How close is the stem cell cure to the Alzheimer's disease

Future and beyond?☆

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Abstract

Alzheimer's disease, a progressive neurodegenerative illness, is the most common form of dementia. So far, there is neither an effective prevention nor a cure for Alzheimer's disease. In recent decades, stem cell therapy has been one of the most promising treatments for Alzheimer's disease patients. This article aims to summarize the current progress in the stem cell treatments for Alzheimer's disease from an experiment to a clinical research.

Key Words: Alzheimer's disease; stem cells; cell replacement; neurogenesis; neural regeneration; reviews

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INTRODUCTION

Neurodegeneration is known as the progressive loss of structure or function of neurons, including death of neurons[1]. Many neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease occur as a result of neurodegenerative processes. Regenerative therapy could be a promising approach as the regeneration of lost or altered cellular functions, which could significantly reverse functional decline to an extent that raises the patient's survival rate and physiological function.

As a representative of the neurodegenerative disorders, Alzheimer's disease (AD) is pathologically characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. Neurofibrillary tangles, and senile plaques involve the basal forebrain cholinergic system, amygdala, hippocampus, and cortical areas. Both amyloid plaques and neurofibrillary tangles are clearly visible in brains of AD patients. Neural loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus[2].

AD patients usually have many common symptoms. The National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) established the most commonly used criteria for AD[3]. According

to these criteria, the presence of cognitive impairments (learning & memory, language, perceptual skills, orientation), and a suspected dementia syndrome confirmed by neuropsychological testing are contributed to the clinical diagnosis of AD. The observable symptoms are often mistakenly thought to be "age-related" concerns, or manifestations of stress. In the early stage, the most common symptom is unable to acquire new memories, observed as difficulty in recalling recently observed events. When AD is suspected, the diagnosis is usually confirmed with behavioral assessments and cognitive tests, often followed by a brain scan if available. Nowadays, specific treatments for particular symptoms of AD are available. However, specific "disease-modifying" treatments aimed at preventing or reversing the basic pathophysiologic processes of AD still remain under investigation. In this literature, the recent research advances in stem cell treatments of AD are reviewed from an experiment to a clinical research.

STEM CELL STRATEGY

Currently, there is no proven cure for AD. In terms of drug therapy, available drugs can only improve cognitive symptoms temporarily. No drug treatment can reverse, stop, or even slow this inexorable neurodegenerative process[4]. Also, non-drug treatments, including gene therapy[5-6] and behavioral interventions[7-8],

can only bring temporary symptomatic relief and not result in halting the progression of these diseases. In recent decades, neurogenesis has been proved to exist in restricted regions of the adult brain in many kinds of species including humans[9]. This continuing neurogenesis in the subventricular zone, olfactory bulb, and hippocampal dentate gyrus is supported by the identification of neural stem cells (NSCs)[10], suggesting that the adult central nervous system (CNS) may be amenable to the cell intervention.

A variety of animal models have been developed to examine the etiology of AD and to determine the responses of endogenous and/or transplanted stem cells to the pathological microenvironment of the brain. Under many conditions, adult neurogenesis is impaired, and the dysfunctional neurogenesis, both decreased and increased, has been reported for AD transgenic models[11-13].

Increasing research evidences in recent decades may promise a bright future for stem cell based neuroreplacement therapies for AD. The adult-born neurons in the diseased brain seem to be a good candidate for those lost or apoptotic neurons. Because stem cells can be genetically modified in vitro and have high migratory capacity after transplantation into the brain, they can be an efficient way to delivery neurotrophic factors or enhance gene expression that can modify the course of the disease[14].

Endogenous neural progenitor/stem cells

Since neural progenitor/stem cells have been proven to exist in the adult CNS and to be involved in the neurogenesis process, the activation of endogenous neural progenitor/stem cells populations that can migrate to the injured regions, proliferate and functionally integrate into the existing circuit represents a significant strategy to promote neural regeneration in the diseased brain[15]. This activation within the brain is to protect the remaining tissues and prevent secondary neuron loss through the production of neurotrophic and neuroprotective factors, such as brain derived neuronal factor (BDNF) and vascular endothelial growth factor[16]. A recent research has demonstrated that the self-repair in the adult brain can be augmented by the infusion of growth factors to activate endogenous neural precursor cells that contribute to new tissue formation and functional recovery after stroke[17].

