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Neuroreplacement therapy and stem cell biology under disease conditions

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Abstract. Recent advances in stem cell technology are expanding our ability to replace a variety of cells throughout the body. In the past, neurological diseases caused by the degeneration of neuronal cells were considered incurable because of a long-held 'truism'; neurons do not regenerate during adulthood. However, this statement has been challenged, and we have now found much evidence that the brain is indeed capable of regenerating neurons after maturing. Based on this new concept, researchers have shown neural differentiation of stem cells and recovery of function following transplantation of these cells into the brain. These results may promise a bright future for clinical applications of stem cell strategies in neurological diseases; however, we must consider the pathophysiological environments of individual diseases that may affect stem cell biology. Before we begin to develop clinical applications, we must consider environmental factors that have not been discussed in the current preclinical studies. Here, we study cases of Alzheimer's disease and schizophrenia and discuss the effects of environmental factors under disease conditions.

Key words. Stem cells; Alzheimer's disease; schizophrenia; transplantation; amyloid-β precursor protein; reelin.

Introduction

The discovery of multipotent neural stem cells (NSCs) in the adult brain [1, 2] has brought revolutionary changes in the theory of neurogenesis, which currently posits that regeneration of neurons can occur throughout life, opening a door for the development of novel therapies to treat neurological diseases by neuronal regeneration using stem cell transplantation. While pluripotent stem cells, such as embryonic stem cells, can develop into any of the three major tissue types [endoderm (interior gut lining), mesoderm (muscle, bone, blood) or ectoderm (epidermal tissues and nervous system)], the ability of multipotent stem cells to become a variety of cells is limited by their individual characteristics. These include their partial commitment to a specific differentiation pattern. Each stem cell contains distinctive information that would allow it to become a discreet type of cell in a tissue-specific environment. Neural stem cells, capable of spontaneously differentiating into neurons and glia, are the most promising candidates for neuroreplacement therapies.

Neural stem cells have been isolated from the embryonic and adult mammalian [3, 4] as well as the human [5] central nervous system (CNS) and propagated in vitro in a variety of culture systems [6]. Although the ideal positive selection marker has not yet been discovered, phenotypical CD133+, CD34– and CD45– cells from human fetal brain tissue seem to possess the ability to differentiate into neural cells [7].

Successful stem cell transplantation is dependent on the ability of these cells to migrate and to integrate into the host CNS. Hence, the source and culture conditions of stem cells are crucial factors. An inability to grow NSCs

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in vitro in the absence of complex and undefined biological fluids (i.e., serums) has long been a major obstacle in understanding the biology of these cells. Now, however, NSCs can be maintained or expanded in serum-free defined media containing the basic fibroblast growth factor (bFGF) and the epidermal growth factor (EGF) [6, 8, 9]. Although human NSCs (HNSCs) are reported to express nearly undetectable telomerase after a certain number of passages than their rodent counterparts [10], some researchers have been able to continuously expand these cells in vitro and differentiate them into βIII-tubulin and glial fibrillary acidic protein (GFAP)-immunopositive cells without the addition of any factors [8], suggesting a spontaneous differentiation of HNSCs into neurons or astrocytes. This ability to expand multipotent HNSCs in vitro and to produce neural cells under defined conditions offers a well-characterized and replicable source of transplantable cell types for neuroreplacement therapies.

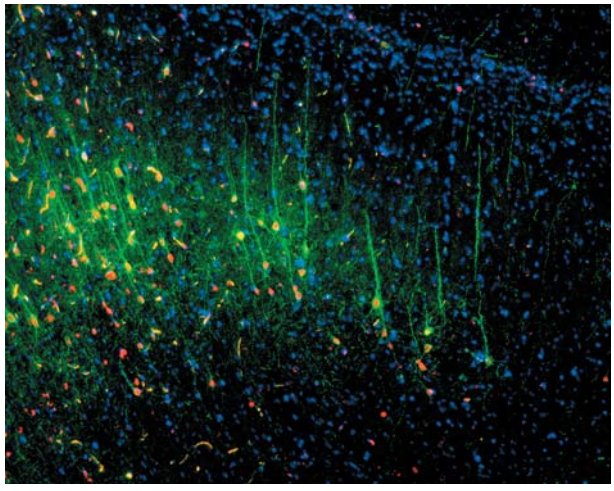

Figure 1. Typical fluorescent immunohistochemical pictures in the aged rat brain 30 days after HNSC transplantation. HNSCs migrated into the cortex and differentiated into neurons as indicated by the βIII-tubulin (green)-positive cells, which have morphologies typical of pyramidal cells in layers IV and V of the cingulate and parietal cortices. Apical dendrites were pointed toward the edge of the cortex.

