Stem cell therapy for Alzheimer’s disease and related disorders: current status and future perspectives

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Underlying cognitive declines in Alzheimer’s disease (AD) are the result of neuron and neuronal process losses due to a wide range of factors. To date, all efforts to develop therapies that target specific AD-related pathways have failed in late-stage human trials. As a result, an emerging consensus in the field is that treatment of AD patients with currently available drug candidates might come too late, likely as a result of significant neuronal loss in the brain. In this regard, cell-replacement therapies, such as human embryonic stem cell- or induced pluripotent stem cell-derived neural cells, hold potential for treating AD patients. With the advent of stem cell technologies and the ability to transform these cells into different types of central nervous system neurons and glial cells, some success in stem cell therapy has been reported in animal models of AD. However, many more steps remain before stem cell therapies will be clinically feasible for AD and related disorders in humans. In this regard, cell-replacement therapies, such as human embryonic stem cell (ESC)- or induced pluripotent stem cell (iPSC)-derived neural cells, hold potential for treating AD patients who may be beyond the help of pharmacological therapies. We will briefly review the current state of research in AD pathogenesis and new stem cell technologies. Additionally, the potential challenges and strategies for using cell-based therapies for AD and related disorders will be discussed.

INTRODUCTION

Alzheimer’s disease (AD) is clinically characterized by progressive loss of memory and other cognitive functions. Typically, several years pass between the initial onset of symptoms and eventual death. AD is estimated to have cost the US $172 billion and the world $604 billion in 2010 alone. These costs are staggering in light of predictions that the number of AD cases worldwide, currently estimated at 36 million, will triple by 2050. Therefore, there is a pressing need to identify novel mechanisms and develop new therapeutic strategies for AD. The complexity and multifactorial nature of AD poses unique challenges for pathogenic studies and therapeutic developments. Efforts to target AD-related pathways have shown promise in animal studies only to fail during human trials. An emerging consensus in the field is that treatment of AD patients with currently available drug candidates comes too late, likely as a result of significant neuronal loss in the brain. In this regard, cell-replacement therapies, such as human embryonic stem cell (ESC)- or induced pluripotent stem cell (iPSC)-derived neural cells, hold potential for treating AD patients who may be beyond the help of pharmacological therapies. We will briefly review the current state of research in AD pathogenesis and new stem cell technologies. Additionally, the potential challenges and strategies for using cell-based therapies for AD and related disorders will be discussed. We will also highlight recent studies that have obtained or developed promising cell types that could be used to defeat this devastating disease in the future.

ADVANCEMENT OF RESEARCH IN AD PATHOGENESIS

Genetics of AD pathogenesis

It is well known that the brains of AD patients accumulate two types of classically misfolded proteins. The first is amyloid-beta (Aβ), which is the pathological cleavage product of the amyloid precursor protein (APP). Aβ accumulates into plaques and smaller oligomers. Mutations in APP or in proteins involved in APP processing are well documented as being linked to inherited familial AD, an early-onset autosomal-dominant form of the disease that begins before the age of 65 years but only accounts for <2% of all AD cases. Many of the failed drugs in clinical trials directly or indirectly target this pathway with small molecules or antibody therapies to decrease Aβ production or promote Aβ clearance. The second of the misfolded proteins in AD is tau, a microtubule-associated protein that accumulates intracellularly as neurofibrillary tangles, a pathological feature that most closely correlates with...
cognitive decline in AD. However, mutations in tau usually cause frontotemporal dementia but not AD.

