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Stem cell Therapies in Alzheimer's disease: applications for disease modeling

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Abbreviations

AD	Alzheimer's disease
APP	amyloid precursor protein
ApoE	Apolipoprotein E
BDNF	brain-derived neurotrophic factor
CASS4	Cas scaffolding protein family member 4
CSF	Cerebrospinal fluid
EVs	extracellular vesicles

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FGF	fibroblast growth factor
IL	Interleukin
iNOS	Inducible nitric oxide synthase
iPSCs	Induced pluripotent stem cells
MSCs	Mesenchymal stem cells
NSCs	Neural stem cells
Oct4	Octamer-binding transcription factor 4
INPP5D	Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1
Sox2	sex determining region Y-box 2
TNF	Tumor necrosis factor
TREM2	triggering receptor expressed on myeloid cells 2

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease with complex pathological and biological characteristics. Extracellular β-amyloid deposits, such as senile plaques, and intracellular aggregation of hyperphosphorylated tau, such as neurofibrillary tangles, remain the main neuropathological criteria for the diagnosis of AD. There is currently no effective treatment for the disease, and many clinical trials have failed to prove any benefits of new therapeutics. More recently, there has been increasing interest in harnessing the potential of stem cell technologies for drug discovery, disease modeling, and cell therapies, which have been utilized to study an array of human conditions, including AD. The recently developed and optimized induced pluripotent stem cells (iPSCs) technology is a critical platform for screening anti-AD drugs and understanding mutations that modify AD. Neural stem cells (NSCs) transplantation has been investigated as a new therapeutic approach to treat neurodegenerative diseases. Mesenchymal stem cells (MSCs) also exhibit considerable excitement to treat neurodegenerative diseases by secreting growth factors and exosomes, attenuating neuroinflammation. This review highlights recent progress in stem cell research and the translational applications and challenges of iPSCs, NSCs, and MSCs as treatment strategies for AD. Even though these treatments are still in relative infancy, these developing stem cell technologies hold considerable promise to combat AD and other neurodegenerative disorders.

Keywords: Alzheimer's disease; stem cells

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Significance

Alzheimer's disease (AD) is a neurodegenerative disease that results in learning and memory defects. Although some drugs have been approved for AD treatment, only less than 20% of AD patients benefit from these drugs. Stem cell-based therapies, including iPSCs, NSCs, MSCs, provide promising therapeutic strategies for AD.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia, named first in 1906 by Alois Alzheimer. Currently, the presence of extracellular β-amyloid deposits, such as senile plaques, and the intracellular accumulation of hyperphosphorylated tau, such as neurofibrillary tangles, are still the main neuropathological criteria for the diagnosis of AD (Kent et al., 2020; Scearce-Levie et al., 2020). Early-onset AD emerges in patients younger than 65 years of age, accounting for less than 5% of all cases, and most cases of late-onset AD occur after the age of 65 (Sabayan and Sorond, 2017). Patients with AD will inevitably die within 5-12 years of the onset of AD symptoms (Bruni et al., 2020). The clinical manifestations of AD are progressive. Typical features are early neuroinflammation, learning and memory impairments, followed by complex attention, visuospatial function, executive function, praxis, language, gnosis, behavior, and/or social impairment (Scheltens et al., 2016; Arvanitakis et al., 2019; Lempriere, 2019). In recent years, although significant progress has been made in clarifying key aspects of the biology, the etiological mechanisms of AD are still far from being fully understood (Jafari et al., 2020; Kim et al., 2020). New treatment strategies and drugs attempting to slow or halt cognitive

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deficiency and neuronal loss of AD are proposed every year (Roberson and Mucke, 2006; Benek et al., 2020). The USA Food and Drug Administration has only approved five drugs for the clinical treatment of AD, and these include the cholinesterase inhibitors tacrine, galantamine, donepezil, rivastigmine, and the glutamate receptor antagonist memantine. However, these five pharmacological agents can only relieve symptoms without affecting the main pathological features of AD (Kumar et al., 2015; Stakos et al., 2020). In addition, the effects of these drugs vary from person to person; no more than 20% of patients have a moderate efficiency, while more than 60% of patients have tolerance, non-compliance, and side effects (Serretti et al., 2007; Zetterberg and Bendlin, 2020). Therefore, effective therapeutic strategies for AD are of great priority.

In the past years, there has been increasing interest in harnessing the potential of stem cell technology for drug discovery, disease modeling, and cell therapies (Mancuso et al., 2019; Lee et al., 2020; Yang et al., 2020). The most commonly used stem cell types in AD research are induced pluripotent stem cells (iPSCs), brain-derived neural stem cells (NSCs), and bone marrow mesenchymal stem cells (MSCs) (Yang et al., 2013; Chen et al., 2014; Penney et al., 2020). Stem cell-based therapies might be a better approach than traditional therapies, as it could reduce neuronal loss, increase synaptic connections, and improve the microenvironment in the brain fundamentally. The mechanisms of action (Figure 1) include 1. Replacement of injured or lost neuronal cells: stem cells can differentiate into cholinergic neurons, which could integrate with the host, repair neural circuits, and eventually replace the lost neurons