Functional differentiation of HNSCs after transplantation into the brain

The successful transplantation of HNSCs into aged rats with subsequent improvement of cognitive function reported by Qu et al. [11] reinforces the potential feasibility of HNSC transplantation therapy. In this study, HNSCs expanded without differentiation under the influence of mitogenic factors in supplemented serum-free media and labeled by the incorporation of bromodeoxyuridine (BrdU) into nucleus DNA, were injected into the lateral ventricle of mature (6-month-old) and aged (24-month-old) rats. Cognitive function of the animals was assessed by the Morris water maze both before and four weeks after the transplantation of HNSCs. Before HNSC transplantation, some aged animals (aged memory-unimpaired animals) cognitively functioned in the range of the mature animals, while others (aged memory-impaired animals) functioned entirely below the cognitive range of the mature animals. After HNSC transplantation, most aged animals had cognitive function in the range of the mature animals. Strikingly, one of the aged memory-impaired animals showed dramatic improvement in behavior, functioning even better than the mature animals. Statistical analysis showed that cognitive function was significantly improved in both mature and aged memory-impaired animals. These behavioral results show the beneficial effects of HNSC transplantation into the host brain in most animals tested. After the second water-maze task, postmortem brains were further analyzed by immunohistochemistry for βIII-tubulin and GFAP, markers for neurons and astrocytes, respectively. There was no sign of ventricular distortion, no evidence of tumor formation, and further, no strong host anti-graft immunoreactivity was observed. Intensely and extensively stained with III-tubulin, neurons with BrdU-positive nuclei were found in the bilateral cingulate and parietal cortices and in the hippocampus. The βIII-tubulin-positive neurons found in the cerebral cortex were typified by a dendrite pointing toward the edge of the cortex (fig.1). In the hippocampus, donor-derived neurons exhibited multiple morphologies varying in cellular size and shape in one or more processes and in branching.

Recently, Englund et al. reported that by using patch-clamp techniques, grafted neural progenitors can differentiate into morphologically mature pyramidal projection neurons, establish appropriate long-distance axonal projections, exhibit normal electrophysiological properties, and become functionally integrated into host cortical circuitry [12]. These neuronally differentiated cells established corticothalamic and contralateral hippocampal connections, respectively, as revealed by retrograde tracing. Whole-cell patch-clamp recordings from grafted cells with morphological characteristics of pyramidal neurons showed that they were able to generate action potentials and that they received functional excitatory and inhibitory synaptic inputs from neighboring cells. Although these authors used the immortalized neural progenitor cell line RN33B prelabeled with GFP by using a lentiviral vector, the cells they studied displayed morphological features of cortical or hippocampal pyramidal neurons that were quite similar to the neuronally differentiated HNSCs in Qu‘s study. Thus, it is clear that NSCs differentiated into region-specific neurons in the adult brain can develop the physiological properties of mature neurons and can become functionally integrated into host neural circuitry.

Considerations for neuroreplacement therapies

Even following Qu's study [11], many researchers continue to experiment by initially differentiating stem cells into certain types of neuronal cells in vitro and by then transplanting these cells into the target area of brain tissues [13]. This approach seems to be natural because it is quite similar in concept to the transplantation of fetal dopaminergic tissue into basal ganglia of Parkinson's patients; however, we must consider several crucial issues regarding the so-called 'natural' approach. First, fully differentiated neuronal cells do not migrate; thus they cannot be integrated into the host brain. Second, a 'cure' for neurodegenerative disease is conceivable only if we replace degenerating neurons in the brain. For example, dopaminergic neurons transplanted into the basal ganglia may not be functionally regulated by the host brain and may cause side effects [14]. Third, if an injection is made into the brain, concomitant tissue destruction will cause monocyte recruitment, and the ensuing immune response will eliminate the transplanted cells. To avoid these problems, I suggest injecting undifferentiated stem cells directly into brain ventricles.

Carvey et al. [15] isolated midbrain precursor cells that respond to a combination of interleukin-1, interleukin-11, leukemia inhibitory factor, and glial cell line-derived neurotrophic factors with a tyrosine hydroxylase-immunoreactive phenotype. The clone selected for the highest percentage of tyrosine hydroxylase-immunoreactive phenotypes expressed several phenotypic characteristics of dopaminergic neurons and also nuclear receptor-related-1 (Nurr1). Transplantation of these cells into the striatum of a rat model of Parkinson's disease provides significant functional benefits, perhaps even to the same extent as transplantation of freshly harvested embryonic dopaminergic neurons, indicating that clonally expanded mesencephalic progenitor cells can be differentiated into functional dopaminergic neurons. Although the cloning step is tedious and NSC proliferation may not be rapid enough to use this technology in clinical applications, transplantation of undifferentiated stem cells that are already committed to the target cells may be advantageous.