The vast majority (>98%) of AD cases, which do not involve mutations in genes of APP-processing pathways, are sporadic with onset beginning over the age of 65 years. For this population, the strongest predictor of developing AD, aside from age, is the genetic risk factor apolipoprotein (apo) E4. Each individual carries two copies of the apoE gene that exists in three allelic forms, e2, e3 and e4, that encode three corresponding isoforms: apoE2, apoE3 and apoE4, respectively. Importantly, apoE4 carriers make up 60–75% of AD cases although those individuals only represent approximately 25% of the normal population. AD patients with apoE4 have a younger age of disease onset relative to non-carrier patients. All well-conducted genome-wide association studies on late-onset AD from different populations around the world have identified, with extremely high confidence, apoE4 as the top late-onset AD gene. Remarkably, the lifetime risk estimate of developing AD for individuals with two copies of the apoE4 allele (approximately 2% of the population) is approximately 60% by the age of 85 years and for those with one copy of the apoE4 allele (approximately 25% of the population), approximately 30%. In comparison, the lifetime risk of AD for those with two copies of the apoE3 allele is approximately 10% by the age of 85 years. Thus apoE4 should be considered a major gene with semi-dominant inheritance for late-onset AD. Interestingly, carriers of apoE2, the rarest isoform, have a decreased risk for developing AD compared with homozygous carriers of apoE3. Genome-wide association studies also identified other genes that modulate the risk of late-onset AD, including CLU, CRI, PICALM, BIN1, SORL1, GAB2, ABCA7, MS4A4/MS4A6E, CD2AP, CD33, EPHA1 and HLA-DRB1. However, the relative contribution of these genes to AD is modest compared with apoE4.

Aβ and AD pathogenesis

Diverse lines of evidence suggest that APP and Aβ contribute causally to the pathogenesis of early-onset familial AD, although to what extent they also contribute to late-onset sporadic AD is still unclear. Overexpression of APP in humans through duplication of its gene or trisomy of chromosome 21, which harbors the APP gene, causes early-onset AD, whereas partial trisomy 21 excluding the APP gene does not. The catalytic subunit of the γ-secretase protein complex, involved in releasing Aβ peptides from its precursor, is formed by presenilin 1 (PS1) or PS2. Autosomal-dominant mutations in APP, PS1 or PS2 that alter APP processing and the production or self-aggregation of Aβ also cause early-onset AD. Neuronal expression of mutant human APP (hAPP), either alone or in combination with mutant PS1 in transgenic rodents, causes several AD-like alterations. Aβ also causes synaptic dysfunction and other neuronal impairments when added to acute brain slices or primary neuronal cultures. Biochemical and animal studies have suggested that insoluble Aβ fibrils found in amyloid plaques and monomeric Aβ are less pathogenic than soluble, nonfibrillar assemblies of Aβ, such as Aβ dimers, trimers and larger oligomers. How exactly the different Aβ assemblies cause synaptic and neuronal dysfunction has been a topic of intense study and debate. They may act inside or outside the cells and engage proteins as well as lipids.

ApoE4 and AD pathogenesis

Emerging evidence suggests that apoE4 has both Aβ-dependent and -independent roles in AD pathogenesis. In vivo, apoE is associated with amyloid plaques, and in vitro, it can form complexes with Aβ peptides. Studies in apoE-deficient mice expressing mutant hAPP demonstrate that apoE is actually required for the formation of fibrillar amyloid plaques. Interestingly, decreasing apoE’s lipidation status by knocking out ATP-binding cassette transporter A1 (ABCA1), a major regulator of cellular cholesterol and phospholipid homeostasis, in mutant hAPP mice significantly increases brain Aβ loads, whereas increasing apoE lipidation status by overexpressing ABCA1 decreases brain Aβ levels (for a review, see Kim et al.). Thus, altering apoE lipidation changes its ability to mediate Aβ clearance or deposition in the brain. Furthermore, in hAPP transgenic mice, human apoE stimulates Aβ clearance, and apoE2 and apoE3 clear more Aβ than apoE4, which may be related to apoE isoform-dependent effects on astroglial degradation of Aβ deposits. Microdialysis measurements of Aβ clearance rates in the brains of mutant hAPP transgenic mice expressing apoE3 or apoE4 reveal that apoE4 decreases Aβ clearance by approximately 40% compared with apoE3. Although apoE4 clearly increases Aβ accumulation and amyloid plaque formation in both humans and transgenic mouse models, it is still uncertain whether this process actually contributes to cognitive deficits in AD. As reported, plaque loads determined histopathologically or radiologically do not correlate well with cognitive impairments in humans. Furthermore, in the very oldest population (>90 years of age), the presence of apoE2 is associated with a reduced risk of dementia but an increased amyloid burden relative to apoE3.

