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REVIEW ARTICLE

Therapeutic potentials of neural stem cells in Alzheimer's disease

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Abstract

The commonest cause of dementia among the elderly population is Alzheimer's disease (AD). It is a health concern globally as the number of people affected by dementia worldwide is rapidly increasing. Several genes have been linked to AD and the pathogenesis of the disease has been extensively and vigorously examined. Thus far, only a few drugs have been approved by the Food and Drug Administration (FDA) for the pharmacological treatment of AD and a growing body of research has turned to alternative options such as stem cell therapy. This review will give an overview of the pathological and clinical aspects of AD. Although researchers have explored the suitability and feasibility of using various types of stems cells to treat AD, this review will focus mainly on neural stem cells (NSCs)/ neural progenitor cells (NPCs). The behaviour and properties of NSCs will be described, accompanied by a comprehensive discussion of the therapeutic strategies involving the use of NSCs/NPCs in the treatment of the disease.

Keywords: Alzheimer's disease, neurogenesis, neural stem cells, neural progenitor cells

INTRODUCTION

Dementia can be regarded as a clinical syndrome affecting one's intellectual functions in a progressive manner. In the World Alzheimer Report 2015 published by Alzheimer's Disease International,[1] the worldwide new cases of dementia in 2015 were estimated to be 9.9 million (49%, 25%,18% and 8% in Asia, Europe, the Americas and Africa respectively) with approximately a total of 46.8 million people living with dementia globally in the same year. This number is expected to escalate to 74.7 million in 2030 and 131.5 million in 2050. Dementia incurred a US$ 604 billion cost worldwide in 2010, and was estimated to increase 35.4% to US$818 billion in 2015, which amounted to 1.09% of the global gross domestic product (GDP). On the other hand, the commonest cause of dementia is Alzheimer's disease (AD), which is a progressive disorder of neurodegeneration. Among the characteristics of AD is diminishing cerebral cortical neurons and synapses, leading to a significant and gradual reduction in brain mass,[2] as well as ongoing cognitive decline and impairment.

Due to the enormous economic impact of dementia on the global economy, researchers in the field have carried out many studies to tackle the problem. However, not only are there no specific tests to definitively diagnose AD, especially in the early, preclinical stage, but there is also no cure to the disease currently. To date, pharmacological treatment of AD is mainly symptomatic rather than disease-modifying. The lack of effective disease-modifying drugs thus far explains the complexity of the disease and the many challenges faced by researchers. Despite the numerous studies on AD, pharmacotherapy mainly involves a few Food and Drug Administration (FDA) - approved drugs, which include the cholinesterase inhibitors (e.g. rivastigmine, donepezil, tacrine and galantamine) and memantine.[3] While there is a growing body of research on other drugs such as the non-steroidal anti-inflammatory drugs (NSAIDs), which have been observed to be associated with a reduction of AD risk,[4] other scientists in this field have turned their attention to stem cell-based therapy in AD.[5]

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As an alternative option to the conventional drug treatment in AD, many types of stem cells have been explored in the past, these include mesenchymal stem cells (MSCs),[6] embryonic stem cells (ESCs),[7] induced pluripotent stem cells (IPSCs)[8] and neural stem cells (NSCs).[9] These different types of stem cells have their unique properties and there are pros and cons of using them as potential candidates in cell-based therapy. For example, there exist many ethical concerns regarding the use of ESCs. Due to their extensive differentiation capacity, these cells have the potential risk of developing into teratomas.[10] IPSCs are cells derived from somatic cells and they share similarities with the ESCs with regards to their self-renewal and differentiation ability.[11] Although they are free from ethical issues in comparison with ESCs, they share a similar risk of tumourigenesis[12] due to their pluripotent nature.