Currently, it is widely accepted that neurogenesis in the hippocampus are involved in learning and memory formation[18-19]. The hippocampus-dependent learning tasks can significantly increase the proliferation of endogenous neuronal progenitors, survival of new neurons and, the task performance by animals correlates positively with the amount of adult born neurons[20]. Alterations in the microenvironment where the endogenous NSCs reside play an important role in NSC activation. In AD brain, the pathogenesis causes drastic biological responses soon after the lesion. For example, immune responses include the activation of microglia

and astrocytes around the senile plaques area and T-lymphocyte infiltration into the damaged brain. These cells produce cytokines and other molecules that promote or inhibit the neurogenic function of the NSCs[21]. Therefore, it is possible for damaged cells to be replaced from endogenous NSC pools. However, the capacity of self-repair is obviously not enough. Studies have also suggested that the capacity of endogenous NSCs to compensate for lost cells is limited. In spite of the activated proliferation, NSCs become gliogenic rather than neurogenic[22]. Obviously, most of the new migrated neurons in and near the injured area die before differentiating into functional neurons, possibly because of a lack of factors and stimulation to support their survival and differentiation; thus, only 0.2% of the dead neurons are replaced[23].

However, it has not been determined whether these effects depend directly on the promotion of neuronal regeneration by NSCs, or whether accompanying events, such as enhanced glial regeneration and other types of trophic support, are more important. Moreover, a key issue in the field of neuronal regeneration is that newly generated neurons need to make the appropriate connections, although the details of this process are still largely unknown. Further studies are needed to clarify how newly generated neurons are associated with neurological improvement and to elucidate the comprehensive mechanism regulating the endogenous regeneration system.

Exogenous neural progenitor/stem cells

The success of isolation and easy gene-engineered modification of embryonic stem cells (ESCs) and NSCs *in vitro* profoundly provides researchers a promising tool to replace the "injured" neurons in the AD brain. Therefore, the cell transplantation strategy has given rise to hopes for clinical application of these *in vitro* produced neuronal cells in the cell replacement procedures for AD. Since chronic inflammation is a characteristic property of AD brain, transplantation of neuronal precursor cells (NPCs) has been proven to particularly inhibit ongoing inflammatory reactivity. Researchers have tested that the intrahippocampal transplantation of NPCs is effective in attenuating inflammatory responses and plays a neuroprotection role in beta-amyloid 42 (Aβ-42) peptide-injected rat hippocampus, indicating effects of NPCs transplantation in AD models are consistent with cellular actions to attenuate inflammatory reactivity[24]. The progressive degeneration of cholinergic neurons occurs in the forebrain cholinergic projection system especially in the nucleus basalis of Meynert. Moghadam *et al* [25] used ESCs-derived NPCs to treat Ibotenic acid-induced AD models in order to investigate the production of cholinergic neurons derived from engrafted cells. After transplantation, not only a significant behavioral improvement in memory deficits was observed, but also the majority (about 70%) of the NPCs retained neuronal phenotype and about 40% of them had a cholinergic cell phenotype with no tumor formation.

Similarly, in the Aβ-induced AD model, the transplantation of ESCs-derived NPCs into the injured hippocampus can also improve the memory dysfunction of the AD models[26]. Furthermore, the transplanted cells demonstrate characteristics of proper synapse formation between host and grafted neural cells[27]. Recently, another group reported the *in vivo* functional integration of the human ESCs-derived NSCs after cell transplantation. At 6 months after transplantation, human axons identified with the human-specific middle-weight neurofilament protein antibody were found inside the stratum radiatum. Alongside these projections, small patches of human synaptophysin immunoreactivity were detected, suggesting the formation of presynaptic terminals[28]. Hippocampal grafts placed in the dentate gyrus were projected to both the ipsilateral and contralateral pyramidal cell layers, while axons of donor neurons placed in the motor cortex extended *via* the external and internal capsules into the cervical spinal cord and *via* the corpus callosum into the contralateral cortex. Their data indicate that neurons derived from human pluripotent stem cells (PSCs) are endowed with a remarkable potential to establish orthotopic long-range projections in the adult mammalian brain. Meanwhile, in another mouse AD model, the transplantation of NSCs was reported to improve cognition function mediated by the neurotrophic factor BDNF[29].

The differentiation of transplanted NSCs in response to local cues in the brain suggests that environmental cues have the ability to direct the fate of stem cells to become the specific, terminally differentiated cells that are required to restore functions. Alternatively, stem cells are differentiated prior to transplantation and then directed to the correct areas through surgery. However, care should be taken with the characterization of these cells in regard to their multipotentiality and genetic stability with increasing passages in culture, as transformed cells may contribute to the formation of tumors[30].