Both Aβ and apoE4 cause inhibitory interneuron impairments, contributing to learning and memory deficits

The GABAergic system is important in shaping learning and memory, especially in the hippocampus, a critical structure for the encoding of new episodic memories and spatial learning and navigation. The dentate gyrus (DG), a subregion of the hippocampus, functions as a signaling gatekeeper between the entorhinal cortex and hippocampus in the processing of learning and memory tasks. Learning triggers rapid increases in inhibitory synaptogenesis and gamma-aminobutyric acid (GABA) content at inhibitory synapses, which accompanies enhanced synaptic inhibition of excitatory neurons. Spatial learning also triggers a lasting increase in GABA release from hippocampal GABAergic interneurons. Genetically enhancing GABAergic innervation in the DG of the hippocampus or increasing GABA levels by knocking down GABA transporter 1 improves learning and memory, whereas decreasing GABA levels by overexpressing GABA transporter 1 is detrimental. Furthermore, optogenetically...
inhibiting the activity of even a small population of GABAergic interneurons in the DG of the hippocampus impairs learning and memory. 33

Several lines of evidence suggest that Aβ regulates neuronal and synaptic activities and that accumulation of Aβ in the brain causes an intriguing combination of abnormally elevated network activity and synaptic depression. 11 Impairment of inhibitory interneurons and aberrant stimulation of glutamate receptors, which can result in excitotoxicity, appear to have important upstream roles in this pathogenic cascade. 2,11,34–36 Excessive neuronal activity might trigger a vicious positive feedback cycle by augmenting Aβ production, which is regulated, at least in part, by neuronal activity. This further destabilizes the network. 37

ApoE4 impairs GABAergic inhibitory interneurons, contributing to AD pathogenesis. 2 ApoE4 knock-in (KI) mice show an accelerated age-dependent decrease in hilar GABAergic interneurons, which correlates with the extent of apoE4-induced impairments of adult hippocampal neurogenesis and with learning and memory deficits. 38–40 Interestingly, the detrimental effect of apoE4 on GABAergic interneurons is cell autonomous, 41 which is important for potential stem cell transplantation therapy in AD patients with apoE4 (see below). In transgenic mice expressing neurotoxic apoE4 fragments, the loss of hilar interneurons is more pronounced and also correlates with learning and memory deficits. 38 Tau removal prevents these adverse effects but not when GABA signaling is blocked with a low dose of picrotoxin. 38 These findings strongly suggest that apoE4 causes age- and tau-dependent impairment of hilar GABAergic interneurons, leading to decreased neurogenesis in the hippocampus and learning and memory deficits. Recently, it has been reported that age-dependent hilar GABAergic interneuron impairment also correlates with learning and memory deficits in aged wild-type rats and mice. 42,43

Dysfunction of the GABAergic system may also contribute to cognitive impairment in humans. AD patients have decreased GABA and somatostatin (SST) levels in the brain and cerebral spinal fluid 44–48 and these alterations are more severe in apoE4 carriers. 49 ApoE4 is associated with increased brain activity during rest and in response to memory tasks, 50,51 possibly reflecting impaired GABAergic inhibitory control. With functional magnetic resonance imaging activation paradigms, mild cognitive impairment patients demonstrate hyperactivity in the medial temporal lobe; 52–55 and high-resolution functional magnetic resonance imaging indicates that hippocampal hyperactivity in mild cognitive impairment localizes to the DG/CA3 region of the hippocampus, 56 paralleling findings in mice with apoE4-induced GABAergic hypofunction in the DG. 38–41 Treatment of apoE4-KI mice with the GABA_A receptor potentiator pentobarbital or transplantation of mouse inhibitory neuron progenitors restores normal hippocampal activity and learning and memory, while blocking GABAergic signaling promotes the damaging effects of apoE4. 38,57 Likewise, reducing hippocampal hyperactivity with the antiepileptic levetiracetam improved cognition in patients with amnestic mild cognitive impairment and in a mouse model of AD. 38,59 These studies support the hypothesis that apoE4 contributes to AD pathogenesis, at least partially, by causing and exacerbating age-dependent impairment of GABAergic interneurons, leading to learning and memory deficits. 2

