Alzheimer’s disease (AD) is a progressive neurological disorder characterized by the aggregation of two proteins, amyloid-β and hyperphosphorylated tau, and by neuronal and synaptic loss. Although some drugs have been shown to slow the progression of the disease, at present no treatment has been developed that can stop or reverse the progression of the pathology. Recently, new therapeutic strategies have been proposed for the treatment of the disease. Among these, the development of stem cells and gene-modified cells is an especially promising therapeutic approach for AD. In this review we highlight the experimental and preclinical studies that have been focused on stem cell-based and gene-modified cell-based uses as potential therapies for AD. The potential clinical applications are also discussed.

Introduction

Alzheimer’s disease (AD) is a neurodegenerative disease and the most common form of dementia. The disease is clinically characterized by a progressive impairment of memory and other cognitive functions that interfere with mood, reason, judgment, and language, leading to dementia and death [1]. An estimated 10% of Americans over the age 65 are diagnosed with AD, with an incidence of 50% in people aged 85 years or older [2]. In some cases, early-onset Alzheimer’s can occur between ages 40 and 50, with a mean life expectancy following diagnosis of approximately 7 years [3].

The earliest damage in AD occurs in the entorhinal cortex, hippocampus, and basal forebrain; these brain structures play a critical role in memory [4]. The pathophysiology of the disease involves the accumulation and aggregation of two proteins, amyloid-β (Aβ) and hypersphosphorylated tau, which are the principal components of extracellular plaques and intraneuronal neurofibrillary tangles [5]. Aβ is produced by the cleavage of amyloid precursor protein (APP), a transmembrane protein present in neurons [6]. APP is cleaved first by β-secretase and subsequently by γ-secretase, releasing the soluble Aβ peptide [7]. There are two forms of Aβ peptide, Aβ1–40, which is more common, and Aβ1–42, which is associated with early onset of AD [8].

The second hallmark of AD is the tau protein, a microtubule-associated protein (MAP) that plays a key role in microtubule stabilization [9]. In AD, the accumulation of Aβ and hyperphosphorylated tau affects the connection between certain groups of neurons that form synaptic partners, causing them to function improperly and eventually die [10]. The disruption of neuronal circuits and the synaptic loss in the human cortex and hippocampus contribute to the cognitive decline observed in AD [11] suggesting that the synaptic loss represents a critical event in AD pathology.

Currently, there are no drug treatments that can provide a cure for AD. However, different treatments have been developed that can improve symptoms or slow down the neuropathology [12,13]. The main types of drugs used to treat AD patients are cholinesterase inhibitors and NMDA receptor antagonists. Other forms of treatment that have been explored by preclinical researchers were immunotherapeutic strategies aimed to reduce Aβ and tau deposits [14–16]. Although these treatments reduce the progression of the disease, they do not provide a definite cure for AD. One possible treatment option that has been proposed is the use of stem cells and gene-modified cells in therapeutic strategies for AD and other neurodegenerative disorders.

This review describes the various types of stem cells and gene-modified cells that have been explored for potential treatments and reviews the recent experimental and preclinical studies performed on the subject.

Stem-Cell Therapy in AD

Embryonic stem cells for AD

Embryonic stem cells (ESCs) are derived from the inner cell mass of the developing blastocyst and they have the capacity to produce every type of cell and tissue in the body [17,18]. ESCs cannot serve direct treatment themselves because extrauterine ESCs transplantation commonly generates teratomas or teratocarcinomas and can also be rejected immunologically [19]. Nevertheless, ESCs can still be of use for cell replacement therapy after full differentiation toward cells such as neural stem cells (NSCs), mesenchymal stem cells (MSCs), or other types of cells.
Neural stem cells

The identification of NSCs created high expectations for clinical application of these cells in cell replacement therapeutic procedures of AD. NSCs are defined as self-renewing multipotent cells that can differentiate into neurons, astrocytes, and oligodendrocytes [20]. NSCs were first discovered in the subventricular zone (SVZ) and in the subgranular layer (SGL) of the hippocampal dentate gyrus in adult brains of mice [21]. NSCs produce neuroblasts that migrate from the SVZ to the olfactory bulb where they form mature neurons involved in the sense of smell [22]. NSCs from the SGL migrate and differentiate into neurons in the hippocampus, implying that neurogenesis in the SGL may be important for memory [23]. The impairment in memory and the deterioration in odor identification [24] often observed in AD patients might indicate that the development of the pathology affects neurogenesis. Even so, the fact that there are NSCs in the adult brain implies that inducing neurogenesis in the AD brain can be a potential strategy for cell replacement therapy [25].