Another question would be to examine the specific methods by which the transplanted cells migrate to the target area. Aleksandrova et al. described the ability of NSCs to extensively and site-specifically migrate [16]. Furthermore, there are reports that NSCs injected into the ventricle were extensively transported by cerebrospinal fluid (CSF), survived, and notably, migrated into neural tissues [17–19]. Since NSCs are reported to migrate into damaged areas of the brain [20, 21], dying cells may trigger an environmental cue for the migration of stem cells. In addition, neurons are continuously dying in neurodegenerative diseases; therefore, we may not need to consider the development of a special technology to guide the migration of stem cells directly to the brain area affected by a specific pathology. In summary, undifferentiated stem cell transplantation may be able to overcome the problems associated with terminally differentiated cells, since transplanted cells may migrate to their proper positions and be incorporated into the host brain without further alterations.

HNSCs may be the most promising candidates for neuroreplacement therapy; however, ethical issues and the risk of immunological rejection limit their value. Tissue rejection may not be particularly problematic in neuroreplacement strategies since the brain does not produce a significant level of immune response unless traumatic damage has occurred. Nonetheless, if the brain's immune system is activated or if monocytes or other immune-responsive cells are recruited to brain tissue under disease conditions, heterologous transplantation may pose significant problems. Although alterations in the character or personality of an individual have not been considered an important issue with fetal neural transplants in Parkinson's disease, some patients may have psychological difficulties accepting brain tissue from outside sources. Thus, an ideal biological source of cells for replacement therapies would be autologous transplantation of stem cells derived from the patient's own tissues.

Alternative sources of stem cells for neuroregeneration

Some researchers are trying to find an autologous transplantable cell source in embryonic stem (ES) cells isolated from the inner cell mass of the human embryo or of blastocysts [22]. ES cells proliferate extensively and, theoretically, can differentiate into any type of somatic cell. On the other hand, we must develop methods of guiding the differentiation of cells of interest, because ES cells do not have the information necessary to become the specific cell type that may be needed in individual cases. Recently, McKay et al. reported that a highly enriched population of midbrain NSCs can be derived from mouse ES cells [23]. They reported that dopamine neurons generated by these stem cells show the electrophysiological and behavioral properties expected of neurons from the midbrain, encouraging us to consider the use of ES cells in cell replacement therapy for Parkinson's disease. These authors focused on the fact that midbrain precursor cells express Nurr1, which is a transcription factor with a role in the differentiation of midbrain precursors into dopamine neurons. Further, they established a stable Nurr1-expression ES cell line using cytomegalovirus plasmid processed by the five-stage method [24] that leads to an efficient differentiation of ES cells into neurons. They found that the majority of ES cells differentiated into tyrosine hydroxylase-positive cells in vitro.

Then they grafted these terminally differentiated ES cells into the rat striatum after 6-hydroxy dopamine lesioning. The animals that received grafts of the ES cell-derived dopaminergic neurons showed functional recovery in amphetamine-induced rotation behavior and also showed that the transplanted cells responded to electrical stimuli, similar to host striatal neurons. Although these results are promising for neuroreplacement strategies using ES cells for treatment of neurological diseases, long-term safety and efficacy issues need to be addressed. For example, ES cells are not committed to become neural cells as NSCs are, and without that commitment, unwanted cells may be developed along with midbrain dopamine neurons. In addition, tumor formation is a problem associated with ES cell grafting [25]. Even if we were able to completely control the cell fate of ES cells and the ethical issues regarding the use of human embryonic tissue were resolved, the problem of tissue-specific epigenetic modifications would have to be overcome before an autologous cell therapy using ES cells could be developed. Cloning by nuclear transfer is an inefficient process in which most clones die before birth and survivors often display growth abnormalities [26]. This may be due to the tissue-specific DNA methylation patterns from the somatic nuclei used in cloning [27]. Thus, cloned ES cells may also receive tissue-specific epigenetic modifications and may not fully function as neural cells.