On the other hand, MSCs are relatively easier to obtain, as evidenced by the many studies using these cells in AD research. MSCs can be obtained from many locations in the body, including the bone marrow[13] adipose tissue,[14] umbilical cord blood[15] etc. They are easily cultured in the lab and have been reported to possess immunomodulatory effects[16]. However, these multipotent stem cells mainly differentiate into cells that originate from the mesoderm like the osteoblasts, chondrocytes and adipocytes,[17] although they have also been shown to transdifferentiate into neural tissues.[18] Nevertheless, there is still no clear evidence that transdifferentiated cells can function like neurons.[19] However, MSCs have been shown to act indirectly in animal models via its paracrine effects. For instance, the release of soluble intracellular molecule-1 (sICAM-1) by human umbilical cord blood-derived (hUCB)-MSCs, have been reported to decrease Aβ plaques through the induction of microglial expression of Aβ-degrading neprilysin 1 via the sICAM-1/LFA-1 signalling pathway.[20]

On the contrary, NSCs have been shown to differentiate into astrocytes, neurons and oligodendrocytes[21] readily in cultures. Therefore, the use of NSCs in neurodegenerative diseases like AD may be a plausible therapeutic option. This review will give an overview of Alzheimer's disease, and the properties and behaviour of NSCs in general. It will also discuss in-depth, the role of NSCs in AD by examining recent promising experimental and human studies that raise hope in the treatment of the disease.

1. Alzheimer's disease

According to the data published by the Alzheimer's Association in 2016, 84,767 deaths from AD were recorded on the official death certificates in 2013. While there was a 71% increase in deaths from AD between 2000 and 2013 in the United States (US), in contrast, a decrease in deaths as a result of pancreatic cancer, heart disease and stroke was observed in the same period. The same report also gave an estimate that one new case of AD will develop every 33 seconds by 2050, giving rise to approximately 1 million new cases in the US each year.[22] Therefore, AD is a growing and pressing health concern, not just in the US, but also in other parts of the world.

1.1 Genes associated with AD

AD is generally accepted as a common disease of ageing, although there are patients who experience an early onset of the disease before 65 years of age, which comprise of only approximately 5% of all AD cases.[23] After 65 years of age, the estimated age-related prevalence of AD doubled every 5 years.[24] Based on the prevalence and incidence of AD, age can be considered as one of the key risk factors for the disease. The exact cause of AD remains largely unknown, even though the neuropathological changes in the brain of AD patients have been unfolded over decades of research. Approximately 13% of patients with early-onset AD have familial AD (also referred to as early-onset familial AD [EOFAD]) which has an autosomal dominant pattern of inheritance. Mutations involving three genes have been associated with EOFAD, namely presenilin 1 (PSEN1), presenilin 2 (PSEN2) and amyloid precursor protein (APP). The PSEN1, PSEN2 and APP genes are located on chromosomes 14, 1 and 21 respectively.[25]

The remaining 95% of patients with AD have late-onset AD (LOAD), with a later age of onset after 65 years. These cases are also known as sporadic AD, which is associated with the apolipoprotein E (APOE) gene, a major risk gene located on chromosome 19.[25] The APOE gene has three alleles, i.e. epsilon 2 (ε2), epsilon 3 (ε3), and epsilon 4 (ε4). Those who have the ε4 allele have an increased risk of AD,[26] compared to those who are ε4 negative. However, sporadic AD is a complex multifactorial disease that involves a blend of genetic and environmental factors. Previous research showed a higher

prevalence of AD in females, suggesting the role of gender in the disease.[27] However, findings on the role of gender in AD are inconsistent in different studies. A lower educational level has been linked to an increased risk of AD[28] whereas vascular risk factors like hypertension, stroke, diabetes, hypercholesterolemia and smoking are also associated with AD, linking cerebrovascular dysfunction with the pathology of the disease.[29]

1.2 Pathogenesis

The pathogenesis of AD involves an array of factors in a complex relationship. Two key pathological features of AD are the presence of beta-amyloid (Aβ) plaques and neurofibrillary tangles. Aβ plaques are deposited extracellularly around the neuronal cells as well as in the blood vessels in the brain (cerebral amyloid angiopathy) whereas neurofibrillary tangles are found intracellularly in the neurons.[30] According to the Aβ cascade hypothesis, the accumulation of Aβ deposits in the form of amyloid plaques is the primary pathology of the disease. This is due to an imbalance between Aβ production from amyloid precursor protein (APP) and Aβ clearance,[31] which is viewed as the initial toxic event in the pathogenesis of AD, followed by a cascade of events such as neurofibrillary tangle formation, loss of neurons and eventually, dementia. This hypothesis is supported by genetic studies and is linked to the mutations of the PSEN1, PSEN2 and APP genes.[32]