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(Telias and Ben-Yosef, 2015); 2. Secretion of neurotrophic factors: stem cells can secrete neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and fibroblast growth factor (FGF), to promote cell survival, increase synaptic connections, and improve cognitive function (Blurton-Jones et al., 2009); 3. Anti-amyloid protein production: stem cell transplantation reduces amyloid-beta (Aβ) levels and reduces Aβ toxic reactions, which is beneficial for the survival of transplanted cells and cognitive recovery (Bae et al., 2013); 4. Anti-inflammatory response: stem cell transplantation reduces the expression of proinflammatory factors interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS), and exerts neuroprotective effects (Lin et al., 2018); 5. Promote the activation of endogenous stem cells: transplantation of exogenous stem cells improves the microenvironment of brain, which facilitates the survival of endogenous stem cells and stimulates their activation (Philips and Robberecht, 2011); 6. Improve the metabolic activity of neurons in the brain: stem cell transplantation increases the connection and metabolism between neurons and improves cognitive function (Blurton-Jones et al., 2014). Progress in stem cell-based therapy provides us a new perspective for treating neurodegenerative disease, especially in AD. In this review, we underline some key insights into the disease mechanisms derived from studies of iPSCs, NSCs, and MSCs, discuss the pros and cons of these stem cell types as therapeutics. Additionally, we review new research to track stem cell therapy, highlight the most relevant stem cell trials in AD and other neurological disorders, and discuss the potential applications, as well as the major challenges and future

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directions in cell-replacement therapy of AD.

The pathogenesis of AD

AD is the most common neurodegenerative disorder causing dementia and is characterized by memory deficit and cognitive decline (Hampel et al., 2018; Ong et al., 2018). Early-onset familial AD (FAD) occurs in people aged 40-60, and sporadic late-onset AD (SAD) occurs after the age of 70 (Jeong, 2017). For the neuropathological diagnosis of AD, cerebrospinal fluid (CSF) or positron emission tomography (PET) imaging biomarkers can be used as surrogate markers for Aβ and tau deposition in brains (Brier et al., 2016; Leuzy et al., 2016). Studies of cognitive function and changes in CSF and neuroimaging biomarkers in FAD and SAD have determined that the disease is at a preclinical stage at least 10 to 20 years before the onset of clinical symptoms (Olsson et al., 2016; Tarawneh et al., 2016). The disease is characterized by the early deposition of Aβ in early-onset nerves and other cortical areas, including the default pattern network, followed by regional cortical hypometabolism, decreased hippocampal volume, accumulation of tau pathology, and the onset of symptomatic cognitive impairment. Plasma neurofilament light chain (NfL) and CSF are emerging biomarkers that track the general level of neurodegeneration in all forms of neurodegenerative dementia (Di Stefano et al., 2016; Han et al., 2016; Rabinovici, 2016; Pascoal et al., 2017).

Apolipoprotein E (ApoE) may affect amyloid pathology by directly binding Ab in the plaque, regulating AD risk. ApoE can have a regulatory effect on tau pathology and tau-related neurodegeneration and may independently affect neurons and neuronal

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networks (Li et al., 2019; Mentis et al., 2020). Reactive astrocyte and microglia hyperplasia are prominent pathological features of the AD brain, and the activation of the immune system is a critical regulator of AD pathology (Sabatino et al., 2019; Heneka, 2020).

Although significant progress has been made in the understanding of the pathology of AD, we have yet to discover disease-relief therapies that are effective in humans. The pathological biology of AD is very complicated. The older the age, the greater the possibility that other age-related diseases and AD pathology will cause cognitive decline (Congdon and Sigurdsson, 2018; Si et al., 2018; Teipel et al., 2018; Cummings, 2019). The ongoing in-depth research in this area is critical to making discoveries that will eventually reveal novel treatments that can truly change the course of the disease.

Stem cell treatment in AD modeling

Some drugs have been approved to slow down cognitive deficiency and neuronal loss in AD. Although these drugs can improve some AD symptoms, only less than 20% of AD patients will benefit, while over 60% of patients develop tolerance and side effects (Serretti et al., 2007; Zetterberg and Bendlin, 2020). With rapid growing achievements in stem cell research, stem cell-based therapy provides a new option for AD treatment.

Applications of iPSCs in AD modeling

Mouse fibroblast cells were first shown to be reprogrammed into iPSCs in 2006 by applying four transcription factors, including sex-determining region Y-box 2 (Sox2),

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cMyc, Octamer-binding transcription factor 4 (Oct4), and Kruppel-like factor 4 (Takahashi and Yamanaka, 2006). The next year, this technology was applied to human somatic cells to generate iPSCs successfully (Takahashi et al., 2007). Since then, considerable efforts have followed to optimize this technology and reprogram cells by newly defined or fewer factors and more efficient delivery systems (Chuah and Zink, 2017; Di Lullo and Kriegstein, 2017; Pournasr and Duncan, 2017; Devalla and Passier, 2018). Lineage specifiers involved in the ectodermal specification and mesendodermal specification can synergistically induce pluripotency without Oct4 and Sox2 (Aoi, 2008; Nakagawa et al., 2008; Chia et al., 2010; Buganim and Jaenisch, 2012). A growing number of novel reprogramming factors have been identified as maternal and pluripotency-associated factors, such as Esrrb, Tet1, Sall4, and PR domain-containing 14 (Maherali et al., 2008; Doege et al., 2012; Moon et al., 2012; Hu et al., 2014; Chen et al., 2015). Manipulation of microRNAs (miRs) can replace traditional reprogramming factors to increase the efficiency of reprogramming somatic cells into iPSCs (Judson et al., 2009; Anokye-Danso et al., 2011). Besides, the differentiation into iPSCs has been extended to various cell types, including human keratinocytes, fibroblasts, mature B lymphocytes, liver and stomach cells, human amniotic fluid-derived cells, glia cells, pancreatic-β cells, as well as microglia, neurons, astrocytes, endothelial cells, oligodendrocytes, and brain pericytes (Stadtfeld et al., 2008; Tsai et al., 2010; Watanabe et al., 2011; Zhou et al., 2011; Meng et al., 2012; Montserrat et al., 2012). Moreover, co-culture models of multiple brain cell types have been developed to simulate the complex interactions between neuronal

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cells *in vivo*. Improvements of differentiation methods to increase the maturity, yield, and purity of brain cell types, and the developments of co-culture and three-dimensional (3D) models to better imitate the pathologies of AD remain in development (Choi et al., 2014). Further improving these reprogramming strategies and models has promising potential to facilitate neurodegenerative disease research and clinical applications (Figure 2).