Bone marrow contains stemlike cells used not only for hematopoiesis but also for production of a variety of nonhematopoietic tissues. A subset of stromal cells in bone marrow, which has been referred to as mesenchymal stem cells (MeSCs), is capable of producing multiple mesenchymal cell lineages, including bone, cartilage, fat, tendons, and other connective tissues [28–31]. Recent reports show that human MeSCs (hMeSCs) also have the ability to differentiate into a diverse family of cell types, that may include muscle and hepatocytes, that may be unrelated to their phenotypical embryonic origin including muscle and hepatocytes [32–37]. Since hMeSCs can be isolated from a patient‘s bone marrow, these cells would be good candidates for autologous transplantation material. Although the potential therapeutic use of hMeSCs in the CNS has been discussed [38, 39] and several in vivo transplantation studies showed neural and glial differentiation of hMeSCs [40–44], technologies to induce neural lineage from hMeSCs are not fully established. Adult stem cells continue to possess some multipotency, but cell types produced from adult stem cells are limited by their tissue-specific character. To overcome this barrier of stem cell lineage, alterations are necessary. However, the regulatory mechanisms of tissue-specific stem cell fate decisions remain unclear, and differentiation of MeSCs into neural cells may require alteration of their epigenetic information before transplantation. Verfaillie et al. recently identified multipotent progenitor cells that copurify with MeSCs in adult bone marrow [45]. They hypothesized that these cells contribute to most, if not all, somatic cell types, suggesting that this subpopulation of hMeSCs may be primarily responsible for neural differentiation. Other issues associated with MeSCs are the inability to grow the cells in vitro without serum and the absence of positive selection markers for definitive isolation of these cells. Thus, neuroreplacement therapy by MeSC transplantation must clear some hurdles before it can be considered for clinical use.

Particular issues for stem cell therapy for Alzheimer's disease

Researchers have succeeded in recovering brain function in adult animal models by transplantation of stem cells, indicating the existence of a regulatory mechanism for stem cell biology in the adult brain. Nonetheless, these studies did not take into account the effect of pathological changes that may occur in the diseased brain and that may prevent the normal differentiation or migration of stem cells. In thinking about neuroreplacement therapy for Alzheimer's disease (AD), we must consider several relevant issues.

In AD, memory deterioration involves the degeneration of basal forebrain (BF) cholinergic neurons, making them the specific neuron population that should be replaced. Long projections and the expression of nerve growth factor receptors of these cells make cholinergic neurons phenotypically different from dopaminergic neurons, leading some researchers to declare BF cholinergic neurons irreplaceable. However, it has been shown that BF cholinergic neurons transplanted into the striatum and the nucleus basalis of Meynert (NBM, which provides major cholinergic input to the neocortex) of the adult rat brain not only survived but also expressed the cholinergic phenotype [46]. HNSCs transplanted into nonneurogenic areas of the adult brain also displayed the cholinergic phenotype in a region-specific manner [47]. Furthermore, engrafted cholinergic-rich (but not noncholinergic) cell suspensions reversed the deficits in radial-arm maze performance previously caused by NBM excitotoxic lesions [48] in an animal model. Although these results hint that it may be possible to replace BF cholinergic neurons, it will be necessary to show that degenerating BF cholinergic neurons in AD can be replaced by HNSC transplantation.

Amyloid-β peptide (Aβ), which is heavily deposited in the brains of AD patients, is derived from the amyloid-β precursor protein (APP) as a result of cleavage by β- and γ-secretases. APP is a type I integral-membrane protein that spans the endoplasmic reticulum, Golgi apparatus, and cell surface. Furthermore, APP undergoes proteolytic processing. Cleavage by β-secretase occurs in the extra-

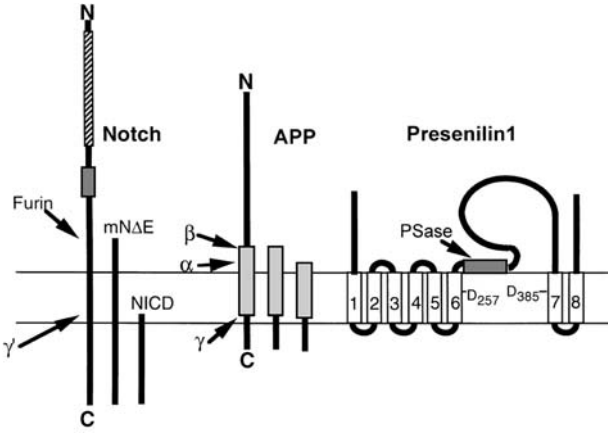

Figure 2. Amyloid-β peptide (Aβ), which is heavily deposited in the brains of AD patients, is derived from APP as a result of the cleavage of APP by β- and γ-secretases. β-Secretase cleavage occurs in the extracellular domain, producing a soluble ectodomain and a membrane-associated carboxy-terminal fragment. Heterogeneous γ-secretase catalyzes an intramembranous cleavage of this membrane-associated fragment, resulting in generation of Aβ and production of a C-terminal fragment of APP. α-Secretase cleaves in the middle of Aβ and prevents Aβ deposition.

cellular domain, producing a soluble membrane-associated carboxy-terminal ectodomain fragment, and heterogeneous γ-secretase catalyzes an intramembranous cleavage of this fragment, resulting in the generation of Aβ and the production of a C-terminal fragment of APP (fig. 2). The prevalence of Aβ neurotoxicity theory in AD pathology and the absence of a phenotype in the APP knockout mouse tend to limit our focus on the physiological functions of APP. Previous studies have shown that APP may be involved in neurite outgrowth [49, 50], cell proliferation [51–53], neuronal migration [54] and neuronal differentiation [55]. APP expression is increased by brain injury [56, 57], and amyloidogenic secretions increase in apoptotic cells [58]. APP may also be involved in cell survival [59–61]. Although these facts may indicate the involvement of APP in neuroplasticity, the physiological functions of APP are not clear.

