, age-related memory impairment may be reversible through re-activation of endogenous stem cells.

Stem cells may actually play a limited role in age-related diseases of the brain, such that part of the neural damage observed during the disease process may be attributed to the loss of stem cells' ability to divide. Stem cells are vulnerable to many of the same age-related factors which affect somatic cells, such as stress and free radicals. Preliminary evidence indicates that NSCs may play a role in the pathogenesis of AD. For example, amyloid beta-peptide, which plays a role in the pathology of AD, also inhibits proliferation and promotes apoptosis of human cortical stem cells in culture [66]. Interestingly, the most common predictor of onset of AD is loss of smell [67, 68]. Patients with early-onset AD exhibit as much as a 75% loss of neurons in the anterior olfactory nuclei neurons [69]. Under normal conditions, NSCs in the SVZ migrate to the olfactory bulb to become interneurons, thus the loss of smell may result from loss of proliferation of these precursor cells. If a general feature of AD is the loss of proliferation of NSCs, then loss of proliferation in the SGL of the hippocampus may contribute to the observed loss of memory in AD. This is suggested by the decreased proliferation of neural precursors in models of AD, which may be exacerbated by the general loss of proliferation in the SGL which accompanies aging. However, the exact role of NSCs in AD has yet to be fully elucidated.

Stem cells may also play a limited role in the response to injury or disease in the aging brain. Division of NSCs has been implicated as contributing to the recovery period following a stroke [70]. Recent evidence suggests that latent stem cells begin proliferating following an ischemic episode, and in fact may explain the partial functional recovery following blood vessel occlusion. In rats receiving a middle cerebral artery occlusion, cells proliferate in the SVZ and migrate to the site of injury, where they express neuronal markers and send projections to appropriate target regions [71]. Although the number of cells which are replaced is small (approximately 0.2% of total striatal neurons), this finding gives hope that methods which promote

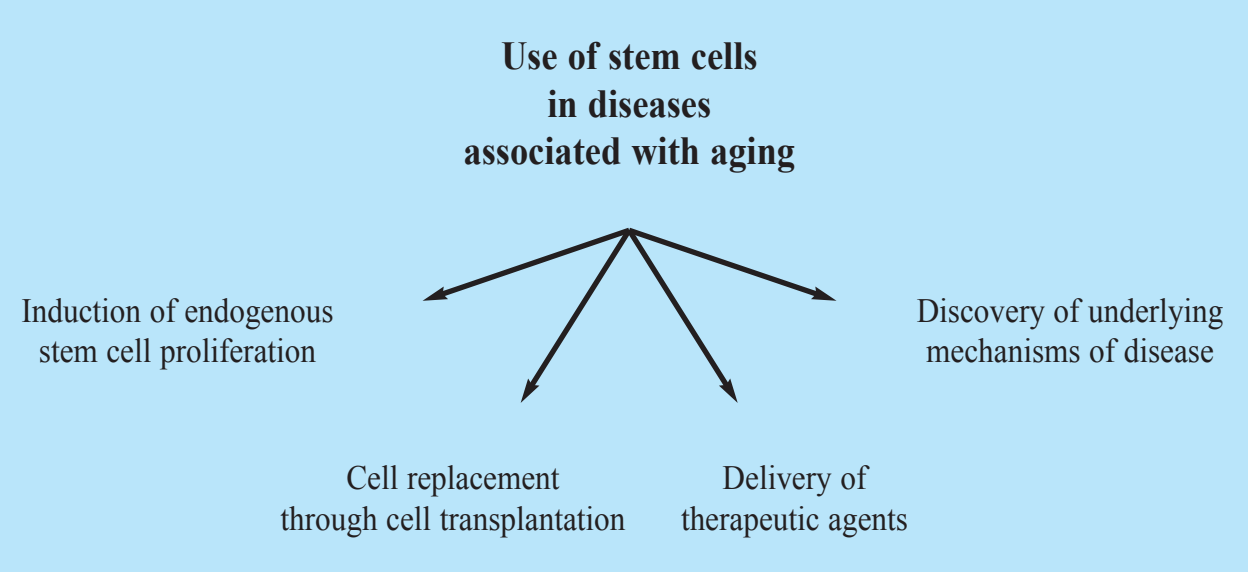

Fig. 3 Stem cells have several potential uses for treating neurodegenerative disorders. Stem cells themselves can replace damaged cells through division of endogenous NSCs or transplantation of exogenous NSCs. Stem cells that have been genetically engineered to secret protective and/or growth-promoting factors, such as bFGF, BDNF and GDNF, can activate new growth while slowing the disease progression. Stem cells can also be used to study the mechanisms underlying the disease process itself.

proliferation of these cells may aid in the recovery of neuronal number and function following stroke. To date, there is no evidence that loss of stem cell proliferative capacity contributes to the onset of PD, or that endogenous NSCs proliferate in response to cell loss associated with PD. However, neural stem cells have recently been identified in the adult substantia nigra which produce glia *in vivo*, and neurons, astrocytes and oligodendrocytes *in vitro* [72]. These cells can differentiate into neurons when transplanted into the hippocampus but not the substantia nigra, suggesting that manipulation of the environment in the substantia nigra to favor neurogenesis may allow endogenous stem cells to divide and replace neurons lost in PD. However the reality of this hypothetical scenario has not yet been examined.

Stem-cell based therapeutics

Stem cells have a number of potential applications in treating neurodegenerative diseases (Figure 3). Cells lost during disease can be replaced by division of stem cells already present in the brain (endogenous source), or by transplantation of new cells into the damaged region (exogenous source). Stem cells which have been genetically engineered to secrete factors which promote cell survival and/or growth, such as growth factors or other protective agents, can be transplanted into the damaged area to slow the rate of degeneration and promote growth of new neurons. Finally, stem cells may actually play a role in the disease process of some neurodegenerative disorders, making them a useful tool for studying the mechanisms underlying the disease process itself. In the next section, we will examine the potential use of stem cells in the aging brain.

Stem-cell based therapeutics: exogenous

When the body cannot replace damaged tissue through generation of new cells, one therapeutic approach is the transplantation of exogenously produced cells. For this method to be successful, the

transplanted cells must 1) survive the transplantation; 2) differentiate into appropriate cell phenotype(s); and 3) make appropriate, functional connections with host tissue. In the case of cells transplanted into the diseased brain, the cells must also be able to survive and function in the dysfunctional environment created by the disease itself. Compared to transplantation into more homogenous tissue with better proliferative capacity, such as the liver and the hematopoietic system, transplantation into the CNS is even more complicated, given the intricate nature of connections between various populations of neurons. In the diseased brain, transplantation is complicated even further by the inhospitable environment created by the pathology of the disease itself, and the often diffuse nature of neurodegenerative diseases.

One common approach is to culture stem cells prior to transplantation to increase the number of cells available for transplantation. Stem cells can be grown for prolonged periods of time, during which time their genetic makeup can be manipulated to add or delete genes. Once transplanted, stem cells can migrate a great distance in both intact and damaged brains [73], and can differentiate into appropriate cells for the host tissue. However, there are several issues which must be resolved before cell replacement using stem cells can become a standard therapy. Once cells have become transplanted, it is difficult to regulate the final phenotype of the cells following differentiation *in vivo*. In instances where a specific cell type is needed for functional recovery (such as the need for new dopaminergic neurons in Parkinson's disease), it may be more appropriate to introduce a more restricted precursor which will develop into a neuron rather than a multipotent stem cell which could potentially develop into glia. Additionally, there is increasing evidence that stem cells themselves vary between populations, depending on the source tissue, thus necessitating tissue-specific lineages of stem cells for cell replacement therapy.