NSCs can be directly isolated from fetal or adult nervous tissue or derived from ESCs [26,27]. Apart from being harvested from external sources, commercial NSC lines such as HB1.F3 are also of potential use [28] and have been explored as a potential therapeutic option for Parkinson’s disease [29] and AD [30].

There are abundant examples in which NSCs have been successfully used for cell-based therapies [31,32]. In one such example, presented by Qu et al. [33], the transplantation of NSCs into the lateral ventricle of aged and memory-impaired rats, improved their cognitive function. In addition, the authors showed that these transplanted NSCs have successfully differentiated into neurons and glia and migrated to different brain areas. Another example, performed by Blurton-Jones et al. [34], elucidated some of the factors needed for the proper integration of transplanted NSCs. In their study, they showed that NSCs that have been transplanted into the hippocampus of aged triple transgenic mice (3xTg-AD) rescued their spatial learning and memory deficits. In addition, the researchers observed a highly significant increase in axonal outgrowth and synaptic density with high levels of BDNF, leading them to discover that the NSC-derived BDNF is essential for the cognitive benefits induced by the NSC transplantation. These results are in accordance with other studies that have suggested that NSC therapy is most likely to be successful when used in conjunction with a continuous supply of trophic factors with the aid of genetically modified NSCs [35,36].

When grown in vitro, NSCs generate structures called neurospheres, which are nonadherent spherical clusters of cells [37]. These neurospheres can be directly transplanted into specific brain sites, leading to their utilization in AD. For example, Wang et al. [38] have transplanted neurospheres derived from mouse ESCs into the frontal cortex and barrel field of C57BL/6 mice after including a lesion of nucleus basalis of Meynert and have demonstrated that these transplanted neurospheres can differentiate into neurons that can form functional connections capable of improving motor and cognitive function in mice.

NSCs have also been used for the differentiation of specific types of neurons that have clinical applicability. Cholinergic neurons are one such type of neuronal cells, that are especially vulnerable in AD [39,40]. NSCs can be differentiated specifically toward cholinergic neurons and can serve as a source pool for these cells that might be advantageous for developing cell-based treatments for AD. Differentiation into cholinergic neurons can be achieved in vitro by the combination of basic fibroblast growth factor, heparin, and laminin [41] or by transplanting NSC cell lines in specific brain sites [42].

The effect of Aβ and APP on NSCs

Several articles have demonstrated both in vitro and in vivo that Aβ plays a role in the proliferation and differentiation of NSCs in AD [43–45]. It should be noted, however, that the form of Aβ (oligomeric or fibrillar) can produce contrasting results. A few studies described Aβ as having a disruptive effect on proliferation and migration of NSCs in the SVZ. In a study that used adult APPSwe single-transgenic mice, a decrease in hippocampal neurogenesis was observed [46]. Conversely, a study performed by Jin et al. [47] found an increase in proliferation and neuronal differentiation in young PDGF-APP (Sw, Ind) transgenic mice. These researches point that Aβ oligomers induce neurogenesis and that as pathology progresses, Aβ aggregates into fibrils and these fibrils lead to the apparent decrease in proliferation and cell survival.

These findings can reflect the expected implications when treating pathology at different stages. At a late stage of pathology the therapeutic reduction of Aβ aggregation could enhance endogenous cell proliferation and differentiation while an opposite effect may be received at an early stage. One way that has been demonstrated as capable of reducing Aβ aggregation is immunotherapy [48]. Biscoaro et al. [49], showed that Aβ immunotherapy using an antibody against Aβ decreased Aβ plaque burden and promoted survival of newly formed neurons in 8–9-months-old APP/PS1 mice, supporting the notion that a reduction in Aβ accumulation by immunotherapy can restore neuronal functions in the AD brain. Translating these findings into the clinical setting, however, has been so far unsuccessful – in a phase III clinical trial, for example, no efficacy was found when patients were treated with Bapineuzumab, an anti Aβ monoclonal antibody [50].