Induced-PSCs (iPSCs)

Efforts to investigate the pathophysiology of human AD are hampered by the lack of genuine in-vitro models. Stem cells generated by induced direct reprogramming of adult somatic cells using are termed as iPSCs, offering paradigm shifting opportunities by providing specific/personalized models for studying AD, and personalized renewable source of cells for practical autologous cell therapies and regenerative medicine applications, that avoid immune rejection. The possibility of using iPSCs as a tool for development of such AD patient specific model systems, however, remains at best challenging.

In 2006, Takahashi *et al* [31] discovered that four transcription factors could reprogram mouse fibroblasts to a pluripotent state. Ever since then iPSCs have created excitement among researchers, as iPCs are more available, easier to make, and less ethically conflicted than ESCs. This has been widely regarded as a milestone advance in stem cell research, as it may allow researchers to obtain PSCs without the controversial use of embryos. Because iPSCs are developed from an adult somatic cell, it is believed that iPSCs can avoid immunogenic responses.

iPSCs are a specific cell type compromised by disease, even lost in patients, that can be recreated in culture. Furthermore, iPSCs have the potential to provide an unlimited source for any desired cell type. Ultimately, aside from being an exciting research tool to probe embryogenesis and disease pathogenesis, iPSCs are so-called "disease modeling" for drug screening, identifying novel drugs to treat diseases and patient-tailored cell therapy.

Most (90‒95%) of AD patients are sporadic populations (sAD) while 5‒10% patients are diagnosed as having early-onset AD, half of whom are familiar AD (fAD). fAD is caused by a mutation in at least one of three genes: presenilin 1/2 and amyloid precursor protein (APP)[32]. Obviously, iPSCs technology contributes to capture the genomes of AD patients and to generate live cellular models of both the fAD and sAD. These models allow us to identify the earliest events of AD and to investigate aspects of AD pathogenesis that are not replicated in animal models.

Yagi's group[33] recently pioneered to generate iPSCs from fibroblasts of fAD patients with mutations in presenilin 1 (A246E) and presenilin 2 (N141I), and characterized the differentiation of these cells into neurons. Their remarkable data showed that fAD-iPSC-differentiated neurons increased $A\beta_{42}$ secretion, recapitulating the molecular pathogenesis of mutant presenilins. Furthermore, $A\beta_{42}$ secreted from these neurons sharply responded to γ-secretase inhibitors and modulators, indicating the potential for identification and validation of candidate drugs. This finding significantly demonstrates that the fAD-iPSC-derived neuron is a valid model of AD and provides an innovative strategy for the study of age-related neurodegenerative diseases. Marchetto *et al* [34] in their study of Rett syndrome using iPSCs, reported the in vitro differentiation of iPSCs into neurons that contained glutamatergic synapses and were capable of generating spontaneous synaptic activity. The spontaneous synaptic activity observed in the differentiated neurons hinted that iPSC technology can be used to study not only human neurons but also patient-specific neural networks[35].

However, the utility of iPSCs-derived neurons remain unresolved. Previous research has reported the marked differences in differentiation propensity between PSC lines, even between iPSC lines generated from the same individual[36]. And furthermore, differentiation variability has become an important issue. This issue becomes more complex if this novel method is to investigate a disease with unclear developmental changes. Thus, it is unclear if iPSCs and iPSC-derived NPCs from AD patients would generate neurons differently than control cells, such as embryonic stem cells and neural stem

cells.