We have found [62] evidence that APP signaling is one of the regulatory systems involved in the differentiation of NSCs and that the overexpression of APP caused glial rather than neural differentiation of these cells, indicating that the pathological alteration of APP metabolism in AD induces glial differentiation of neural stem cells and leads to the exhaustion of the stem cell population, which may be an important function in the ongoing neurogenesis of the adult brain. Furthermore, even if NSCs are transplanted into the brain of a patient with AD, under pathological conditions these cells may differentiate into astrocytes rather than efficiently produce neurons.

APP function in stem cell biology

We have previously shown that in serum-free unsupplemented media, HNSCs grown as neurospheres migrate and differentiate into neural cells [8], suggesting that HNSCs are capable of producing endogenous factors necessary for their own differentiation in vitro. During the early stages of serum-free differentiation, we observed that differentiating HNSCs appear to reach out to nearby apoptotic cells, that HNSCs initially become apoptotic under serum-free differentiation conditions [63, 64], and that these cells subsequently express migration and/or differentiation factors to influence the fate of neighboring cells.

While many factors are released following apoptotic cell death, we found that APP expression is elevated in apoptotic cells. Damaged neurons and neurons committed to apoptosis demonstrate signals strongly immunopositive for APP [65, 66]. Moreover, amyloidogenic fragments produced from APP are reported to be released into the extracellular space from neuronal cells under serum-deprived conditions [67]. The expression of APP is also reported to increase during retinoic acid-induced neuronal differentiation [68]. The mRNA expression of β-amyloid precursor-like proteins (APLP-1 and APLP-2) is also up-regulated during retinoic acid-induced differentiation of human SH-SY5Y neuroblastoma cells [69]. The increase in APP expression levels during neuronal differentiation in various cell culture systems suggests an important cellular function for APP during the differentiation process.

APP is also known to be up-regulated during the developmental stage [70] and after brain damage [57], events that involve migration and differentiation of NSCs. Together with our findings, these facts indicate that one of the physiological functions of APP may be the regulation of NSC biology to allow for the successful formation and replacement of crucial developing structures and neuronal circuits. A possible scenario to reconstruct neuronal circuits under the guidance of NSCs could be that sAPP released from damaged or dying cells may preferentially induce glial differentiation in a population of NSCs. These NSC-derived glial cells could then produce factors that may support surrounding damaged cells [71] and promote neuronal migration and differentiation of other NSCs in this area. It is possible that under normal physiological conditions, APP is necessary to recover from brain damage.

We have found evidence that glial differentiation is induced by the addition of sAPP or the overexpression of APP by gene transfection to HNSCs [62]. Recently, Bahn et al. reported that stem cells from subjects with Down's syndrome (DS) differentiated into astrocytes rather than into neurons [72]. Since DS patients have inherited three copies of APP on chromosome 21, this abnormal differen-

tiation may be the result of an overdose of APP [73]. In addition to characteristic physical manifestations, DS patients often exhibit early-onset AD. Arai et al. suggested that APP plays a role in neuronal development and that the earlier appearance of AD in adult DS patients is associated with an abnormal regeneration process related to aging [74]. Bondolfi et al. also found that aged transgenic APP mice exhibit neuronal loss and extensive gliogenesis in the neocortex [75]. Thus, in cases of familial AD and DS, the increased levels of APP fragments produced in the brains of these subjects may modify the biological equilibrium of HNSCs in such a way that a pathological shift in the proper ratio of neurons and glia brings about a premature or glial differentiation of HNSCs, thereby exhausting the HNSC population. Since the effective natural replacement of degenerating neurons in the adult brain during aging or disease processes may be important in maintaining normal brain function, malfunction of neuroreplacement by NSCs would pose serious problems.