Some believe that the accumulation of Aβ can be due to microglial and immune cell (e.g. monocyte) dysfunction, leading to failure of Aβ clearance. However, it is noteworthy that Aβ clearance is a complex process involving various cell types, including microglial and infiltrating immune cell engulfment and degradation[33]. In murine models, an inflammatory and patrolling subtype of monocytes has been identified. The former is shown to play a role in parenchymal Aβ clearance, while the latter is associated with the vasculature.[34,35] Earlier studies have demonstrated that the patrolling monocytes play a crucial role especially in perivascular Aβ clearance, as a deficiency in this type of monocytes led to an increase of Aβ in the vasculature,[36] cortex and hippocampus.[37] On the other hand, it has been demonstrated that ageing and progression of AD decrease the ability of microglia to clear Aβ[38] whereas young microglia have been shown to restore the ability of aged microglia to clear Aβ in an *ex-vivo* model.[39]

Alternate splicing of the microtubule-associated protein tau (MAPT) gene (found on chromosome 17) produces six isoforms of tau proteins.[40] These proteins are found abundantly in the neurons and central nervous system (CNS) and less commonly elsewhere in the body. Under normal physiological conditions, tau proteins, which are microtubule-associated proteins (MAPs) function to stabilise microtubules in the neurons.[41] According to the tau hypothesis, normal tau turns into paired helical filament (PHF) tau and neurofibrillary tangles as a result of abnormal and uncontrolled phosphorylation of tau. Supporting this hypothesis is the fact that tau pathology takes place before Aβ plaque formation and the disease progression and severity of AD are more closely correlated to tau pathology than Aβ plaque load.[42]

Some researchers propose that amyloid and tau pathologies alone cannot explain for all of the disease processes in AD. It is believed that inflammation is induced by Aβ deposits and later enhanced by tau aggregates, which gives rise to the inflammatory hypothesis in the pathogenesis of AD.[43] Activated microglia have been shown to be involved in the pro-inflammatory process, leading to the release of substances such as cytokines and chemokines that are potentially toxic to the neurons.[44] The inflammatory hypothesis is further supported by epidemiological research that shows that the use of NSAIDs is inversely associated with the risk of AD.[4] Besides, Aβ plaques, tau proteins and inflammation, oxidative stress[45] induced by reactive oxygen species (oxidative stress hypothesis) as well as vascular risk factors (vascular hypothesis)[29] are also proposed to be contributing factors in the disease process of AD.

1.3 Clinical features

A German neuroanatomist and psychiatrist, Dr Alois Alzheimer first described AD in his landmark conference lecture in 1906, which was subsequently documented in an article in 1907[46]. Alzheimer gave an account of a 51-year-old woman named Auguste D, who had a "peculiar disease of the cerebral cortex" and presented with the clinical features of gradual cognitive, psychosocial and behavioural impairments. After more than a century, these descriptions of Alzheimer are still very consistent with our current understanding of the clinical presentation of AD today. Clinically, the symptoms of AD are categorised into three main groups. The first group involves cognitive dysfunction

(e.g. memory loss, difficulties with language, impairment of intellectual skills), the second group are mainly psychiatric symptoms and disturbances in behaviour (e.g. depression, delusions, hallucinations) and the last group affects the ability to carry out daily activities (e.g. driving, dressing and eating unaided, shopping). The disease usually begins with symptoms of mild memory loss, gradually progressing to those of severe dementia (reviewed by Burns & Iliffe).[47]

1.4 Diagnosis

The definitive diagnosis and staging of AD are made through autopsy and pathological evaluation of findings such as the observation of amyloid plaques and neurofibrillary tangles.[48] However, in the clinical setting, diagnosis mainly relies on a thorough medical history, physical examination and neuropsychological tests. Guidelines that are used commonly in the diagnosis of AD include International Classification of Diseases (ICD), Diagnostic and Statistical Manual of Mental Disorders (DSM), the International Working Group for New Research Criteria for the Diagnosis of AD criteria (IWG), the United States National Institute of Aging-Alzheimer's Association (NIA-AA) diagnostic guidelines for Alzheimer's disease (reviewed by Cece & Shifu).[49] Some of these guidelines rely more on clinical features while others support the use of both clinical features, biomarkers and imaging. Each of these guidelines has their strength and short-comings and a detailed comparison between the guidelines is beyond the scope of this review.