Despite some successes in animal models, iPSCs technology is not yet ready for human trials. The chief concern is safety[37]. Current iPSCs protocols cannot efficiently eliminate undifferentiated cells and, tend to be oncogenic and form teratomas, just like ESCs. Additionally, most patient-specific iPSCs have been generated using integrating vectors, which may not get silenced efficiently or can disrupt endogenous genes that are a potential impediment in human iPSCs therapy[38]. Other questions include lack of efficient targeting strategies to repair mutant alleles. Many mouse iPSCs harbor epigenetic abnormalities are reported in recent studies, which may develop genetic mutation on prolonged culture and continue to retain epigenetic memory of their donor cells[39]. In terms of the iPSCs technique, human patient adult cells-derived stem cells still face ethical and scientific hurdles. (1)Ethical hurdles: Undoubtedly, it turns out that iPSCs are not entirely problem-free. Researchers working with iPSCs still must countenance certain ethical concerns, and they may also face newly discovered scientific hurdles. Some of the ethical issues are not unique to the iPSCs. Scientists have been prohibited from introducing PSCs into human or nonhuman primate blastocysts. (2)Scientific hurdles: Whether iPSCs are truly equivalent to ESCs? Some studies have raised the possibility of significant differences. Lanza group compared differentiated cells derived from a series of iPSCs lines and ESCs lines, and found thatboth cells differentiated to form blood cells, vascular cells or retinal cells. However, the iPSCs did so at a significantly lower rate and had a higher rate of cell death[40]. Another study raised a question about whether iPSCs can serve as tools for modeling disease. Researchers compared ESCs with iPSCs that carried the mutation for the mental impairment disorder fragile X syndrome. They found that while the ESCs expressed the mutation, the iPSCs did not[41]. Some scientists hope that the retroviruses which these problems arise from can be used to generate iPSCs. Some teams are trying to explore virus-free modes of producing the cells[42-44].

PROSPECTIVE QUESTIONS

Although stem cell-based replacement strategies carried out in animal models have shown promising results, there are still many hurdles to overcome before these approaches can be translated into the AD patients. One major challenge is the development of a safe method to deliver stem cells to the injury region. In addition, the stage of differentiation of those cells needs careful consideration: fully differentiated cells are associated with a smaller efficiency due to poor viability, while undifferentiated cells present a higher risk of undirected differentiation and uncontrolled proliferation.

Adult neurogenesis is important for cellular therapy and physiopathology of the CNS, as for development and pharmacology of the adult brain. However, some studies are of controversies and remain to be confirmed. Hence, the role, contribution and significance of newborn neurons in the adult brain remain to be fully elucidated and understood. One of the main limitations in determining and understanding the role of newborn neurons in the adult brain is the use of bromodeoxyuridine (BrdU) for studying neurogenesis. BrdU is a thymidine analog that incorporates DNA of dividing cells. However, BrdU is not a marker for cell proliferation and neurogenesis. It is a marker for DNA synthesis. Furthermore, BrdU is a toxic and mutagenic substance. As such, BrdU labeling is not without pitfalls and limitations, when studying adult neurogenesis[45]. The microenvironment in which stem cells are placed also needs consideration, as local soluble factors are likely to affect differentiation events in the tissue[46]. Environmental factors need to be included: pro-inflammatory cytokines are associated with a negative effect on neural differentiation, while anti-inflammatory cytokines may have the opposite effect. The inflammatory status of the brain and the possible activation of inflammatory responses therefore need to be considered with cell replacement strategies[47]. In all, developing our understanding of the processes controlling the activation, migration and differentiation of stem cells will be a critical step towards the usage of stem cells for new regenerative therapies.

CONCLUSIONS

It has been a century since the first description of AD. In the past decades, tremendous advances in understanding of the molecular pathogenesis of AD have been springing out. However, no proven effective treatment is able to delay the onset or slow the progression of AD. It continues to rob millions of their memories and their lives. Now, many new therapies directly targeting the mechanisms underlying AD are now in the pipeline. A combination of psychosocial, behavioral, and pharmacologic strategies aims at slowing the process of AD and preserving quality of life for as long as possible. Until medical research discovers definitive disease modifying treatments for patients with AD, we must continue to maximize all available resources to provide the best possible individualized patient-centered and family care.

Despite a better understanding of the pathology of cognitive impairments and clinical features of AD, which have aided diagnosis and management of the disease, further work is required to improve screening and decrease the burden of care on healthcare systems and families. Furthermore, more research is needed to elucidate the mechanisms of the disease to enable new drug targets to be developed based on biological models, which may provide novel treatments for the prevention and management of AD.

The situation for neuronal replacement aiming at functional restoration in AD is extremely complex

because the stem cells have to be pre-differentiated in vitro to many different types of neuroblasts for subsequent implantation in a large number of brain areas. However, to give long-lasting symptomatic benefit, a cholinergic cell replacement approach will require intact target cells and host neurons that the new cholinergic neurons can act on. Stem cell-based cell replacement strategies are very far from clinical application in AD. But the neuroreplacement strategy will undoubtedly become more feasible as we advance our understanding of the pathogenesis of AD and foster creativity in research aiming to elucidate the physiological role of NSCs in the adult brain. Also, the NSCs indeed exist in the complexity and intricacy of the architecture of the human brain, the mechanisms and therapeutic potential of NSCs just need to be further explored. Challenges and hope always exist together.

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