It is proposed that we attempt to control sAPP levels in the brain to develop an NSC-transplantation therapy for AD, because a therapy that does not control these levels may not be effective in an environment where APP metabolism is altered because of excessive gliogenesis. Incidentally, this approach raises the question of whether Aβ immunization, which may also reduce APP fragments, is helpful in maintaining stem cell function in AD. Our opinion is that Aβ immunization is not helpful because it may not be able to properly control APP levels, which would result in the elimination of APP function in the brain. In our study, HNSCs transplanted into APP knockout mice [76] did not migrate or effectively differentiate into neurons in the cerebral cortex [62], indicating that there were insufficient environmental factors to properly guide the migration and differentiation of HNSCs in APP knockout mice and, further, that environmental or secreted APP may be an important factor in regulating the cell fate and migration of HNSCs in vivo. Since HNSCs may play an important role in neuroregeneration, and if APP is indeed involved in the regulation of HNSCs, as we propose, destruction of the APP system may jeopardize the maintenance of brain function.

Following some early findings of APP mutations in familial AD (FAD) [77, 78], it was subsequently well recognized that the total number of FAD cases was far greater than could be accounted for by mutations in the APP gene, which led to investigations into other genes. Mutations of the presenilin-1 (PS1) gene located on chromosome 14 were found to be present in about 70% of FAD cases [79]. Additionally, a mutation of presenilin-2, which is located on chromosome 1 and was identified by its homology to PS1, has been reported [80]. Dominant mutations in either of these PS genes appear to increase the amount of Aβ fragments in vitro [81] and in vivo [82]. In recent studies, mice with a null allele of PS1 were reported to display selectively lower γ-secretase activity [83]. These observations indicate that PS either regulates the activity of γ-secretase or is itself a component of γ-secretase. PS1-deficient mice also show developmental abnormalities and reduced NSC populations, presumably caused by premature differentiation of these cells consistent with altered Notch signaling [84]. Furthermore, genetic interactions between the Notch homologues glp-1 and lin-12 and the PS homologues sel-12 and hop-1 in *Caenorhabditis elegans* [82] indicate the involvement of presenilins in the Notch signaling pathway. Notch is involved in critical intercellular signaling in a wide array of developmental processes that control the patterning of tissues. As an embryo develops, progenitor cells must differentiate and acquire distinct characteristics, a process that involves communication and coordination among the emerging cells. The Notch family of genes encodes large proteins that contain segments in the membranes of these cells. These proteins act as receptors for extracellular ligands that specify cell fate, leading to tissue organization during development. Notch undergoes proteolytic processing similar to the β- and γ-secretase cleavages of APP (fig. 2). Signaling through Notch requires a ligand-induced cleavage occurring in the transmembrane domain that releases the Notch intracellular domain, which translocates to the nucleus, modifies the transcription of the target genes and regulates the differentiation of NSCs [85]. Thus, if a deficiency of adult neurogenesis is a factor in AD pathology, the role of FAD-linked PS mutations in the regulation of NSC biology should also be considered.

Reelin deficiency and stem cell biology

Twin studies indicate polygenic transmission and prenatal epigenetic vulnerability in schizophrenia [86]. Petronis et al. suggest the etiological importance of environmental factors, inherited and acquired epigenetic defects, and epimutations in schizophrenia [87]. Although protein and gene expression levels of reelin have been reported to be decreased in patients with schizophrenia and autism [88–91], the mechanistic relationship between the pathology of this disease and the down-regulation of reelin is not clear.

Reelin is a large extracellular matrix (ECM) protein of approximately 400 kDa [92] that binds to the α3 subunit of integrin receptors expressed on neuronal cell surfaces [91, 93] on very low density lipoprotein receptors (VLDLRs) and on apolipoprotein E receptor 2 (ApoER2) [94–96], triggering the adaptor function of the disabled-1 (Dab-1) cytosolic protein [93, 97] (fig. 3). The clustering of integrin receptor subunits following reelin binding activates a tyrosine kinase (focal adhesion kinase) to phosphorylate Dab-1. This phosphorylated Dab-1 binds

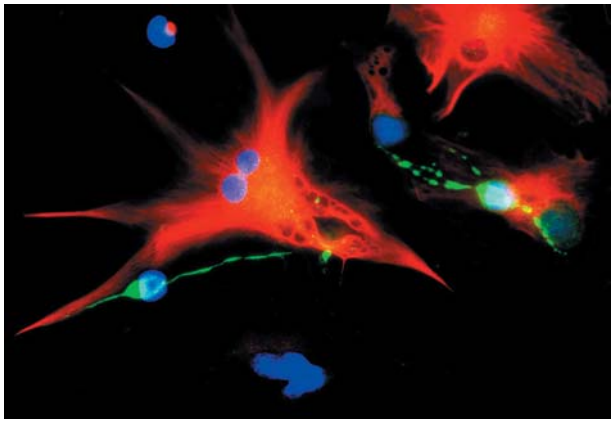

Figure 3. Reelin is a large extracellular matrix (ECM) protein that binds to the $\alpha 3$ subunit of integrin receptors expressed on neuronal cell surfaces and to a very low density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2), triggering the adaptor function of the disabled-1 (Dab-1) cytosolic protein. The clustering of integrin receptor subunits following reelin binding activates a tyrosine kinase (focal adhesion kinase) to phosphorylate Dab-1. This phosphorylated Dab-1 binds and transports soluble tyrosine kinases and transcription factors to functional cellular compartments.