Age is the primary risk factor for neurodegenerative diseases, including AD; therefore, using stem cells to study AD may seem counterintuitive. However, in the very early stages of differentiation, neurons differentiated from iPSCs with FAD mutations, or iPSCs from AD patients, exhibit AD-related phenotypes (Ochalek et al., 2017; Ortiz-Virumbrales et al., 2017; Wezyk et al., 2018). These alterations parallel the stages of AD progression, which are understudied *in vivo*. Genome-wide association studies have shown that alterations in many different genes can promote the development of AD, and different genetic changes in patients with AD shared pathological manifestations in some cases (De Strooper and Karran, 2016). Generating specific individual brain cells of iPSCs has potential applications for patient-specific treatment (Chen et al., 2016; Cota-Coronado et al., 2019).

Neurons. Numerous neurodegenerative diseases that occur during aging attest that brain neuronal cells, as non-dividing cells, face major challenges in maintaining normal function and health during the multiple decades of life. A better understanding of the mechanisms may help ensure the health and survival of neurons. With the development and application of iPSCs technology, more and more literature has been

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published demonstrating FAD or SAD modeling using iPSCs (Chambers et al., 2009). iPSCs can differentiate into neural progenitor cells, after which the neural progenitor cells are patterned to different neuronal lineages (Maroof et al., 2013; Nicholas et al., 2013). There are numerous neuron subtypes, including dopaminergic neurons, glutamatergic neurons, GABAergic neurons, and cholinergic neurons (Soldner et al., 2009; Zhang et al., 2013; Begum et al., 2015; Sun et al., 2016). Glutamatergic neurons harboring mutated APPV717I were observed to have elevated β-secretase cleavage of APP and increased levels of both Aβ and tau phosphorylation (Muratore et al., 2014). In contrast, neurons harboring mutated APPA673T were found to have reduced β-secretase cleavage of APP and production of Aβ (Maloney et al., 2014). Neurons expressing mutated APPK670N/M671L or APPV717I exhibited impaired low-density lipoprotein endocytosis, reduced mitophagy, cellular uptake defects, and degradation pathway impairment compared to neurons carrying APP duplications (Knappenberger et al., 2004; Israel et al., 2012; Fang et al., 2019). Patients with Down syndrome (DS) develop early-onset dementia. The DS-iPSC neurons accumulate tau hyperphosphorylation and Aβ deposits, similar to that caused by mutations in FAD (Shi et al., 2012; Chang et al., 2015; Dashinimaev et al., 2017; Ovchinnikov et al., 2018). Glutamatergic neurons derived from PSEN1null and PSEN1ΔE9 mutations iPSCs have been shown to gain γ-secretase function via loss- or gain-of-function without loss of other functions (Knappenberger et al., 2004; Wang et al., 2018). Neurons from iPSCs harboring mutated PSEN1V89L, PSEN1A246E, and PSEN1L150P showed to be more sensitive to oxidative stress and Aβ-induced toxicity

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than those from healthy individuals (Armijo et al., 2017; Ochalek et al., 2017). iPSCs derived from SAD often share the same phenotypes with those with FAD mutations. In addition to the elevated tau phosphorylation and Aβ accumulation, the SAD- iPSC neurons show activation of ER, elevated DNA damage, enlarged endosomes, activation of oxidative stress pathways, and mitochondrial dysfunction (Duan et al., 2014; Birnbaum et al., 2018).

Astrocytes. Astrocytes are the most abundant cell type in brains, which play essential roles in providing energetic, trophic, physical, and metabolic support to other brain cells (Molofsky and Deneen, 2015; Weber and Barros, 2015; Liddelow et al., 2017). Multiple protocols have been developed to differentiate iPSCs into astrocytes (Krencik et al., 2011; Emdad et al., 2012). Altered marker protein localization and decreased morphological complexity were exhibited in astrocytes harboring both SAD-linked APOE4 and FAD-linked PSEN1M146L mutations (Jones et al., 2017). Increased reactive oxygen species production, impaired fatty acid oxidation, elevated release, and reduced uptake of Aβ42 were observed in astrocytes carrying the PSEN1ΔE9 mutation (Oksanen et al., 2017; Konttinen et al., 2019). When co-cultured with human neurons, astrocytes derived from iPSCs promote the maturation and survival of neurons. However, the effects can be impaired by APOE4 and PSEN1ΔE9 mutations (Kuijlaars et al., 2016; Zhao et al., 2017). Moreover, the APOE4 mutated astrocytes exhibit a reduced ability to internalize Aβ42 and extensive gene expression alterations compared with APOE3-mutated astrocytes (Zhao et al., 2017; Lin et al., 2018).