Despite the potential obstacles to using transplanted cells to replace lost tissue, there is evidence that such methods can produce significant recovery in rodent disease models. For example, transplantation of passaged stem cells results in recovery of physiological and behavioral function in rodent models of Parkinson's disease and stroke, as discussed below.

Transplantation of embryonic cells and tissue

Much of the controversy over stem cell replacement therapy centers on the use of ES cells to replace lost tissue. ES cells are pluripotent cells isolated from the inner cell mass of blastocysts, which give rise to every cell in the mature organism. ES cells differ from stem cells, which are multipotent but are generally considered to have a more restricted potential than ES cells, such that the development of stem cells tend to be restricted to the types of cells usually present in the donor organ. While there have been tentative advances in the use of adult-derived stem cells of the hematopoietic system, the ability of cells to transdifferentiate (*i.e.*, de-differentiate into a more primitive cell, then differentiate into a completely different type of cell) remains controversial. In fact, recent reports in *Nature* suggest that transdifferentiation is actually a result of stem cells fusing with more mature cells to create an abnormal hybrid [74, 75]. More promising work has been done using ES cells to replace cells lost in Parkinson's disease. More mature cells, such as fetal midbrain precursor cells, can integrate into the CNS, form functional dopaminergic neurons and alleviate Parkinson's-like symptoms in the rat [76]; however, the ability of these cells to generate dopamine-secreting neurons is variable and unreliable. ES cells, on the other hand, have the capacity to proliferate extensively and can generate functional dopaminergic neurons *in vitro* [77]. When an enriched population of neural stem cells is transplanted into the striatum of rats lesioned with 6-OHDA, a commonly used animal model for Parkinson's disease, the cells integrate into the injured area and develop into neurons which exhibit properties expected of midbrain neurons [77].

Transplant studies in humans with PD have had varying degrees of success and failure. Clinical trials have focused on transplantation of human fetal mesencephalic tissue, which is highly controversial given the need for six to seven human fetuses needed *per* patient. Transplantation of less tissue is generally unsuccessful in terms of therapeutic effect, thus there is a need for a large amount of tissue for transplantation. The success of symptom amelioration also

varies from improved function to no change to worsening of symptoms. While transplantation of human fetal tissue can reduce bradykinesia and rigidity, there is little to no improvement in gait and tremor [78, 79]. Disturbingly, some patients receiving grafts develop abnormal movements (termed "runaway dyskinesias or dystonia") as a complication following surgery [80]. Additionally, the survival rate of cells following transplantation is generally poor, and is usually not sufficient to reach the minimum number of surviving cells to have a positive effect [81]. Given the recent success in rat models of PD [77], future clinical trials are likely to focus on use of human ES cells as an alternative to using fetal tissue to replace cells lost in PD.

Transplantation of exogenous cells in animal models is also showing some promise in treating ischemia following stroke. Following injury, NSCs can travel great distances from the site of implantation to the site of disease or injury. For example, transplanted NPCs rapidly migrate to the site of active gliomas in the adult rodent brain, even when the transplantation site is in the contralateral hemisphere [82]. A number of studies suggest that transplantation of NSCs in rodents results in partial replacement of cells lost during ischemic injury, and can result in partial recovery of motor and cognitive function. Functional recovery following transplantation of NSCs has been observed in both young [83] and adult [84] animals, as evidence by increased numbers of neurons in the infarct site, and improved performance in tasks such as the Morris water maze.

In contrast to the large number of studies of NSCs in PD, there have been no studies to date which examine the feasibility of using NSCs to repair AD-associated neuronal loss. One problem associated with cell replacement strategies in AD is the diffuse nature of cellular damage as the disease progresses. A more significant problem is the hostile environment created by the disease. AD patients exhibit elevated oxidative stress, which can induce apoptosis in NSCs derived from the SVZ [85]. Amyloid beta-peptide, a self-aggregating protein present in amyloid plaques associated with AD, inhibits NSC proliferation and differentiation [66], suggesting that exogenous cells transplanted into the AD brain may not survive to replace damaged cells.

Stem-cell based therapeutics: endogenous

Mobilization of endogenous stem cells to replace damaged tissue represents a less invasive route of therapy than transplantation of foreign cells/tissue and avoids the problems associated with a limited supply of donor cells. Several lines of evidence suggest that this approach is possible. First, many studies point to the existence stem cells throughout the brain [86, 87]. Second, there is substantial evidence that that neural stem cells respond to environmental cues which stimulate neurogenesis, such as dietary restriction, exercise, hormone therapy and growth factor infusion. While studies have focused on the two primary neurogenic regions (the SVZ and SGL), neurogenesis has been observed in the cortex; however, some regions, such as the spinal cord, appear to be inherently less responsive.

Growth factors

The adult brain is continuously generating new neurons [88, 89], although the capacity to generate new neurons diminishes with age. One strategy for cell replacement in neurodegenerative disorders is promoting proliferation of endogenous stem cells by infusing biologically active agents *in vivo*, or promoting the survival of stem cells of the constitutively dividing population and/or the cells that are induced to divide upon injury. In order for this method to succeed, endogenous stem cells must not only proliferate and travel to the point of injury, they must also differentiate into an appropriate phenotype which makes appropriate, functional connections and survive in the host tissue. As with transplanted tissue, these cells must overcome the additional complication of survival in diseased tissue.

NSCs respond to a variety of growth factors and cytokines which affect proliferation, differentiation and survival of these cells. In particular, growth factors have been examined as potential "activators" of cell replacement. One growth factor, basic fibroblast growth factor (bFGF), promotes neurogenesis both *in vitro* and *in vivo*. Infusion of bFGF into the ventricles for 14 days results in proliferation of progenitor cells in the SVZ without evidence of proliferation in the hippocampus [90]. Four weeks fol-

lowing factor withdrawal, many of the newborn cells had migrated into the olfactory bulb and differentiated into phenotypes resembling local interneurons, suggesting that bFGF promoted neurogenesis. Interestingly, NSCs which appear early in development respond only to FGF; as development proceeds, loss of FGF or FGF receptors leads to a loss of stem cell proliferation [22, 91, 92]. NSCs which appear later in development require either FGF or epidermal growth factor (EGF) to proliferate [93, 94]. As cells become more restricted in their developmental potential, the growth factor requirement tends to become more specialized; for example, GRPs respond to platelet-derived growth factor (PDGF) [95], while NRPs respond to factors such as sonic hedgehog (Shh), FGF and neurotrophin-3 (NT-3) [96-98]. Thus, cell proliferation may be stimulated by administration of appropriate growth factor; however, some growth factors, such as FGF, act on multiple cell types, which may cause both the desired proliferation and proliferation of non-desirable cells.