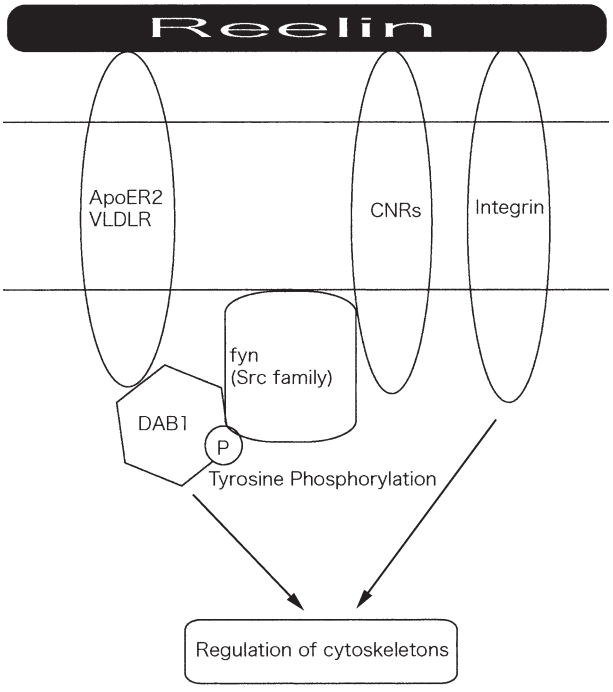

Figure 4. Human neural stem cells express reelin immunoreactivity in neuronal morphological cells after differentiation in vitro.

and transports soluble tyrosine kinases and transcription factors to functional cellular compartments [98]. In the null reeler mouse, migrating neurons fail to penetrate the subcortical plate, perhaps because of a deficiency of serine protease activity associated with reelin [99]. While much attention is focused on the role of reelin during corticogenesis, the existence of reelin expression in several neuronal populations of the adult brain [100] may point to other important functions for this protein. Studies have suggested that in addition to its essential role during neuronal migration, the reelin pathway may have a role in neuroplasticity.

Neurogenesis, including corticogenesis, is also dependent on NSCs in the fetal brain. Since similar NSCs exist in the anterior subventricular zone (SVZ) and in the hippocampal dentate gyrus of the adult brain [2, 101, 102], a regulatory system for NSC migration, similar to the system found in the fetal brain, may also exist in the adult brain.

We have observed that, in vitro, neuronal cells migrate along the processes of astrocytes, which are morphologically similar to radial glia. This finding suggests that HNSCs may express factor(s) to self-regulate migration. We observed an association of donor-derived radial glia-like astrocytes and neuronal processes in our HNSC transplantation study [11]. Host glial cells are reported to respond to transplantation of NSCs [103] and lesions [104] and also to become radial glia-type cells. These facts indicate that mechanisms for neural migration are similar to corticogenesis during the embryonic developmental process.

We found evidence that human NSCs express reelin (fig. 4), $\alpha 3$-integrin subunits, and DAB-1 and that they are able to physically respond to exogenous recombinant reelin in vitro. The addition of recombinant reelin to differentiating HNSC cultures with a defined medium caused an immediate retraction of their processes and migration of the cells back to the neurospheroid. We also observed that reelin-positive NSCs could migrate after transplantation into the brain of the reeler mouse, which has a deficit in functional reelin expression due to a mutation in the reelin gene, while other NSCs did not [18]. In this study, HNSCs transplanted to reeler homozygous mice did not migrate, while HNSCs transplanted to wild-type mice showed symmetrical migration and incorporation into the host brain. The only cells that migrated in reeler homozygous mice were reelin-positive HNSCs. Since transplantation studies indicate poor stem cell migration patterns in reeler mice, we hypothesized a similar inefficient migration pattern for endogenous stem cells. We found a significantly reduced level of proliferating cells in the hippocampus of both homozygous and heterozygous reeler mice compared to that of wild-type mice, whereas stem cell populations in SVZ of homozygous and heterozygous reeler mice were preserved, indicating that the proliferation of stem cells was not affected. We also found that stem cell migration from the SVZ to the hippocampus was dramatically diminished when reelin was not present, indicating that reelin may be involved in the migrational process of stem cells and that a deficit in this process may be related to modest neuronal loss in the reeler brain [105] or to the loss of γ-aminobu-

tyric acid-ergic (GABAergic) interneurons in schizophrenia [106].