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Microglia. Microglia are brain immune cells that play numerous roles, including clearance of dying cells, synaptic pruning, and regulation of neuroinflammation in the brain (Salter and Beggs, 2014; Heppner et al., 2015). In the past ten years, numerous microglial genes have been identified as risk factors for AD by improved sequencing technologies. These genes include Cas scaffolding protein family member 4 (CASS4), triggering receptor expressed on myeloid cells 2 (TREM2), Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1 (INPP5D), sialic acid-binding immunoglobulin-like lectin (Siglec) 3, SPI1, and HLA-DRB1 (Guerreiro et al., 2013; Jonsson et al., 2013; Lambert et al., 2013; Huang et al., 2017). These findings indicate that microglial dysfunction might contribute to the development of AD. Microglia induced from healthy individuals iPSCs are capable of Aβ uptake, synaptic pruning, and phagocytosis. After exogenous Aβ treatment, the microglia showed altered gene expression and secreted various cytokines (Muffat et al., 2016; Abud et al., 2017). Microglia derived from SAD patient iPSCs exhibited elevated release of specific cytokines and altered phagocytosis after lipopolysaccharide treatment (Xu et al., 2019). Microglia carrying mutated APOE4 showed an impaired ability to internalize Aβ, extensive gene expression, and reduced morphological complexity compared with isogenic APOE3 controls (Lin et al., 2018).

Oligodendrocytes. The function of oligodendrocytes is to generate a myelin sheath that wraps the axons of nerve cells and forms the white matter of the brain. Oligodendrocytes also mediate inflammation, providing trophic support and contributing to metabolism regulation in the brain (Simons and Nave, 2015; Dimou

and Simons, 2017). Oligodendrocyte dysfunction and white matter loss were observed in mice and patients with AD, which could lead to neuronal dysfunction (Desai et al., 2010; Bartzokis, 2011). Recent studies have reported the induction of oligodendrocytes from 3D organoid models (Hu et al., 2009; Wang et al., 2013; Madhavan et al., 2018). However, there are no studies on human systems to examine AD-related oligodendrocyte function. Selected critical AD studies utilizing iPSCs are summarized in Table 1.

Applications of NSCs in AD modeling

Transplantation of NSCs has been investigated as a prospective therapeutic approach for neurodegenerative diseases, including AD. NSCs are one type of multipotent stem cells that can differentiate into neurons, oligodendrocytes, microglia, and astrocytes (Massirer et al., 2011; Martinez-Morales et al., 2013; Berger et al., 2020; Si et al., 2020). NSCs can be extracted from brain tissues, differentiated from iPSCs and embryonic stem cells, or reprogrammed from somatic cells (Hermann and Storch, 2013; Wen and Jin, 2014; Shahbazi et al., 2018). Transplanted NSCs are capable of secreting neurotrophic factors and replacing damaged neural circuitry to alter lesion protein levels or counter symptomatic deterioration (Liu et al., 2013; Liu et al., 2014; Telias and Ben-Yosef, 2015; Kim et al., 2018). The neurotrophic factors NSCs secrete have been shown to improve memory function, and NSCs overexpressing β-degrading enzyme have been shown to reduce the aggregation of Aβ (Tang et al., 2008; Wu et al., 2016; Marsh and Blurton-Jones, 2017).

The 3xTg mouse is a FAD-related triple-transgenic mouse model, which carries three

mutations: APP Swedish, PSEN1 M146V, and MAPT P301L (Oddo et al., 2003; Billings et al., 2005). After human-derived NSCs transplantation in 3xTg mice, Aβ and tau protein levels remained unchanged, but the memory function and synaptic density improved, indicating that the transplantation of human-derived NSCs may only reverse symptoms (Chen et al., 2014; Ager et al., 2015). Mathew *et al*. showed that mouse-derived NSCs transplantation in 3xTg mice produced similar results as human-derived NSCs. After transplantation, cognitive impairment was rescued and synaptic density was enhanced without altering the Aβ and tau levels (Blurton-Jones et al., 2014). Transplantation of modified NSCs carrying Neprilysin showed to be more effective in delivery than vector-delivered Neprilysin, indicating NSCs can act as effective delivery vehicles (Kim et al., 2013; Blurton-Jones et al., 2014). A different source of NSCs may release various neurotrophins and have a distinct neurogenesis. BDNF is a member of the neurotrophin family and is involved in the mouse-derived NSCs recovery of synaptic connectivity, but it remains unknown which trophic factors are involved in synaptogenesis of human-derived NSCs (Ager et al., 2015). Studies on mouse-derived NSCs showed that cognitive improvement depends mainly on the precise differentiation of NSCs, whereas lineage-specific differentiation of human-derived NSCs had limited effect on cognitive function (Blurton-Jones et al., 2014; Chen et al., 2014). Tg2576 mice harbor the human Swedish APP mutation (isoform 695; KM670/671NL) (Hsiao et al., 1996; Kawarabayashi et al., 2001; King and Arendash, 2002), and reduced Aβ production and acetylcholinesterase were observed after NSCs transplantation into these mice.