**STEM CELL-BASED THERAPIES IN ANIMAL MODELS OF AD**

As previously mentioned, multiple factors are involved in the pathogenesis of AD; these factors have not been successfully targeted by pharmaceutical or immunological agents. 2 With the advancement of stem cell technologies and the ability to generate different types of neuronal and glial cells from stem cells, there is hope for stem cell therapeutics as novel treatments for AD. Toward this goal, some success with stem cell therapies has been made in various animal models of AD as a proof-of-concept. 50–72

**Neural stem cell (NSC)-based therapies in animal models of AD**

The ability of multipotent NSCs to differentiate into a variety of cell types, such as neurons, astrocytes and oligodendrocytes, at transplantation sites is especially promising for cell-replacement therapy. NSCs can be derived from primary tissues, including fetal, postmortem neonatal or adult brain tissues, 72 or from ESCs and iPSCs. 4,73,74

In mouse models of AD, studies have shown that transplanted mouse NSCs differentiate into mature cell types within the brain and improve learning and memory. 75,76 One study shows improvement of cholinergic neuron number and memory in fimbria-fornix-transected AD rats after transplantation with rat NSCs. 77,78 However, it is not clear whether this is due to differentiation, maturation and integration of the transplanted NSCs or whether their secreted factors and signaling molecules stimulate cholinergic neurogenesis and/or prevent further loss. 70 Indeed, it has been shown that NSC grafts increase brain-derived neurotrophic factor levels and lead to behavioral rescue without changing Aβ or tau pathologies in a mutant hAPP-overexpressing mouse model of AD. 79 It seems that secretion of brain-derived neurotrophic factor from the transplanted NSCs is required for rescuing cognitive function in AD transgenic mice, because shRNA-mediated brain-derived neurotrophic factor knockdown abolishes the rescue. 63,79

Grafted NSCs can also be significantly influenced in their migration and differentiation by the microenvironment in recipient brains. For example, overexpression of hAPP causes grafts to yield more astrocytes rather than neurons. 80 Thus the pathogenic process of AD may have a negative impact on the therapeutic effect of NSC transplantation. On the contrary, nerve growth factors are thought to promote survival and differentiation of transplanted NSCs. NSCs stably transduced with human nerve growth factor genes survive and integrate into the cerebral cortex of AD rats upon transplantation and enhance cognitive performance; this survival and integration is not observed in the same rat model transplanted with NSCs without genetic modification. 64,81
Transplantation of NSCs is also used as a vehicle to deliver potential therapeutic agents, including neprilysin, insulin-degrading enzyme, plasmin and cathepsin B, to decrease Aβ levels in AD mouse models. It has been reported that fibroblast-delivered neprilysin reduces amyloid plaques in AD mice. Interestingly, delivery of the same gene by grafted NSCs leads to a more efficient reduction of amyloid plaques in mice. Thus, it is suggested that future NSC-based therapy in AD should focus on such indirect mechanisms, in lieu of primary neuronal replacement, for the delivery of neurotrophic factors.

GABAergic interneuron precursor-based therapies in animal models of AD

Cortical GABAergic interneurons are primarily produced in the embryonic medial ganglionic eminence (MGE). The MGE is a transient embryonic structure in the ventral telencephalon from which immature progenitors of cortical interneurons originate, migrate and distribute throughout the cortex and hippocampus via tangential migration into the radially developing brain. A number of studies demonstrate that this structure can be micro-dissected from developing rodent embryos and heterochronically transplanted into postnatal and adult animals. In these recipients, the transplanted MGE-derived interneuron progenitors migrate and integrate throughout recipient brains to alter ocular dominance plasticity or rescue models of stroke, anxiety, schizophrenia, Parkinson’s disease or epilepsy, which have been reviewed elsewhere.