Apart from Aβ, it appears that alterations in APP may itself affect the faith of NSC differentiation. A study performed by Marutle et al. [51] showed that transplanted NSCs in APP23 transgenic mice exhibited a higher tendency toward glial differentiation when compared with wild-type mice. Similarly, in a previous in vitro study performed by researchers an increase in glia differentiation was noted when NSCs were grown in the presence of high concentrations of APP [52]. In addition, the researchers found that reduction of the APP levels by Phenserine, a cholinesterase inhibitor, led to an increase in the neuronal differentiation of NSCs. It was therefore proposed that a combination of NSC transplantation and a pharmacological approach to regulate APP levels may be a potential strategy for the treatment of AD and other neurological disorders [51].

MSCs for AD

MSCs are multipotent cells found in various tissues [53] that can differentiate in culture into osteoblasts, chondrocytes, adipocytes, fibroblasts, myoblasts, and cardiomyoblasts [54,55]. MSCs have also been demonstrated as capable of having the ability to differentiate in vitro and
in vivo into neuronal and glial cells [56,57], although certain cell modifications such as the expression of specific stem cell genes [58] or epigenetic modifications [59] need to be performed before these cells acquire this ability. MSCs have been considered as a potential therapeutic option because they are readily available in various tissues, are capable of differentiating into a variety of cell types, and can be easily grown in large numbers [60]. Additionally, MSCs can be used for autologous transplantation [61], circumventing the need for immunosuppressant.

MSCs from various sources have been used in different studies: bone marrow-derived MSCs were used by Lee et al. [62] for transplantation into the hippocampus of APP/PS1 mice. Transplantation led to endogenous microglia/macrophage activation. A reduction in Aβ depositing and tau hyperphosphorylation, and an improvement in spatial learning and memory was also observed. These effects were associated with the restoration of microglial neuroprotective function, as evidenced by increased Aβ-degrading factors, decreased levels of neurotoxic cytokines, and increased levels of neuroprotective cytokines.

Human umbilical cord-derived MSCs (hUCB-MSCs) have also been transplanted in an acute AD mouse model. Transplantation of these cells into double-transgenic mice (APP/PS1) reduced Aβ and tau deposition and improved spatial learning and memory decline. These effects were associated with a decrease in levels of inflammatory factors secreted by activated microglial cells [63,64]. Although a therapeutic potential certainly exists for these cells, there have been little comparative research between hUCB-MSCs and other types of MSCs for treatment efficacy in AD models. Future research will need to address this question.

**Induced pluripotent stem cells for AD**

The generation of induced pluripotent stem cells (iPSC) from somatic cells by Takahashi et al. [65] demonstrated that adult mammalian cells can be reprogrammed by the expression of a few embryonic genes. Yamanaka and Takahashi first identified four key genes known as “Yamanaka factors” that are essential for the reprogramming of adult cells—klf4, sox2, c-Myc, and Oct4. Concurrently, Yu and colleagues achieved similar results using oct4, sox2, nanog, and lin28 [66].

iPSC offer the ability of investigating the different phenotypes present in AD by generating pluripotent cell lines from patients with inherited forms of the disease, thus maintaining their endogenous genome and transcriptional feedbacks intact [67]. For example, Israel et al. [68] reprogrammed fibroblasts from patients with sporadic and familial AD into iPSC and differentiated them into neurons. These neurons showed similar functional activity and biochemical changes associated with the disease. Similar results have been achieved by other researchers [69,70]. A collection of fibroblast cell lines from patients with tau mutations is in fact available for the generation of iPSC with the same mutation, providing a new model for the study of alternative splicing in tau that occurs only in humans [71].

**Cell Therapy Using Astrocytes for AD**

AD treatment may be achieved by transplanting supportive cells such as astrocytes [72]. Astrocytes are responsible for maintaining an optimal environment for neurons by phagocytosis of cell debris [73], secretion of neurotrophic factors [74], uptake of Glutamate [75], and maintaining the balance and regulation of potassium and calcium ions [76]. Further, in vitro studies have shown that astrocytes can improve neuronal differentiation, maturation, and synapse formation in rodents [77]. However, similar studies using human astrocytes and human neurons demonstrated that human astrocytes do not enhance differentiation of human NSCs (hNSCs), although the addition of astrocytes or astrocyte-conditioned media enhanced the long-term in vitro survival of hNSC-derived cholinergic neurons [78].