Growth factors may also be used to prevent the cognitive decline associated with aging. One interesting approach has been to transplant NPCs which have been genetically engineered to secrete growth factors which promote neurogenesis. In one model, rat NSCs engineered to overexpress nerve growth factor (NGF) were transplanted into rats following middle cerebral artery occlusion. Forty-eight hours following the insult, the transplanted cells had migrated to the area of the infarct. More importantly, the loss of neurons due to ischemic damage was less severe in the animals that had received transplants, as compared to control animals [99]. In another study, nerve growth factor (NGF) was used to prevent the cognitive decline observed in aging. Aged rats demonstrate cognitive deficits which are accompanied by atrophy of forebrain, reminiscent of changes observed in humans. NPCs engineered to secrete NGF were transplanted into middle-aged rats with no observable cognitive defects. When the cognitive ability of the rats receiving the transplanted cells was compared to age-matched controls at 9 months following the surgery, the rats receiving the NSCs had cognitive function similar to younger adult rats, and showed no evidence of forebrain cholinergic neuron atrophy [100]. Growth factors can also be used to create a favorable environment for the survival of cells which are replaced by new cell divisions. For example, brain-derived neurotrophic factor (BDNF) has been examined for its potential neurogenic activity. Following intraventricular injection of BDNF, newly formed neurons were observed in the striatum, septum, thalamus and hypothalamus; however, the phenotype and functional ability of these cells has not yet been investigated [101]. Similarly, transplantation of cells overexpressing glial cell-line derived neurotrophic factor (GDNF) protects against loss of dopaminergic neurons in the 6-hydroxydopamine mouse model of PD [102].

Promoting survival of stem cells which are activated by the injury itself may prove an effective therapy for patients who have suffered a stroke. Several recent papers suggest that following occlusion of a cerebral artery, the endogenous stem cell population is activated, resulting in division and migration of cells into the damaged area. Following ischemic brain injury in the rat, there is extensive division of NPCs in the SGL and extensive replacement of hippocampal pyramidal neurons, suggesting that the capacity to replace damaged tissue already exists [70]. Infusion of bFGF and EGF into the lateral ventricle did not protect against cell death following the ischemic event, but did increase the number of neurons in the CA1 region which were replaced by endogenously recruited stem cells. Most importantly, the newly generated neurons displayed similar electrophysiological properties as cells normally found in that region, and the animals treated with neurogenesis-promoting growth factors exhibited enhanced recovery of cognitive ability.

While treatment with growth factors is a potential therapeutic approach, it is limited by the fact that most cells will proliferate for a certain number of divisions, then cease dividing despite continued stimulation, a limitation termed the Hayflick limit [103]. NSCs exhibit a slowed proliferation rate with prolonged time in culture (our unpublished observations). The limited responsiveness to growth factor stimulation is true not only for NSCs but for restricted precursors as well. For example, oligodendrocyte restricted precursors proliferate in response to platelet-derived growth factor (PDGF) but will eventually differentiate into mature oligodendrocytes despite continued exposure. NSCs in the aging brain exhibit a decreased ability to proliferate under normal conditions [64], thus the ability

of growth factors to stimulate proliferation may also be inhibited in the aging brain. In the peripheral nervous system, adult NSCs are less responsive than embryonic NSCs to proliferation stimulated by growth factors [104]. However, the ability of growth factors to promote proliferation in the aged brain has not yet been examined sufficiently.

Telomeres as control point in number of cell divisions

An alternative approach to using growth factors to stimulate NSC proliferation is activation of processes which normally participate in cell proliferation, such as cell cycle proteins. This approach targets proteins normally involved in cellular proliferation and differentiation, such as cell cycle proteins. Potential targets are telomeres and the enzyme which catalyzes telomere lengthening, telomerase. Telomeres have long been recognized as being important for maintaining genomic stability by capping the ends of chromosomes and preventing degradation of DNA. However, it has become increasingly clear that telomere length provides a biological clock which regulates the number of cell divisions a cell can undergo before becoming quiescent. The number of cell divisions which can occur *in vitro* depends on the number of cell divisions which have occurred rather than the actual passage of time, which translates into a molecular mechanism for regulating the loss of proliferative capacity. In fibroblasts in culture, progressive shortening of telomeres results in a biological clock which regulates the entry into replicative senescence [105]. Telomere length is maintained by the enzyme telomerase, which adds a six-base DNA sequence (TTAGGG) to the ends of telomeres. Telomerase is comprised of a catalytic subunit (TERT) and an RNA template component (TR). Telomerase activity is high throughout development and is maintained during adulthood in highly proliferative cells, such as germline and cancer cells, but is otherwise inactive or absent in most adult somatic tissue following differentiation. Forced expression of hTERT in normal, telomerase negative cells causes the cells to become immortalized, while causing elongation of telomeres and extension of cellular lifespan [106], suggesting a tight relationship between TERT activity and proliferation. As aging progresses, repression of telomerase activity results in telomere shortening and replicative senescence, thereby limiting the number of cell divisions to a finite number before permanent growth arrest. Thus, telomere length regulates cellular senescence, making telomerase a crucial component in the aging process.

Indirect evidence suggests that stem cells may have higher telomerase activity than senescent cells. Most cells in the body reach replicative senescence after a certain number of divisions, thereby limiting the amount of time a cell line can be maintained in culture. However, some stem cells can undergo cell division for a much longer time period, replicating much beyond the normal lifespan of somatic cells. ES cells in particular have been observed to have extremely long lifespans *in vitro*, maintaining the ability to divide for several years in culture. In the hematopoietic system, stem cells express low to moderate levels of telomerase which is theoretically linked to their ability to divide when other somatic cells have become senescent in the adult [107]. Theoretically, NSCs in the adult brain would have higher telomerase activity than non-dividing neurons, however this question has not yet been addressed. While the developing brain has high telomerase activity, mature neurons and glia lack telomerase activity. The embryonic murine brain has significant levels of telomerase activity [108-110] which is down-regulated by day 16 post-birth [111]. However, recent studies indicate that stem cells retain telomerase activity, even in the nervous system. In cortical stem cells, telomerase activity appears to be restricted to neural progenitors, with no measurable activity in glial precursors [112]. Further, telomerase activity is highly correlated with periods of neurogenesis in the developing cortex and cerebellum, and is undetectable in primary cultures of cortical astrocytes, suggesting a role in neurogenesis but not gliogenesis [112]. Interestingly, telomerase activity is up-regulated in cortical neural progenitor cells in response to bFGF [112], suggesting a link between growth factors which regulate neural development and telomerase activity. This is substantiated by the correlation between the telomerase activity and bFGF expression during brain development.

Several studies suggest that changes in telomere length and telomerase activity can drastically alter the onset and maintenance of an “aging phenotype”.

Telomeres in certain tissues are shorter in older people than younger people [105, 113, 114]. In several human diseases which are characterized by early onset of aging and reduced lifespan, there is evidence of accelerated shortening of telomeres (*i.e.*, Werner's syndrome and Down's syndrome) or shortened telomere length (Hutchinson-Gilford syndrome). In Werner's syndrome, forced expression of telomerase causes dermal fibroblasts derived from patients with Werner's syndrome to become immortalized and exhibit lengthened telomeres [115]. There is also substantial evidence that cellular senescence is a naturally occurring phenomenon during human aging *in vivo*. Several lines of evidence suggest that cellular senescence contributes to many of the pathological changes observed in aged individuals [116, 117]. In normal human fibroblasts obtained from older adults, there is evidence of decreased growth capability [118] and shortening of terminal restriction fragments of telomeres [114]. Experimental models substantiate a role for telomere length in the aging process. Mice engineered to have no telomerase activity (though knockout of the mTR gene) have short telomeres, and exhibit many symptoms normally associated with aging, including cell growth arrest and decreased capacity to respond to stress in highly proliferative organs [119, 120]. In these mice, telomere shortening in hepatic cells contributed to end-stage organ failure in an experimental model of cirrhosis which was partially reversed using adenovirus-mediated gene therapy resulted in improved hepatic function [120], indicating a direct link between telomerase activity and organ health.