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Further, many astrocytes expressing α7 nicotinic receptors were found to repair damaged neurons, and the endogenous neurogenesis was enhanced in the transplant region of Tg2576 mice (Lilja et al., 2015). Anti-inflammatory cytokine levels were significantly higher in microglial cells and could inhibit Aβ production and promote Aβ clearance rate when NSCs were transplanted into Tg2576 mice at early stages of disease. Moreover, synaptic density, VEGF, and neurogenesis were increased after transplantation. Timely intervention is essential since these results were not obtained when NSCs were transplanted into Tg2576 mice at later stages (Kim et al., 2015; Haiyan et al., 2016). APP/PS1 mice are widely used as an AD mouse model and harbor both the Swedish and PSEN1 (L166P) mutations (Maia et al., 2013). Enhanced synaptic formation without a change in Aβ levels was observed after transplantation of NSCs into APP/PS1 mice (Li et al., 2016). In contrast, McGinley's study suggested that NSC transplantation reduces Aβ levels by regulating microglial activation (Zhang et al., 2015; McGinley et al., 2018). Administration of NSCs in APP/PS1 mice also resulted in enhanced levels of tropomyosin receptor kinase B (TrkB) and BDNF. The expression of the NMDA receptor 2B subunit, which plays a critical role in memory and learning function, was also increased, resulting in improved the cognitive function (Zhang et al., 2014). Cholinergic-like neurons derived from NSCs were also introduced into APP/PS1 mice. This showed that cholinergic acetyltransferase's concentration and activity were elevated, and there was an increase in functional dendrites (Gu et al., 2015). In another study, astrocytes and microglia activity was decreased, which regulates the Toll-like receptor 4 signaling pathway, leading to a

decrease in neuroinflammatory response and the improvement of cognitive function (Zhang et al., 2016). Another AD mice model is the 5xFAD mouse, which harbors five mutations: the PSEN1, Florida (I716V), Swedish (K670 N/M671 L), London (V717I), M146 L, and L286 V mutations (Oakley et al., 2006; Jawhar et al., 2012). These mice are immune-deficient, allowing long-term safety and efficacy evaluation of NSCs transplantation. A clinical-grade NSCs line failed to differentiate and had no impact on synapses after successful engraftment into 5xFAD mice for up to five months. The protein levels of BDNF and Aβ did not change and no improvement in behavior impairment was observed (Marsh et al., 2017). In contrast, rapid differentiation and reconstruction of functional neural circuits were observed after transplantation of reprogrammed NPCs from human mononuclear cells. BDNF levels increased, and improvement in behavior impairment was observed after 5 to 6 months (Zhang et al., 2019). These two studies indicate that, compared with normal NSCs, reprogrammed somatic cells might have greater neural lineage-specific differentiation capacity. Selected AD studies utilizing NSCs are summarized in Table 2.

Application of MSCs in AD modeling

MSCs are a type of pluripotent stem cell with self-renewing, immunomodulatory properties, that have limited differentiation capacity (Song et al., 2020). MSCs can differentiate into chondrocytes, osteocytes, fibroblasts, and adipocytes (Ankrum et al., 2014; Si et al., 2019). Unlike iPSCs and NSCs, MSCs are not expected to replace the impaired neurons and incorporate into neuronal networks because it is controversial whether MSCs can differentiate into ectodermal or endodermal cells (Robert et al.,

2020; Varderidou-Minasian and Lorenowicz, 2020). MSCs can be distinguished from other cell types by the expression of CD105, CD90, CD73, and CD44, and by the lack of CD14, CD45, CD19, CD11b, CD34, and human leukocyte antigen DR isotype expression (Bari et al., 2019; Elahi et al., 2020). MSCs can be harvested from many tissues, including adipose tissue, umbilical cord tissue, bone marrow, fetal tissues, placental tissues, dental pulp, and peripheral blood (Keane et al., 2017; Sa da Bandeira et al., 2017). MSCs have neuroprotective effects in addition to antifibrotic, anti-inflammatory, anti-bacterial, anti-tumorigenic, chemo-attractive, anti-apoptotic, pro-angiogenic, and tissue repair effects (Pierro et al., 2017; Naji et al., 2019). There are multiple mechanisms behind the neuroprotective effects of MSCs. MSCs can secrete neurotrophic growth factors such as BDNF and glial cell-derived neurotrophic factor (GDNF) to improve the survival of neuronal cells (Teixeira et al., 2017; Hao et al., 2018). It is well known that MSCs can modulate the immune system, and neuroinflammation has been reported to play a pathomechanistic role in neurodegenerative diseases. When MSCs enter the neuroinflammatory milieu, they will release pro-inflammatory and anti-inflammatory factors, and activated T cells can interact with neuronal cells to reduce neuronal death (Ransohoff, 2016). Secreted biological factors such as messenger RNA, proteins, or microRNA via extracellular vesicles (EVs) are other mechanisms to improve neuronal survival (Richards et al., 2016). Finally, a novel hypothesis to the neuroprotective effects of MSCs is that MSCs improve neuronal health by donating their mitochondria to neurons carrying dysfunctional mitochondria (Zhao et al., 2013; Glenn and Whartenby, 2014).