Inhibitory interneuron impairments are a feature of both AD-related mouse models and human AD patients, and interneuron deficits seem to be a convergence point for apoE4 and Aβ mechanisms of the disease. To determine whether replacing lost cells could restore neuronal network function and behavior, we transplanted embryonic MGE-derived interneuron progenitors into the hippocampal hilus of aged apoE4-KI mice with or without Aβ accumulation. Despite the toxic environment created by apoE4 alone or in combination with Aβ, in both conditions, the transplanted cells developed into mature interneurons, functionally integrated into the hippocampal circuitry and rescued learning and memory. Because the progenitor cells, which expressed wild-type mouse apoE, survived and integrated equally well into apoE3-KI and apoE4-KI mice (including those with significant Aβ plaque buildup), we provide further support for the model that the detrimental effects of apoE4 are cell autonomous. This is important for potential stem cell-based therapy of AD in the future, indicating that transplanted human MGE-like cells without apoE4 expression or Aβ overproduction would have a good chance to survive and functionally integrate in the brains of AD patients.

These studies demonstrate that MGE cells possess attractive characteristics for possible cell-based therapeutics: high capacity of migration, autonomous integration, subtype inhibitory differentiation, and circuit-modulation. A key aspect of GABAergic interneurons is that one such inhibitory neuron could connect to, and thus influence, more than a thousand excitatory neurons. This suggests that the survival and functional integration of even small numbers of transplanted MGE cells could significantly improve learning and memory.

Derivation and transplantation of GABAergic inhibitory neuron precursors from PSCs

Murine MGE allograft transplantation studies are encouraging as proof-of-concept, but one of the ultimate goals for stem cell research is to develop human cell therapies. Correspondingly, one of the next steps for clinical translation would be to develop a reliable source of human MGE-like cells, particularly from PSCs, which could provide a potentially unlimited source of MGE cells for transplantation therapies of AD. Various protocols exist for the differentiation of mouse PSCs into cortical interneuron precursors. In one study, a mouse PSC line with an Lhx6-GFP reporter was differentiated into cells expressing both FoxG1 and NKX2.1 using a modified protocol for the generation of ventral telencephalic cells. By day 12 of differentiation, many of these cells express Lhx6 and possess a differentiation pattern similar to MGE-derived progenitors upon transplantation. Interestingly, the study shows a bias in differentiation of the transplanted MGE-like cells towards SST+ interneurons upon maturation, which is attributed to higher levels of sonic hedgehog (SHH) signaling. Following this study, an enhanced protocol was developed for the generation of mouse PSC-derived cortical inhibitory neurons by the forced expression of NKX2.1 that could eliminate the need for sustained SHH expression. Mouse PSC-derived cortical inhibitory neurons were shown to be able to replace those neurons lost due to pilocarpine administration; indeed, mice that received these MGE-like inhibitory progenitors doubled the density of GABAergic interneurons in the hilus relative to control animals. Taken together, these and other studies provide substantial evidence that PSCs can serve as a renewable source of cortical interneuron progenitors.

Differentiation protocols for human PSCs have also proved encouraging, as several groups report the derivation of cortical interneuron progenitors from both human ESCs (hESC) and iPSCs consistent with studies performed in mouse ESCs (mESCs), SHH signaling is also necessary for efficient patterning into MGE-like progenitors. A highly efficient method for generating MGE-like progenitors from hESCs (up to 93% NKX2.1+ without cell sorting) was developed, which relies on high concentrations of SHH. Upon transplantation, these human MGE-like cells mature into GABAergic interneurons as well as basal forebrain cholinergic neurons; in a mouse model depleted of these neuronal subtypes in the medial septum, the human cells restored short-term behavioral learning and memory deficits. Of note, the group reported no tumor formation in genetically immunodeficient mice transplanted with the hESC-derived MGE-like progenitors, likely due to the high purity of the differentiated cells and the absence of residual undifferentiated hESCs.