The rate of telomere shortening is sensitive to stresses which accompany aging, such that stressed cells experience replicative senescence earlier than unstressed cells, resulting in a shorter replicative lifespan. The triple-G structure of telomere results in high sensitivity to insults such as ultraviolet irradiation [121], alkylation [122] and oxidative stress [123]. Oxidative stress has recently been implicated in promoting the acceleration of telomere loss and reduced lifespan in several biological systems. Experiments suggest that maintenance of a low rate of telomere shortening in human fibroblasts is highly dependent on superoxide dismutase [124]. Interestingly, shortened telomere length is now being linked to several age-related diseases which are believed to have oxidative stress as a causative factor, including vascular dementia [125] and atherosclerosis [126]. Accordingly, it has been hypothesized that telomere length may reflect both the cumulative effect of oxidative stress and normal reduction of telomere length, providing a link between oxidative stress and the aging process [127]. Increased oxidative stress accompanies aging in the human brain, and is associated with Alzheimer's disease. While it has not yet been tested experimentally, one can hypothesize that oxidative stress associated with Alzheimer's disease may underlie at least part of the lack of cell replacement by NSCs observed in this disease. *In vitro* experiments suggest that $A\beta_{1\text{-}42}$, which causes oxidative stress in neurons, can also cause loss of stem cell proliferation [66]; however, the relationship between the oxidative stress and telomerase activity in NSCs has not yet been examined.

While telomere length appears to play a central role in determining cellular aging, it should be kept in mind that telomeres are only part of the overall picture. Mice have longer telomeres (50-150 kb) than humans (10-15 kb), but a much shorter lifespan (~2 years *vs*. ~80 years, respectively). Approximately 70% of immortalized human somatic cell lines [128] and 90% of human cancer cell lines [129] exhibit *in vitro* telomerase activity, suggesting that the progression into cellular senescence cannot be entirely attributed to this enzyme. Additionally, many telomerase-negative cells are capable of maintaining normal telomere lengths [130], indicating potential telomere-lengthening activity in non-telomerase enzymes.

Overcoming obstacles to cell division

Mammalian neurons exit the cell cycle during the embryonic period, entering an extended period of mitotic quiescence termed the G0 phase, during which the cells do not progress through the cell cycle. Once a neuron has matured, it cannot proliferate even when stimulated by compounds or growth factors which promote cell cycle progression in cells which can temporarily enter G0, such as fibroblasts. Stem cells that are quiescent are transiently held at G0 and are capable of re-entering the cell cycle following appropriate stimulation. As noted above, there is a limit to the number of divisions a proliferative cell will undergo before

becoming senescent, differentiating or dying [103]. Cellular senescence results from cells permanently exiting the cell cycle. Once cells have entered G0, they are generally incapable of re-entering the cell cycle and, indeed, activation of DNA replication in terminally differentiated neurons often leads to apoptosis [131]. As previously described, telomere length plays a critical role in determining the number of replications a cell will undergo before withdrawing from the cell cycle. However, there are also a number of proteins and transcription factors which regulate the cell cycle itself which are potential therapeutic targets for maintaining the proliferative capacity of cells [132]. Among the best known pro-proliferation pathways is the ras/raf/mek/erk protein kinase pathway. In rodents, activation of this pathway shortens the G1-S transition time, while in *Drosophila* the same pathway promotes proliferation by affecting the G2-M transition [133]. This pathway is coupled to cell cycle *via* interactions with p53 and the retinoblastoma tumor suppressor (Rb) and E2F families of proteins. Rb proteins target the transcription factor E2F to promote cell proliferation and growth. E2F proteins promote cell cycle progression *via* interaction with the promoter region of many of the genes involved in DNA replication, including dihydrofolate reductase, DNA polymerase α and thymidine kinase, and genes involved in cell growth, including c-myc, cdc2 and E2F1 [132]. In multipotent stem cells, the p120/E2F pathway plays a critical role in maintaining self-renewal capability, as p107 expression levels is upregulated in rapidly growing, undifferentiated NSCs but decline during neuronal differentiation [134]. Several cdk inhibitors, such as p15, p16 and p27, mediate suppression of cell proliferation by inhibiting activation of the Rb protein [135]. Differentiation of the embryonic carcinoma cell line NTera2 into postmitotic neurons is associated with elevated expression of p15 and p16, suggesting that these proteins promote neuronal mitotic quiescence [136]. Thus, a number of proteins are involved in neural proliferation and differentiation which could be targeted to promote neurogenesis.

Theoretically, promotion of NSC division and differentiation would allow for the production of new neurons. This could be achieved by overexpression of proteins which promote growth, such as E2F proteins, or inhibition of cell cycle inhibitors, such as p15 and p16. However, the effect would have to be specifically targeted to NSCs without affecting local, postmitotic neurons. Reentry into the cell cycle induces apoptosis in post-mitotic neurons which involves activation of proteins normally involved in the cell cycle, such as cdk4 and cdk6 [137]. Further, stimulation of proliferation may not be sufficient to replace new neurons if the surrounding environment is not permissive to cell survival. In AD, there is evidence for re-expression of proteins normally expressed during development [138], suggesting that proliferative signals may already be present during the disease, but other factors, such as oxidative stress, may be inhibiting the survival of proliferating NSCs or, conversely, may be pushing cells into apoptosis. Thus, neural stem cells that have become quiescent with age may respond to cell-cycle activation by dying rather than proliferating. Another potential problem with this approach is the potential for generating tumors if cells lose the ability to exit the cell cycle. Loss of cell cycle regulation is a common feature in cancerous cells, and involves many of the same proteins involved in normal cell cycle regulation. Any therapeutic approach utilizing cell cycle proteins will have to address these problems in order to be successful.

Conclusion

Based on current data, several strategies have emerged for repairing damage associated with age-related neurodegenerative disorders. In Parkinson's disease, transplantation of exogenous tissue is a promising strategy, despite the observed problems in human studies to date. The recent discovery of NSCs in the adult substantia nigra offers the potential use of these cells for endogenous repair strategies [72]. Transplantation of exogenous tissue is showing some promise also for treating ischemic damage following stroke. In stroke and AD, each disease causes activation of endogenous NSCs, as indicated by activation of cell cycle proteins and/or proliferation of NSCs. However, in each disease there is limited cell replacement, possibly due to limited survival of new cells, thus any cell replacement strategy will have to address not only cell proliferation but also cell survival. In sum, NSCs offer a potential therapeutic strategy for some of the most

devastating neurodegenerative disorders which afflict the aging brain. As with any disease, the development of new therapies relies heavily on a thorough knowledge of the biology of the system being studied, and the ramifications of alterations of that system during disease. The dynamic nature of the signaling pathways, combined with differences in cell populations based on location and degree of differentiation, creates a complex scenario for determining which pathways are critical for NSC regulation. While the general outline of signaling pathways which affect NSC proliferation, differentiation and survival is beginning to become more clear, there remains a great deal to be learned before basic research can be translated from the bench to bedside. However, the intense amount of research being done in this area offers the promise of valid therapeutics in the foreseeable future for many neurodegenerative diseases associated with aging.