Several studies have associated glial function and the development of AD pathology. One finding pointing to this interaction was the observation that in AD, a large number of activated astrocytes and microglia accumulate around Aβ deposits [79–81] signifying that these cells are involved in the clearance of Aβ deposits. Interestingly, cultured adult mouse astrocytes, but not neonatal mouse astrocytes, showed the ability to degrade Aβ deposits, although when transplanted into the hippocampus of APdE9 mice, both adult and neonatal mouse astrocytes showed Aβ clearance by phagocytosis [81]. Thus, it appears that astrocytes can play a role in the dynamics of pathology and that astrocyte transplantation may be an effective approach for the treatment of AD.

**Gene-Modified Cell-Based Therapy for AD**

Gene modification of stem cells prior to transplantation can be useful for increasing cell survival and making them more effective [82]. In addition, modified cells could be used for the delivery of factors that can ameliorate neurological disorders [83].

As previously mentioned, due to the loss of cholinergic neurotransmitters in AD, some researchers were interested in developing gene-modified cells that can produce acetylcholine (Ach). Primary fibroblast cell line genetically engineered to express choline acetyltransferase showed the capacity to produce Ach after transplantation into the hippocampus of rats [84].

Another example of the use of the facilitation of gene therapy for AD is the over expression of neprilysine (NEP), an Aβ degrading protease that has been shown to ameliorate extracellular amyloids [85]. Transgenic mice (APP/PS1) injected with lentiviral vector expressing NEP showed a reduction in Aβ deposits [86], and MSCs overexpressing the NEP gene demonstrated the ability to degrade Aβ peptides in vitro [87]. Similar results were obtained in vivo with transgenic mice that were transplanted with primary fibroblasts transfected with a lentivirus carrying NEP [88].

NSCs are also able to express growth factors that have been shown to improve memory and cell function in AD [34]. Human nerve growth factor (hNGF) is one such factor and has been shown to possess the ability to rescue cholinergic neurons in the rodent and primate brains, and enhancing cholinergic function of neurons [89,90]. Taking note of these findings, Wu et al. [36] showed that genetically modified NSCs expressing hNGF can integrate into host tissue and replace damaged or lost neuronal cells. In addition, a phase I clinical trial implantation of fibroblasts carrying the hNGF gene into the forebrain of eight AD patients showed improvement in the rate of cognitive decline [91].
phase II clinical trial for the efficacy of NGF delivery aided with adenovirus is currently ongoing [92].

Another growth factor relevant for cell-based therapy is BDNF. BDNF is produced and trafficked in several brain regions, effecting neuronal activity, function, and survival [93]. BDNF gene delivery administrated after the onset of the disease in mice and primates reversed synapses loss, improved cell signaling, and restored learning and memory [94]. Moreover, NSCs transplanted in a rat model with cholinergic neurons loss led to an increase in the number of cholinergic neurons, but the combined use of NSCs with BDNF demonstrated higher efficacy pertaining to spatial learning and memory [95].

Summary

Multiple studies have been done on AD. Although some drugs have been shown to slow the progression of the disease, at present no treatment has been developed that can stop or reverse the progression of the pathology. Different types of stem cells have been investigated for the treatment of AD, showing promising results in animal models and in vitro studies. Cell-based therapy using stem cells or gene-modified cells offers several advantages such as direct targeting of the pathology by incorporating new cells with existing cells or replacing existing functional or supportive cells. Treatment could be achieved even through a single injection, and innovative technologies have allowed generating different types of stem cells from various sources and inducing their differentiation to specific directions. Clinical applications are already in the process of being tested, showing some benefits. Although challenges such as cell survivability, immunorejection, and surgical procedures still need to be addressed, the use of autologous cells from patients for the generation of iPSC or harvesting autologous MSCs may circumvent some of these challenges.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Prof. Daniel Offen
Laboratory of Neuroscience
Sackler Faculty of Medicine
Felsenstein Medical Research Center
Rabin Medical Center
Campus Beilinson
Petah-Tikva 49100
Israel
E-mail: danioffen@gmail.com

Received for publication November 10, 2012
Accepted after revision January 11, 2013
Prepublished on Liebert Instant Online January 16, 2013