Some studies on the efficient production of human inhibitory forebrain neurons utilize strategies, such as an
intermediate MGE-progenitor state\textsuperscript{107} or a small-molecule-based strategy for the direct generation of forebrain inhibitory neurons.\textsuperscript{108} Nicholas et al.\textsuperscript{107} induced the differentiation of MGE-like progenitors from both ESCs and iPSCs into GABAergic interneurons with mature physiological properties, while Maroof et al.\textsuperscript{108} demonstrated the importance of SHH signaling for proper acquisition of the forebrain identity. Both studies display the ability of the transplanted PSC-derived neurons to survive, disperse from the injection site and integrate into mouse brains. Nicholas et al.\textsuperscript{108} also reports the absence of tumors from ESC-derived MGE cells in mice up to 7 months posttransplantation. Both studies emphasize an important facet of human development that is often difficult to mimic in \textit{in vitro} studies: a protracted timeline of neuronal maturation.\textsuperscript{110} Indeed, both studies found the neurons generated to be immature with no fast spiking interneurons or with limited integration upon transplantation.\textsuperscript{107,108} Further studies may be required to accelerate the generation of fully mature human GABAergic interneurons in order for these cells to be used in future AD therapies.

\textbf{CONCLUSIONS, CHALLENGES, AND PERSPECTIVES}

As discussed, cell-replacement therapies hold great potential for treating AD patients. With the advent of stem cell technologies and the ability to turn stem cells into different types of CNS neurons and glial cells, some success in stem cell therapy has been made in animal models of AD. Although these preclinical studies are promising, many more steps remain before stem cell therapies can be successfully used for the treatment of AD and related disorders.

NSCs and MGE-like inhibitory progenitors as candidates for stem cell-based therapies in AD

Requirements for neuronal-replacement therapy would entail the distribution of cells throughout the affected tissue by migration from the injection site while maintaining their intended identity, functional integration into or modulation of the circuits. This requires fewer cells to be transplanted and is not as demanding on the cell source or immediate volume that the circuitry. This requires fewer cells to be transplanted and is not as demanding on the cell source or immediate volume that the recipient tissue needs to accommodate; however, there is a legitimate concern about detrimental overgrowth in the form of tumors.\textsuperscript{62} Although it could be advantageous for cells to differentiate into multiple cell types or subtypes, randomly differentiating transplanted cells could introduce variability among patients and could prove deleterious in some cases.\textsuperscript{60,120,121}

\textbf{Variability of donor cells in stem cell-based therapies}

Although hESCs and iPSCs have provided researchers with powerful tools for testing cell-replacement therapies, there is still much to be learned about their unique properties and culture conditions. Variability among differently established ESC lines has long been reported.\textsuperscript{122,123} Because of non-standardized reprogramming methods and donor-to-donor variation, iPSCs in particular can also vary in their differentiation efficiencies and genetic backgrounds, which can affect downstream applications both for drug testing and transplantation. Comparisons of disease cases have often involved derivations of large numbers of patient-specific iPSC lines, which can be technically challenging and labor intensive. Thus more robust and efficient methods are needed to consistently derive a desired cell type, and each of those cell types needs more thorough characterization.

\textbf{Donor cell and patient compatibility and immune rejection of stem cell-based therapies}

Although the brain is considered to be ‘immune privileged,’ donor cells will have to be human leukocyte antigen haplotype-matched at the very least, and recipients would require some level of immunosuppression to prevent rejection of the transplanted cells. Ideally, there may exist the possibility of having patient-specific, isogenic genome-modified iPSC- or iNSC-derived cells when more reliable and efficient protocols are developed. Interestingly, in a transplant case of fetal
midbrain dopaminergic cells that survived in a Parkinson’s disease patient for over 14 years, only a 6-month-long daily regimen of cyclosporin A was sufficient for prolonged survival. Nevertheless, approaches to enhancing donor cell and patient compatibility and of suppressing immune rejection of transplanted cells are needed for future stem cell-based therapies in AD.

**Regulatory approval and path to clinical use**

Eventually, good manufacturing practices will need to be applied while handling all stages of transplantable cells for clinical use. Before making steps toward the clinic, if able to be manipulated in vitro, all grafted cells would ideally be transgenically equipped with a molecular ‘kill switch’ that could be easily activated in the event of adverse effects. Because AD can be a relatively slow-progressing disease, clinical trials will likely take many years to demonstrate success for cell therapies in halting or reversing disease progression. The safe and ethical future of stem cell therapies, especially for AD, will likely be slow, expensive and tightly controlled. However, due to the uniqueness of stem cell-based therapies, regulatory agents are needed to develop new regulatory policies to foster their appropriate development and success.

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