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References

1. **Kemper T.,** Neuroanatomical and neuropathological changes during aging and in dementia. In: Albert M.,Knoepfel J., eds., Clinical Neurology of Aging, Oxford University Press, New York, 1994, pp. 3-67
2. **Cowell P.E., Turetsky B.I., Gur R.C., Grossman R.I., Shtasel D.L., Gur R.E.,** Sex differences in aging of the human frontal and temporal lobes, *J. Neurosci*., **14**: 4748-4755, 1994
3. **Raz N., Gunning F.M., Head D., Dupuis J.H., McQuain J., Briggs S.D., Loken W.J., Thornton A.E., Acker J.D.,** Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter, *Cereb. Cortex*., **7**: 268-282, 1997
4. **Raz N., Torres I.J., Acker J.D.,** Age, gender, and hemispheric differences in human striatum: a quantitative review and new data from *in vivo* MRI morphometry, *Neurobiol. Learn. Mem*., **63**: 133-142, 1995
5. **Doraiswamy P.M., Na C., Husain M.M., Figiel G.S., McDonald W.M., Ellinwood E.H., Jr., Boyko O.B., Krishnan K.R.,** Morphometric changes of the human midbrain with normal aging: MR and stereologic findings, *AJNR Am. J. Neuroradiol*., **13**: 383-386, 1992
6. **Weis S., Kimbacher M., Wenger E., Neuhold A.,** Morphometric analysis of the corpus callosum using MR: correlation of measurements with aging in healthy individuals, *AJNR Am. J. Neuroradiol*., **14**: 637-645, 1993
7. **Trollor J.N., Valenzuela M.J.,** Brain ageing in the new millennium, *Aust N Z J Psychiatry*, **35**: 788-805, 2001
8. **Morrison J.H., Hof P.R.,** Life and death of neurons in the aging brain, *Science*, **278**: 412-9, 1997
9. **Bertoni-Freddari C., Fattoretti P., Paoloni R., Caselli U., Galeazzi L., Meier-Ruge W.,** Synaptic structural dynamics and aging, *Gerontology*, **42**: 170-180, 1996
10. **Mattson M.P., Pedersen W.A., Duan W., Culmsee C., Camandola S.,** Cellular and molecular mechanisms underlying perturbed energy metabolism and neuronal degeneration in Alzheimer's and Parkinson's diseases, *Ann. N. Y. Acad. Sci*., **893**: 154-175, 1999
11. **Sastre J., Pallardo F.V., Vina J.,** Mitochondrial oxidative stress plays a key role in aging and apoptosis, *IUBMB Life*, **49**: 427-435, 2000
12. **Schapira A.H.,** Oxidative stress and mitochondrial dysfunction in neurodegeneration, *Curr. Opin. Neurol*., **9**: 260-264, 1996
13. **Keller J.N., Mattson M.P.,** Roles of lipid peroxidation in modulation of cellular signaling pathways, cell dysfunction, and death in the nervous system, *Rev. Neurosci*., **9**: 105-116, 1998
14. **Reuter-Lorenz P.,** New visions of the aging mind and brain, *Trends Cogn. Sci*., **6**: 394, 2002
15. **Mattson M.P.,** Brain evolution and lifespan regulation: conservation of signal transduction pathways that regulate energy metabolism, *Mech. Ageing Dev*., **123**: 947-953, 2002
16. **Bartke A., Brown-Borg H.M., Bode A.M., Carlson J., Hunter W.S., Bronson R.T.,** Does growth hormone prevent or accelerate aging?, *Exp. Gerontol*., **33**: 675-687, 1998
17. **Flurkey K., Papaconstantinou J., Miller R.A., Harrison D.E.,** Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production, *Proc. Natl. Acad. Sci. U S A*, **98**: 6736-6741, 2001

18. **Honda Y., Honda S.,** The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*, *Faseb J*., **13**: 1385-1393, 1999
19. **Lin K., Hsin H., Libina N., Kenyon C.,** Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling, *Nat. Genet*., **28**: 139-145, 2001
20. **Wolkow C.A., Kimura K.D., Lee M.S., Ruvkun G.,** Regulation of C. elegans life-span by insulinlike signaling in the nervous system, *Science*, **290**: 147-150, 2000
21. **Kalyani A.J., Mujtaba T., Rao M.S.,** Expression of EGF receptor and FGF receptor isoforms during neuroepithelial stem cell differentiation, *J. Neurobiol*., **38**: 207-224, 1999
22. **Quinn S.M., Walters W.M., Vescovi A.L., Whittemore S.R.,** Lineage restriction of neuroepithelial precursor cells from fetal human spinal cord, *J. Neurosci. Res*., **57**: 590-602, 1999
23. **Ling Z.D., Potter E.D., Lipton J.W., Carvey P.M.,** Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines, *Exp. Neurol*., **149**: 411-423, 1998
24. **Roy N.S., Benraiss A., Wang S., Fraser R.A., Goodman R., Couldwell W.T., Nedergaard M., Kawaguchi A., Okano H., Goldman S.A.,** Promoter-targeted selection and isolation of neural progenitor cells from the adult human ventricular zone, *J. Neurosci. Res*., **59**: 321-331, 2000
25. **Roy N.S., Wang S., Jiang L., Kang J., Benraiss A., Harrison-Restelli C., Fraser R.A., Couldwell W.T., Kawaguchi A., Okano H., Nedergaard M., Goldman S.A.,** In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus, *Nat. Med*., **6**: 271-277, 2000
26. **Song H.J., Stevens C.F., Gage F.H.,** Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons, *Nat. Neurosci*., **5**: 438-445, 2002
27. **Li R., Thode S., Zhou J., Richard N., Pardinas J., Rao M.S., Sah D.W.,** Motoneuron differentiation of immortalized human spinal cord cell lines, *J. Neurosci. Res*., **59**: 342-352, 2000
28. **Sato K., Hayashi T., Sasaki C., Iwai M., Li F., Manabe Y., Seki T., Abe K.,** Temporal and spatial differences of PSA-NCAM expression between young-adult and aged rats in normal and ischemic brains, *Brain Res*., **922**: 135-139, 2001
29. **Scolding N.J., Rayner P.J., Compston D.A.,** Identification of A2B5-positive putative oligodendrocyte progenitor cells and A2B5-positive astrocytes in adult human white matter, *Neuroscience*, **89**: 1-4, 1999
30. **Prabhakar S., D'Souza S., Antel J.P., McLaurin J., Schipper H.M., Wang E.,** Phenotypic and cell cycle properties of human oligodendrocytes in vitro, *Brain Res*., **672**: 159-169, 1995
31. **Rao M.S., Mayer-Proschel M.,** Glial-restricted precursors are derived from multipotent neuroepithelial stem cells, *Dev. Biol*., **188**: 48-63, 1997
32. **Mi H., Barres B.A.,** Purification and characterization of astrocyte precursor cells in the developing rat optic nerve, *J. Neurosci*., **19**: 1049-1061, 1999
33. **Seidman K.J., Teng A.L., Rosenkopf R., Spilotro P., Weyhenmeyer J.A.,** Isolation, cloning and characterization of a putative type-1 astrocyte cell line, *Brain Res*., **753**: 18-26, 1997
34. **Laywell E.D., Rakic P., Kukekov V.G., Holland E.C., Steindler D.A.,** Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain, *Proc. Natl. Acad. Sci. USA*, **97**: 13883-13888, 2000
35. **Kondo T., Raff M.,** Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells, *Science*, **289**: 1754-1757, 2000
36. **Sommer L., Rao M.,** Neural stem cells and regulation of cell number, *Prog. Neurobiol*., **66**: 1-18, 2002
37. **McMahon J.A., Takada S., Zimmerman L.B., Fan C.M., Harland R.M., McMahon A.P.,** Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite, *Genes Dev*., **12**: 1438-1452, 1998
38. **Lim D.A., Tramontin A.D., Trevejo J.M., Herrera D.G., Garcia-Verdugo J.M., Alvarez-Buylla A.,** Noggin antagonizes BMP signaling to create a niche for adult neurogenesis, *Neuron*, **28**: 713-726, 2000
39. **Palmer T.D., Markakis E.A., Willhoite A.R., Safar F., Gage F.H.,** Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS, *J. Neurosci*., **19**: 8487-8497, 1999
40. **Whittemore S.R., Morassutti D.J., Walters W.M., Liu R.H., Magnuson D.S.,** Mitogen and substrate differentially affect the lineage restriction of adult rat subventricular zone neural precursor cell populations, *Exp. Cell Res*., **252**: 75-95, 1999