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In the APP/PS1 mouse model of AD, bone marrow-derived MSCs were transplanted via tail vein injection. These mice were found to have a reduction in microglial numbers without alteriation in the numbers of amyloid plaques (Naaldijk et al., 2017). In contrast, a study by Carter *et al.* showed a significant decrease after intracerebral injection of bone marrow-derived MSCs compared with controls treated with PBS two months post-injection. The synaptic transmission-related proteins such as synapsin 1 and dynamin 1 were considerably enhanced in AD mice brains compared with control groups after treatment with bone marrow-derived MSCs (Bae et al., 2013). In another study, human umbilical cord-derived MSCs were induced to neuron-like cells and transplanted into the APP/PS1 AD mouse model. In this model, increased synapsin 1 levels, improved cognitive function, and reduced Aβ deposition were found. The "alternatively activated" microglia (M2-like microglia) and interleukin-4, an anti-inflammatory cytokine associated with M2-like microglia, were increased. Further, the pro-inflammatory cytokines tumor necrosis factor-α and interleukin-4 were decreased significantly (Yang et al., 2013). Neprilysin and insulin-degrading enzyme, two Aβ-degrading factors, increased after treatment with neuron-like cells from human umbilical cord-derived MSCs (Lee et al., 2012). One study reported that MSC transplantation improved AD cognition, and that the pathology may be mediated through modulating tissue repair factors and inflammatory events (Lee et al., 2010). The Wnt signaling pathway has been reported to be involved in the MSCs-regulated neurogenesis in an AD mouse model. Se *et al*. found that the expression of GFAP, nestin, Ki-67, HuD, and SOX2 significantly

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increased in Aβ-treated neural progenitor cells co-cultured with MSCs as compared to Aβ treatment alone. Additionally, β-catenin and Ngn1 expression were enhanced in Aβ-treated neural progenitor cells co-cultured with MSCs (Oh et al., 2015). In AD mouse models, the MSC's effects on mitochondrial function have not yet been studied. Selected AD studies utilizing MSCs are summarized in Table 3.

Challenges and Future Perspectives

The development of stem cell technologies allows the use of differentiated human cells for mutagenesis and drug screening (Hirschi et al., 2014; Sproul, 2015). In the past few decades, many promising preclinical and early clinical findings were obtained. However, many challenges remain regarding the application of stem cells as therapeutic approaches in AD. The development of stem cell technologies also raises the attractive possibility of personalized and regenerative medicine (Sproul, 2015; Chen et al., 2016; Cota-Coronado et al., 2019). Genomic instability of iPSCs, however, is a serious issue for both experimental studies and regenerative medicine. Limited passage numbers and regular checks of genomic alterations in iPSC lines are the most commonly used methods to prevent issues (Kwon et al., 2017; Zhang et al., 2018). Even though the use of integration-free delivery systems have reduced the genomic alterations in iPSCs, it remains an active topic of investigation to reduce genomic instability (Rebuzzini et al., 2016; Yoshihara et al., 2017). Expanding brain cell subtypes generated from iPSCs, improving differentiation protocols of iPSCs, and developing more suitable and complex 3D co-culture systems are important goals for iPSC research. How to improve the reproducibility and consistency of cell subtypes

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obtained from iPSCs also remains unknown. Despite the use of the same protocol, there is considerable variability in gene expression and cellular morphology (Mills et al., 2013; Volpato et al., 2018). To minimize such variability, better standardization of growth conditions and differentiation techniques, adoption of rigorous statistical analyses, and more thorough reporting of methodologies should be established (Lin, 2011; Sullivan et al., 2018). Another challenge is how to generate iPSC-derived brain cells that can adequately mimic the growth and maturation of various cell types in the brain. Signals from other cell types are critical during this process to shape their identity (Cahoy et al., 2008; Gosselin et al., 2014; Bohlen et al., 2017). To resemble cell counterparts more closely, we need to better understand the critical signals involved in the process. The 3D co-culture systems are important models for stem cell application in neurodegeneration research, promoting the development of hallmark pathologies in AD that cannot be found in 2D cultures (Camp et al., 2015; Sloan et al., 2017). The 3D co-culture systems also provides a platform to help develop a better understanding of the complex, interrelated functions and interactions between all cell types in the brain. An ideal 3D co-culture system for AD should include each type of glial cell, all neuronal subtypes, and the blood-brain barrier components (Choi et al., 2014). In many cases, only some aspects of brain function are established in a reduced system model. In the deeper layers, organoids often show dysfunction and cell death, and the introduction of functional vasculature would likely improve this situation. The "bioreactors" used in culture may also improve the health of the cells (Marion et al., 2009; Corti et al., 2012; Baxter et al., 2015; Huh et al., 2016; Mertens et al., 2018).

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The iPSC models to mimic AD have been questioned since the age- and environment-dependent epigenetic and cellular signatures may be lost during reprogramming (Roessler et al., 2014). To overcome this problem, somatic cells are reprogrammed directly into neuronal cells, bypassing the iPSC stage. The reprogrammed have improved capability to retain age-related transcriptomic and cellular alterations compared to iPSCs (Victor et al., 2018). There are also limitations for direct reprogramming due to the low yield of reprogrammed cells and poor reprogramming efficiency (Mertens et al., 2018). Unlike iPSCs, the beneficial role of NSCs in AD is to increase the levels of neurotrophic factors, restore local neuron populations, and increase synaptic density rather than modulate pathological protein levels (Zhang et al., 2004; Zheng et al., 2017; Omole and Fakoya, 2018). However, how long this phenomenon can persist with altering the pathological protein levels and what role NSCs may play in this process remains unknown. As stem cell techniques continue to be refined, novel stem cell-based therapies can be adequately validated and may reveal effective therapeutics that lead to further targeted drug development for AD, ultimately moving the field forward.

Although the literature is replete with therapeutic interventions pursued based on expert opinion and patient acceptance, stem cell transplantation risks cannot be ignored. There is an successful example that a patient received multiple injections of different source-derived allogeneic stem cells to reduce neurologic deficits from a middle cerebral artery stroke(Berkowitz et al., 2016). Despite fewer safety concerns than allogeneic stem cells, applications of autologous stem cells may raise notably

adverse events. A reported case showed that hematopoietic stem cells injection into kidneys of a patient with renal failure were associated with tumor development and ultimately led to nephrectomy(Thirabanjasak et al., 2010). However, we firmly believe that by resolving the unique challenges in clinic, stem cell therapies can provide an important, safe and effective strategy to patients who need.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Zizhen Si, Xidi Wang.