The mechanism of reelin function in the regulation of NSC migration may not only depend on the activation of the reelin receptor complex but may also affect the serine protease activity of reelin. Quattrocchi et al. reported that reelin expression in HEK 293T cells impairs their ability to adhere to a fibronectin-coated dish surface [99], presumably as a result of excess serine protease activity. These reelin functions may also explain the immediate retraction of HNSCs back into the sphere by the addition of recombinant reelin to the culture, as well as the migration mechanisms of NSCs in the adult brain. ECM proteins, which are required for the adherence of the cells to the culture flask or to brain tissue, may be digested by the serine protease activity of reelin. In addition, NSC motility is increased by the activation of the reelin receptor complex. If our proposed mechanism of reelin function proves resonable, it could suggest that transplantation of stem cells into subjects with low levels of reelin may fail to develop efficient neuronal replacement in the brain because these transplanted cells would have failed to migrate into their proper place. Since we have found that reelin-positive NSCs still continue to migrate in the reeler mouse brain, elevating the expression levels of reelin by gene transfection may be an effective technique. It is not clear whether adult neurogenesis is essential for normal brain function. Furthermore, these results indicate that in patients with a deficit in reelin, NSCs may not be able to migrate or to make the appropriate neural connections. Although the rate of endogenous neuroregeneration in the mature brain may be minimal, a defect in this process might pose significant problems in the maintenance of normal brain function.

Deficit in olfactory sensory function and stem cell biology

Olfactory sensory neurons readily degenerate because of their vulnerability to toxic substances in the environment and are replenished continuously from a population of NSCs at the base of the olfactory epithelium. It has been shown that sensory neurons produced in the olfactory epithelium of adults retain the ability to differentiate and establish synaptic contact with target cells in the mature olfactory bulb [107]. Stem cells originating from the SVZ are known to migrate into the olfactory system [108] (fig. 5). Furthermore, these NSCs migrate into the hippocampus [109] and other parts of the brain [9], and the migration of these cells may be important for proper maintenance of brain function.

It is well established that the olfactory sensory pathway is pathologically affected in AD, and it has been suggested that the deficits seen in this pathology may serve as a tool to differentiate AD from other types of dementia [110]. Severe loss (as much as 75%) of anterior olfactory nuclei neurons in early-onset AD has been reported [111]. Significant impairment in olfactory function has been reported in AD and DS patients [112]. Even normal control patients who tested positive for the APOE4 allele, a known risk factor for AD, showed impaired odor identification compared with allele-negative subjects [113]. The deficit in olfactory sensory function in AD may be due to the reduction of the NSC population in the SVZ by premature differentiation, perhaps caused by pathological APP metabolism in this disease. Olfactory dysfunction in patients with schizophrenia has also been a topic of increasing interest, with deficits in identification, detection threshold sensitivity, discrimination, and memory of odors being reported [114, 115]. Since significant reduction of olfactory bulb volume in patients with schizophrenia is also reported [116], and mature olfactory bulbs

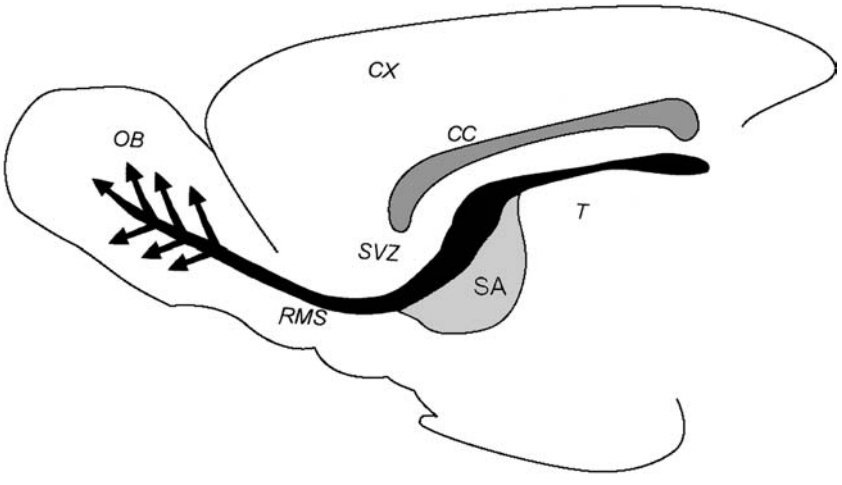

Figure 5. Stem cells originating from the subventricular zone are known to migrate into the olfactory system.

express very high levels of reelin [100, 117], the reduced migration of NSCs from the SVZ to the olfactory bulb because of a reelin deficit may also be responsible for the deficit of olfactory sensitivity in schizophrenia.

Although further studies are required, impairment of olfactory function as observed in neurological diseases may be linked to deficits in adult neurogenesis.

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