41. **Pincus D.W., Keyoung H.M., Harrison-Restelli C., Goodman R.R., Fraser R.A., Edgar M., Sakakibara S., Okano H., Nedergaard M., Goldman S.A.,** Fibroblast growth factor-2/brain-derived neurotrophic factor-associated maturation of new neurons generated from adult human subependymal cells, *Ann. Neurol*., **43**: 576-585, 1998
42. **Shetty A.K., Turner D.A.,** In vitro survival and differentiation of neurons derived from epidermal growth factor-responsive postnatal hippocampal stem cells: inducing effects of brain-derived neurotrophic factor, *J. Neurobiol*., **35**: 395-425, 1998
43. **Smith A.G., Heath J.K., Donaldson D.D., Wong G.G., Moreau J., Stahl M., Rogers D.,** Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides, *Nature*, **336**: 688-690, 1988
44. **Williams R.L., Hilton D.J., Pease S., Willson T.A., Stewart C.L., Gearing D.P., Wagner E.F., Metcalf D., Nicola N.A., Gough N.M.,** Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells, *Nature*, **336**: 684-687, 1988
45. **Conover J.C., Ip N.Y., Poueymirou W.T., Bates B., Goldfarb M.P., DeChiara T.M., Yancopoulos G.D.,** Ciliary neurotrophic factor maintains the pluripotentiality of embryonic stem cells, *Development*, **119**: 559-565, 1993
46. **Yoshida K., Chambers I., Nichols J., Smith A., Saito M., Yasukawa K., Shoyab M., Taga T., Kishimoto T.,** Maintenance of the pluripotential phenotype of embryonic stem cells through direct activation of gp130 signalling pathways, *Mech. Dev*., **45**: 163-171, 1994
47. **Hughes S.M., Lillien L.E., Raff M.C., Rohrer H., Sendtner M.,** Ciliary neurotrophic factor induces type-2 astrocyte differentiation in culture, *Nature*, **335**: 70-73, 1988
48. **Johe K.K., Hazel T.G., Muller T., Dugich-Djordjevic M.M., McKay R.D.,** Single factors direct the differentiation of stem cells from the fetal and adult central nervous system, *Genes Dev*., **10**: 3129-3140, 1996
49. **Koblar S.A., Turnley A.M., Classon B.J., Reid K.L., Ware C.B., Cheema S.S., Murphy M., Bartlett P.F.,** Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor, *Proc. Natl. Acad. Sci. U S A*, **95**: 3178-3181, 1998
50. **Mayer M., Bhakoo K., Noble M.,** Ciliary neurotrophic factor and leukemia inhibitory factor promote the generation, maturation and survival of oligodendrocytes in vitro, *Development*, **120**: 143-153, 1994
51. **Gard A.L., Williams W.C., 2nd, Burrell M.R.,** Oligodendroblasts distinguished from O-2A glial progenitors by surface phenotype (O4+GalC-) and response to cytokines using signal transducer LIFR beta, *Dev. Biol*., **167**: 596-608, 1995
52. **Marmur R., Kessler J.A., Zhu G., Gokhan S., Mehler M.F.,** Differentiation of oligodendroglial progenitors derived from cortical multipotent cells requires extrinsic signals including activation of gp130/LIFbeta receptors, *J. Neurosci*., **18**: 9800-9811, 1998
53. **Gage F.H., Kempermann G., Palmer T.D., Peterson D.A., Ray J.,** Multipotent progenitor cells in the adult dentate gyrus, *J. Neurobiol*., **36**: 249-266, 1998
54. **Garcia-Verdugo J.M., Doetsch F., Wichterle H., Lim D.A., Alvarez-Buylla A.,** Architecture and cell types of the adult subventricular zone: in search of the stem cells, *J. Neurobiol*., **36**: 234-248, 1998
55. **Lemaire V., Koehl M., Le Moal M., Abrous D.N.,** Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus, *Proc. Natl. Acad. Sci. U S A*, **97**: 11032-11037, 2000
56. **Neeper S.A., Gomez-Pinilla F., Choi J., Cotman C.W.,** Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain, *Brain Res*., **726**: 49-56, 1996
57. **Kempermann G., van Praag H., Gage F.H.,** Activity-dependent regulation of neuronal plasticity and self repair, *Prog. Brain Res*., **127**: 35-48, 2000
58. **van Praag H., Kempermann G., Gage F.H.,** Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus, *Nat. Neurosci*., **2**: 266-270, 1999
59. **Kempermann G., Gage F.H.,** Experience-dependent regulation of adult hippocampal neurogenesis: effects of long-term stimulation and stimulus withdrawal, *Hippocampus,* **9**: 321-332, 1999
60. **Young D., Lawlor P.A., Leone P., Dragunow M., During M.J.,** Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective, *Nat. Med*., **5**: 448-453, 1999
61. **Liu J., Solway K., Messing R.O., Sharp F.R.,** Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils, *J. Neurosci*., **18**: 7768-7778, 1998