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Footnotes

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Figure legends

Figure 1. Stem cell mechanisms of action to treat AD. (1) Replace the injured or lost neuronal cells, (2) Secretion of neurotrophic factors, (3) Anti-amyloid protein production, (4) Anti-inflammatory response, (5) Promote the activation of endogenous stem cells, (6) Improve the metabolic activity of neurons in the brain.

Figure 2. Potential applications of developing iPSCs technology in AD modeling and drugs discovery. iPSCs edited from somatic cells can differentiate into multiple neuronal cells, which can simulate the complex interactions between neuronal cells in vivo by 3D co-culture. These reprogramming strategy and models have promising potential to facilitate neurodegenerative disease research, drug discovery and clinical applications.

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Table 1. Summary of selected important AD studies utilizing iPSCs.

Table 1. Summary of selected important AD studies utilizing iPSCs			
Stem cell types	Mutation	Signaficance	*Ref*
iPSCs	sAD, APP^{Dp}	Aβ, p-tau accumulation↑	[87]
iPSCs	Down syndrome	Aβ, p-tau accumulation↑	[90]
iPSCs	Isogenic APOE3/4	Aβ, p-tau accumulation↑; GABAergic neuron↓	[94]
iPSCs	Isogenic PSEN1ΔE9	Oxidative stress and Aβ↑; neuronal function↓	[104]
iPSCs	Isogenic APOE3/4	Aβ clearance↓ Aβ uptake↓	[21]
iPSCs	APPk670N/M671L, APPV7171	Robust Aβ deposition; filamentous tau;	[71]
	PSEN1ΔE9	Aβ caused tau deposition	

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Table 2. Summary of selected important AD studies utilizing NSCs.

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Table 2. Summary of selected important AD studies utilizing NSCs

Stem cell types	Model	Signaficance	*Ref*
NSCs	3xTg mice	endogenous synaptogenesis↑ synaptic density↑	[16]
NSCs	3xTg mice	synaptic density and BDNF↑	[19]
NSCs	3xTg mice	neuronal regeneration↑	[139]
NSCs	3xTg mice	synaptic density↑Aβ↓	[23]
NSCs	Tg 2576 mice	neurogenesis↑	[144]
NSCs	Tg 2576 mice	Aβ production↓ Aβ clearance↑; phosphorylated-tau↓ anti-inflammatory cytokines↑	[145]
NSCs	APP/PS1 tg mice	synaptic density↑ Neuronal metabolism↑	[146]
NSCs	APP/PS1 tg mice	microglia activation↑Aβ↓ proliferation↑ synaptophysin and growth factor↑	[149]
NSCs	APP/PS1 tg mice	mitochondria↑ mitochondrial- related protein ↑	[150]
NSCs	APP/PS1 tg mice	ChAT mRNA↑ ACh concentration↑	[152]
NSCs	APP/PS1 tg mice	neuron expressing protein↑ synaptogenesis and BDNF↑	[151]

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Table 3. Summary of selected important AD studies utilizing MSCs.

Table 3. Summary of selected important AD studies utilizing MSCs			
Stem cell types	Model	Signaficance	*Ref*
MSCs	APP/PS1 Tg mice	microglial numbers↓	[175]
MSCs	APP/PS1 Tg mice	synapsin 1 and dynamin 1↑	[20]
MSCs	APP/PS1 Tg mice	cognitive function↑ Aβ↓ inflammatory cytokine↑	[17]
MSCs	APP/PS1 Tg mice	Aβ degrading↑	[176]
MSCs	Aβ-induced AD model	cognitive function↑ neurotoxicity↓ oxidative stress↓	[177]
MSCs	Aβ-induced AD model	neurogenesis↑ β-catenin and Ngn1↑	[178]