62. **Parent J.M., Yu T.W., Leibowitz R.T., Geschwind D.H., Sloviter R.S., Lowenstein D.H.,** Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus, *J. Neurosci*., **17**: 3727-3738, 1997
63. **Kuhn H.G., Dickinson-Anson H., Gage F.H.,** Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation, *J. Neurosci*., **16**: 2027-2033, 1996
64. **Montaron M.F., Petry K.G., Rodriguez J.J., Marinelli M., Aurousseau C., Rougon G., Le Moal M., Abrous D.N.,** Adrenalectomy increases neurogenesis but not PSA-NCAM expression in aged dentate gyrus, *Eur. J. Neurosci*., **11**: 1479-1485, 1999
65. **Haughey N.J., Liu D., Nath A., Borchard A.C., Mattson M.P.,** Disruption of neurogenesis in the subventricular zone of adult mice, and in human cortical neuronal precursor cells in culture, by amyloid beta-peptide: implications for the pathogenesis of Alzheimer's disease, *Neuromolecular Med*., **1**: 125-135, 2002
66. **Morgan C.D., Nordin S., Murphy C.,** Odor identification as an early marker for Alzheimer's disease: impact of lexical functioning and detection sensitivity, *J. Clin. Exp. Neuropsychol*., **17**: 793-803, 1995
67. **Devanand D.P., Michaels-Marston K.S., Liu X., Pelton G.H., Padilla M., Marder K., Bell K., Stern Y., Mayeux R.,** Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up, *Am. J. Psychiatry*, **157**: 1399-1405, 2000
68. **Esiri M.M., Wilcock G.K.,** The olfactory bulbs in Alzheimer's disease, *J. Neurol. Neurosurg. Psychiatry*, **47**: 56-60, 1984
69. **Nakatomi H., Kuriu T., Okabe S., Yamamoto S., Hatano O., Kawahara N., Tamura A., Kirino T., Nakafuku M.,** Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors, *Cell*, **110**: 429-441, 2002
70. **Arvidsson A., Collin T., Kirik D., Kokaia Z., Lindvall O.,** Neuronal replacement from endogenous precursors in the adult brain after stroke, *Nat. Med*., **8**: 963-970, 2002
71. **Lie D.C., Dziewczapolski G., Willhoite A.R., Kaspar B.K., Shults C.W., Gage F.H.,** The adult substantia nigra contains progenitor cells with neurogenic potential, *J. Neurosci*., **22**: 6639-6649, 2002
72. **Flax J.D., Aurora S., Yang C., Simonin C., Wills A.M., Billinghurst L.L., Jendoubi M., Sidman R.L., Wolfe J.H., Kim S.U., Snyder E.Y.,** Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes, *Nat. Biotechnol*., **16**: 1033-1039, 1998
73. **Terada N., Hamazaki T., Oka M., Hoki M., Mastalerz D.M., Nakano Y., Meyer E.M., Morel L., Petersen B.E., Scott E.W.,** Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion, *Nature*, **416**: 542-545, 2002
74. **Ying Q.L., Nichols J., Evans E.P., Smith A.G.,** Changing potency by spontaneous fusion, *Nature*, **416**: 545-548, 2002
75. **Studer L., Tabar V., McKay R.D.,** Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats, *Nat. Neurosci*., **1**: 290-295, 1998
76. **Kim J.H., Auerbach J.M., Rodriguez-Gomez J.A., Velasco I., Gavin D., Lumelsky N., Lee S.H., Nguyen J., Sanchez-Pernaute R., Bankiewicz K., McKay R.,** Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease, *Nature*, **418**: 50-56, 2002
77. **Widner H.,** The Lund transplant program for Parkinson's disease and patients with MPTP-induced parkinsonism. In: Freeman T.B.,Widner H., eds., Cell transplantation for neurological disorders: toward reconstruction of the human central nervous system., Humana Press, Totowa, 1998, pp. 1-17
78. **Hauser R.A.,** Fetal nigral transplantation in Parkinson's disease: the USF pilot program (12 to 24 month evaluation). In: Freeman T.B.,Widner H., eds., Cell transplantation for neurological disorders: toward reconstruction of the human central nervous system., Humana Press, Totowa, 1998, pp. 19-30
79. **Freed C.R., Greene P.E., Breeze R.E., Tsai W.Y., DuMouchel W., Kao R., Dillon S., Winfield H., Culver S., Trojanowski J.Q., Eidelberg D., Fahn S.,** Transplantation of embryonic dopamine neurons for severe Parkinson's disease, *N. Engl. J. Med*., **344**: 710-719, 2001
80. **Aboody K.S., Brown A., Rainov N.G., Bower K.A., Liu S., Yang W., Small J.E., Herrlinger U., Ourednik V., Black P.M., Breakefield X.O., Snyder E.Y.,** From the cover: neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas, *Proc. Natl. Acad. Sci. U S A*, **97**: 12846-12851, 2000
81. **Park K.I., Teng Y.D., Snyder E.Y.,** The injured brain interacts reciprocally with neural stem cells

supported by scaffolds to reconstitute lost tissue, *Nat. Biotechnol*., **20**: 1111-1117, 2002

82. **Fukunaga A., Uchida K., Hara K., Kuroshima Y., Kawase T.,** Differentiation and angiogenesis of central nervous system stem cells implanted with mesenchyme into ischemic rat brain, *Cell Transplant*., **8**: 435-441, 1999
83. **Li L., Liu F., Salmonsen R.A., Turner T.K., Litofsky N.S., Di Cristofano A., Pandolfi P.P., Jones S.N., Recht L.D., Ross A.H.,** PTEN in neural precursor cells: regulation of migration, apoptosis, and proliferation, *Mol. Cell Neurosci*., **20**: 21-9, 2002
84. **Shihabuddin L.S., Palmer T.D., Gage F.H.,** The search for neural progenitor cells: prospects for the therapy of neurodegenerative disease, *Mol. Med. Today*, **5**: 474-480, 1999
85. **Svendsen C.N., Caldwell M.A., Ostenfeld T.,** Human neural stem cells: isolation, expansion and transplantation, *Brain Pathol*., **9**: 499-513, 1999
86. **Gould E., Gross C.G.,** Neurogenesis in adult mammals: some progress and problems, *J. Neurosci*., **22**: 619-623, 2002
87. **Temple S.,** Stem cell plasticity - building the brain of our dreams, *Nat. Rev. Neurosci*., **2**: 513-520, 2001
88. **Kuhn H.G., Winkler J., Kempermann G., Thal L.J., Gage F.H.,** Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain, *J. Neurosci*., **17**: 5820-5829, 1997
89. **Raballo R., Rhee J., Lyn-Cook R., Leckman J.F., Schwartz M.L., Vaccarino F.M.,** Basic fibroblast growth factor (Fgf2) is necessary for cell proliferation and neurogenesis in the developing cerebral cortex, *J. Neurosci*., **20**: 5012-5023, 2000
90. **Vaccarino F.M., Schwartz M.L., Raballo R., Rhee J., Lyn-Cook R.,** Fibroblast growth factor signaling regulates growth and morphogenesis at multiple steps during brain development, *Curr. Top. Dev. Biol*., **46**: 179-200, 1999
91. **Gritti A., Frolichsthal-Schoeller P., Galli R., Parati E.A., Cova L., Pagano S.F., Bjornson C.R., Vescovi A.L.,** Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain, *J. Neurosci*., **19**: 3287-3297, 1999
92. **Tropepe V., Sibilia M., Ciruna B.G., Rossant J., Wagner E.F., van der Kooy D.,** Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon, *Dev. Biol*., **208**: 166-188, 1999
93. **Lee J.C., Mayer-Proschel M., Rao M.S.,** Gliogenesis in the central nervous system, *Glia*, **30**: 105-121, 2000
94. **Briscoe J., Ericson J.,** The specification of neuronal identity by graded Sonic Hedgehog signalling, *Semin. Cell Dev. Biol*., **10**: 353-362, 1999
95. **Zhu G., Mehler M.F., Zhao J., Yu Yung S., Kessler J.A.,** Sonic hedgehog and BMP2 exert opposing actions on proliferation and differentiation of embryonic neural progenitor cells, *Dev. Biol*., **215**: 118-129, 1999
96. **Sieber-Blum M.,** Growth factor synergism and antagonism in early neural crest development, *Biochem Cell Biol*., **76**: 1039-1050, 1998
97. **Andsberg G., Kokaia Z., Bjorklund A., Lindvall O., Martinez-Serrano A.,** Amelioration of ischaemia-induced neuronal death in the rat striatum by NGF-secreting neural stem cells, *Eur. J. Neurosci*., **10**: 2026-2036, 1998
98. **Martinez-Serrano A., Bjorklund A.,** Ex vivo nerve growth factor gene transfer to the basal forebrain in presymptomatic middle-aged rats prevents the development of cholinergic neuron atrophy and cognitive impairment during aging, *Proc. Natl. Acad. Sci. U S A*, **95**: 1858-1863, 1998
99. **Pencea V., Bingaman K.D., Freedman L.J., Luskin M.B.,** Neurogenesis in the subventricular zone and rostral migratory stream of the neonatal and adult primate forebrain, *Exp. Neurol*., **172**: 1-16, 2001
100. **Akerud P., Canals J.M., Snyder E.Y., Arenas E.,** Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease, *J. Neurosci*., **21**: 8108-8118, 2001
101. **Hayflick L.,** Human cells and aging, *Sci. Am*., **218**: 32-37, 1968
102. **Kruger G.M., Morrison S.J.,** Brain repair by endogenous progenitors, *Cell*, **110**: 399-402, 2002
103. **Harley C.B., Futcher A.B., Greider C.W.,** Telomeres shorten during ageing of human fibroblasts, *Nature*, **345**: 458-460, 1990
104. **Bodnar A.G., Ouellette M., Frolkis M., Holt S.E., Chiu C.P., Morin G.B., Harley C.B., Shay J.W., Lichtsteiner S., Wright W.E.,** Extension of life-span by introduction of telomerase into normal human cells, *Science*, **279**: 349-352, 1998
105. **Allsopp R.C., Weissman I.L.,** Replicative senescence of hematopoietic stem cells during serial

transplantation: does telomere shortening play a role?, *Oncogene*, **21**: 3270-3273, 2002

106. **Greenberg R.A., Allsopp R.C., Chin L., Morin G.B., DePinho R.A.,** Expression of mouse telomerase reverse transcriptase during development, differentiation and proliferation, *Oncogene*, **16**: 1723-1730, 1998
107. **Martin-Rivera L., Herrera E., Albar J.P., Blasco M.A.,** Expression of mouse telomerase catalytic subunit in embryos and adult tissues, *Proc. Natl. Acad. Sci. U S A*, **95**: 10471-10476, 1998
108. **Prowse K.R., Greider C.W.,** Developmental and tissue-specific regulation of mouse telomerase and telomere length, *Proc. Natl. Acad. Sci. U S A*, **92**: 4818-4822, 1995
109. **Blasco M.A., Funk W., Villeponteau B., Greider C.W.,** Functional characterization and developmental regulation of mouse telomerase RNA, *Science*, **269**: 1267-1270, 1995
110. **Haik S., Gauthier L.R., Granotier C., Peyrin J.M., Lages C.S., Dormont D., Boussin F.D.,** Fibroblast growth factor 2 up regulates telomerase activity in neural precursor cells, *Oncogene*, **19**: 2957-2966, 2000
111. **Vaziri H., Dragowska W., Allsopp R.C., Thomas T.E., Harley C.B., Lansdorp P.M.,** Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age, *Proc. Natl. Acad. Sci. U S A*, **91**: 9857-9860, 1994
112. **Lindsey J., McGill N.I., Lindsey L.A., Green D.K., Cooke H.J.,** In vivo loss of telomeric repeats with age in humans, *Mutat. Res*., **256**: 45-48, 1991
113. **Wyllie F.S., Jones C.J., Skinner J.W., Haughton M.F., Wallis C., Wynford-Thomas D., Faragher R.G., Kipling D.,** Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts, *Nat. Genet*., **24**: 16-17, 2000
114. **Faragher R.G., Kipling D.,** How might replicative senescence contribute to human ageing?, *Bioessays*, **20**: 985-991, 1998
115. **Campisi J.,** Cancer, aging and cellular senescence, *In Vivo*. **14**: 183-188, 2000
116. **Goldstein S.,** Replicative senescence: the human fibroblast comes of age, *Science*, **249**: 1129-1133, 1990
117. **Lee H.W., Blasco M.A., Gottlieb G.J., Horner J.W., 2nd, Greider C.W., DePinho R.A.,** Essential role of mouse telomerase in highly proliferative organs, *Nature*, **392**: 569-574, 1998
118. **Rudolph K.L., Chang S., Lee H.W., Blasco M., Gottlieb G.J., Greider C., DePinho R.A.,** Longevity, stress response, and cancer in aging telomerase-deficient mice, *Cell*, **96**: 701-712, 1999
119. **Oikawa S., Tada-Oikawa S., Kawanishi S.,** Site-specific DNA damage at the GGG sequence by UVA involves acceleration of telomere shortening, *Biochemistry*, **40**: 4763-4768, 2001
120. **Petersen S., Saretzki G., von Zglinicki T.,** Preferential accumulation of single-stranded regions in telomeres of human fibroblasts, *Exp. Cell Res*., **239**: 152-160, 1998
121. **Henle E.S., Han Z., Tang N., Rai P., Luo Y., Linn S.,** Sequence-specific DNA cleavage by Fe2+-mediated fenton reactions has possible biological implications, *J. Biol. Chem*., **274**: 962-971, 1999
122. **Sitte N., Merker K., Von Zglinicki T., Grune T., Davies K.J.,** Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part I--effects of proliferative senescence, *Faseb J*., **14**: 2495-2502, 2000
123. **von Zglinicki T., Pilger R., Sitte N.,** Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts, *Free Radic. Biol. Med*., **28**: 64-74, 2000
124. **Samani N.J., Boultby R., Butler R., Thompson J.R., Goodall A.H.,** Telomere shortening in atherosclerosis, *Lancet*, **358**: 472-473, 2001
125. **von Zglinicki T.,** Oxidative stress shortens telomeres, *Trends Biochem. Sci*., **27**: 339-344, 2002
126. **Bryan T.M., Englezou A., Dalla-Pozza L., Dunham M.A., Reddel R.R.,** Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines, *Nat. Med*., **3**: 1271-1274, 1997
127. **Shay J.W., Gazdar A.F.,** Telomerase in the early detection of cancer, *J. Clin. Pathol*., **50**: 106-109, 1997
128. **Bryan T.M., Englezou A., Gupta J., Bacchetti S., Reddel R.R.,** Telomere elongation in immortal human cells without detectable telomerase activity, *EMBO J*., **14**: 4240-4248, 1995
129. **Gobbel G.T., Bellinzona M., Vogt A.R., Gupta N., Fike J.R., Chan P.H.,** Response of postmitotic neurons to X-irradiation: implications for the role of DNA damage in neuronal apoptosis, *J. Neurosci*., **18**: 147-155, 1998
130. **Yoshikawa K.,** Cell cycle regulators in neural stem cells and postmitotic neurons, *Neurosci. Res*., **37**: 1-14, 2000
131. **Edgar B.,** Diversification of cell cycle controls in developing embryos, *Curr. Opin. Cell Biol*., **7**: 815-824, 1995

132. **Callaghan D.A., Dong L., Callaghan S.M., Hou Y.X., Dagnino L., Slack R.S.,** Neural precursor cells differentiating in the absence of Rb exhibit delayed terminal mitosis and deregulated E2F 1 and 3 activity, *Dev. Biol*., **207**: 257-270, 1999
133. **Weinberg R.A.,** The retinoblastoma protein and cell cycle control, *Cell*, **81**: 323-330, 1995
134. **Lois A.F., Cooper L.T., Geng Y., Nobori T., Carson D.,** Expression of the p16 and p15 cyclin-dependent kinase inhibitors in lymphocyte activation and neuronal differentiation, *Cancer Res*., **55**: 4010-4013, 1995
135. **Park D.S., Morris E.J., Padmanabhan J., Shelanski M.L., Geller H.M., Greene L.A.,** Cyclin-dependent kinases participate in death of neurons evoked by DNA-damaging agents, *J. Cell Biol*., **143**: 457-467, 1998
136. **Arendt T.,** Alzheimer's disease as a loss of differentiation control in a subset of neurons that retain immature features in the adult brain, *Neurobiol. Aging*, **21**: 783-796, 2000