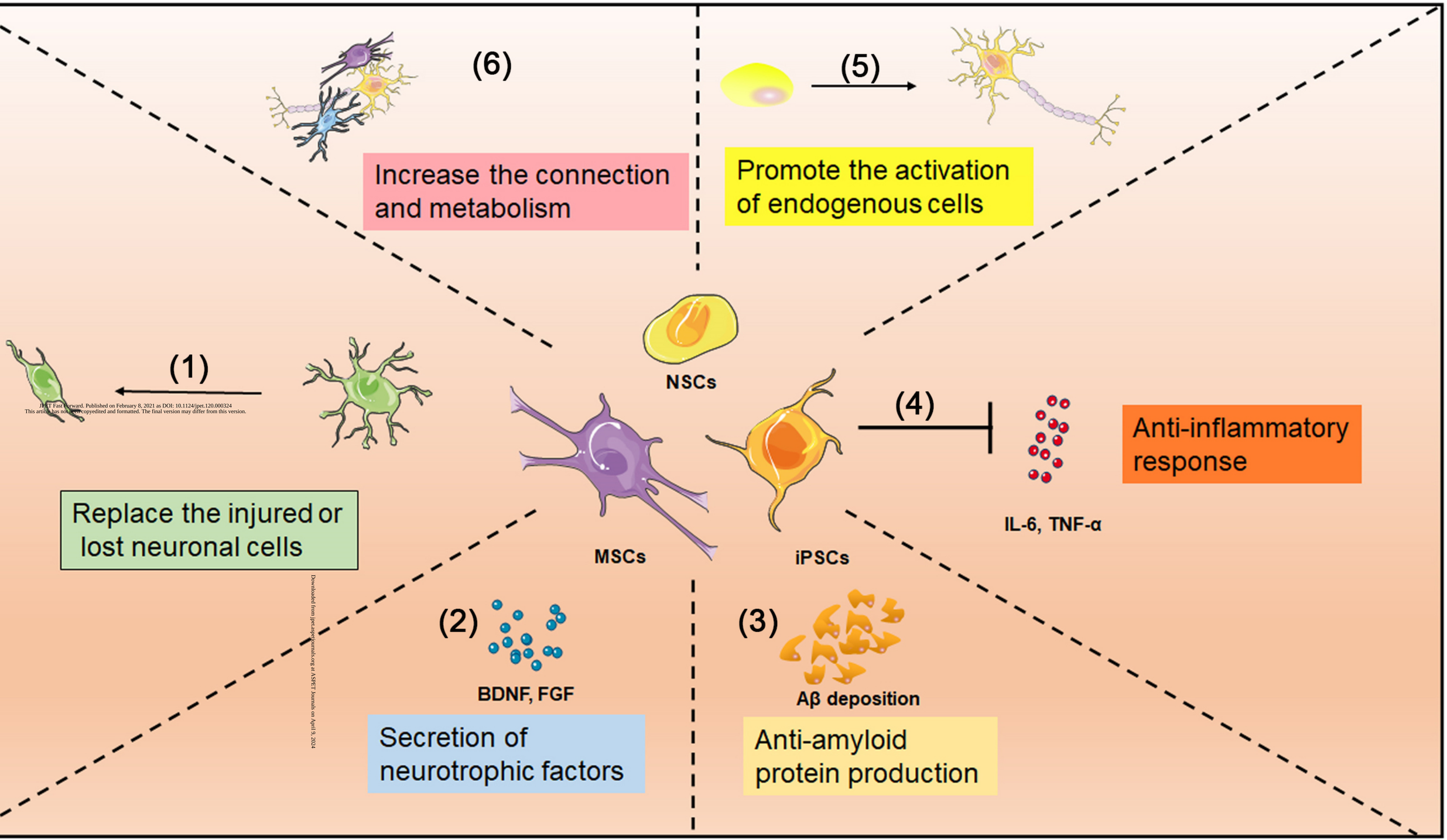
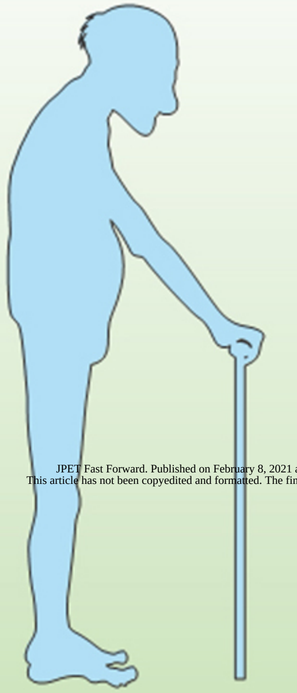


Fig 1

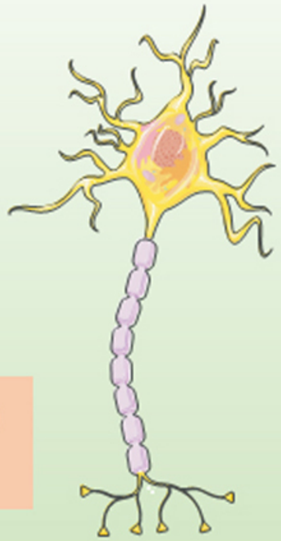


Somatic cells from AD patient or Healthy individual

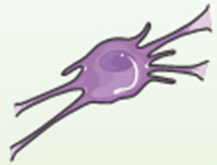
Gene editing

iPSCs

Differentiation to brain cell types

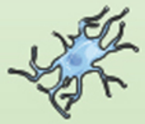


Neuron



Oligodendrocyte

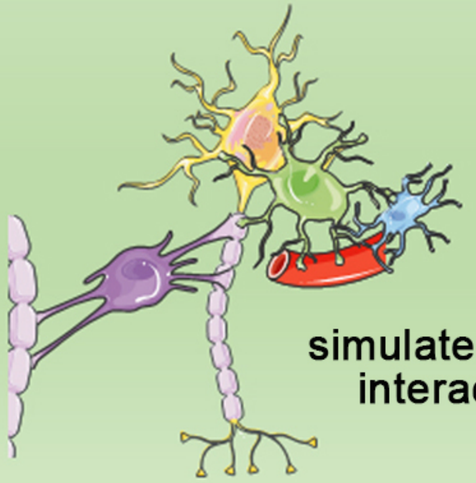
Astrocyte



Microglia

NPCs

3D co-culture



simulate neuronal cells interactions in vivo

AD modeling

Drug discovery

Application

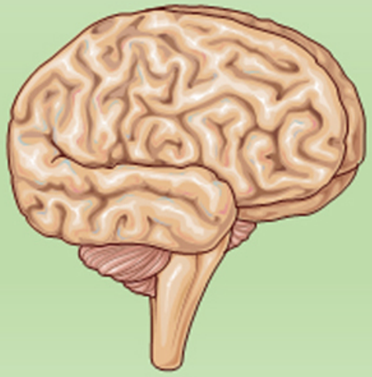


Fig 2