Besides depending on the medical history, physical examination and neuro-psychological tests, biomarkers and imaging also play a part in the diagnosis of AD. One of the goals of using biomarkers and imaging is the early diagnosis of AD, such as in the prodromal stage or when there is a mild cognitive impairment (MCI). Thus far, numerous and different types of biomarkers have been researched on. It is believed that the use of biomarkers greatly enhances the accuracy in the diagnosis of AD as it was reported that of those who were clinically diagnosed with AD, about 10%-30% of them did not have autopsy-based evidence of AD[50] whereas about 30% elderly who were clinically normal were found to have AD at autopsy.[51]

In general, biomarkers used in AD diagnosis can be categorised into neuropsychological, biochemical, neuroimaging and genetic markers. Biochemical markers may be obtained either from the blood or cerebrospinal fluid (CSF) whereas neuroimaging markers are used in either structural or functional imaging.[52] Of the many biomarkers, three are established CSF biomarkers generally and internationally used in the diagnosis of AD, namely amyloid-beta (Aβ),[53] tau protein[54] and phosphorylated tau.[55] However, the establishment of a robust blood biomarker is challenging. For example, blood Aβ proteins show a weak correlation with Aβ amyloidosis in the brain.[56] Similarly, there is also no reliable and definitive blood biomarker for tau pathology to date. As for neuroimaging using positron emission tomography (PET) scan, Fluorine-18 radiolabelled biomarkers such as ^{18}F-flutemetamol (Vizamyl), ^{18}F-florbetapir (Amyvid) and ^{18}F-florbetaben (Neuraceq) have been used as PET tracers for Aβ, which have been shown to have a good correlation with the amyloid plaque deposition in the brain at autopsy.[57]

1.5 Treatment

Acetylcholinesterase inhibitors (AChEIs) and memantine remain the mainstay of pharmacological treatment in AD. The first AChEI approved by the FDA for this purpose was tarcine, which is no longer in use due to poor tolerability and hepatotoxicity.[58] FDA- approved second-generation AChEIs used for the treatment of AD include donepezil, galantamine and rivastigmine. All three drugs are used in mild-to-moderate AD, except for donepezil, which is also approved for moderate-to-severe AD.[59] The use of these drugs is supported by the evidence that neuronal loss and dysfunction in AD involves the cholinergic system.[60] In a recent meta-analysis and meta-regression involving 43 randomised clinical trials, AChEIs were reported to improve functional capacity, cognitive function and global symptomatology. However, an improvement in neuropsychiatric symptoms was not observed. A lower mortality was also reported with AChEIs when compared to placebo.[61]

Another FDA-approved drug, memantine is used to treat moderate-to-severe AD, by acting as an antagonist on non-competitive N-methyl-D-aspartate (NMDA) receptor, which is a subfamily of the glutamate receptor, widely involved in brain function.[62] Besides improvement in cognitive functions, the drug has been demonstrated to improve behavioural disturbances in patients with AD. In a meta-analysis, memantine was also reported to

significantly improve disinhibition, delusion, agitation/aggression and night time/diurnal rhythm disturbances when compared to the control.[63] It is important to note that other than drug therapy, supportive care, which aims at maximizing independence and functioning and minimising behavioural disturbances, also plays an important role in the management of AD.[64] Both patient and caregiver education may be helpful in maintaining social and intellectual activities in patients with AD as much as possible.

2. Neural stem cells (NSCs)

In order to understand the role of neural stem cells (NSCs) in the treatment of AD, it is important to first understand the definition, properties and behaviour of these cells. Generally, the main properties that are characteristic of stem cells include the ability to self-renew and the capacity to differentiate into other different types of more specialised cells. One of the ground-breaking milestones in neuroscience research is the discovery of the existence of NSCs, a type of multipotent stem cells, which can be regarded as the most primitive and least committed cells in the adult nervous system before they develop into more specialised cells of different neural regions. This debunked the old belief that lost neurons and glia in the nervous system could not be replaced by new ones.

To be operationally defined as an NSC, a cell must fulfil at least three criteria: a) multipotency, with the ability to differentiate into cells of all three neural lineages (i.e. astrocytes, neurons and oligodendrocytes), b) the ability to give rise to cells that occupy and populate a developing region or to help regenerate an ablated region in the nervous system and c) the ability to self-renew, so that identical daughter cells can be produced for the perpetuation of the NSCs themselves.[65] On the other hand, the terms "committed stem cells" or "early progenitor cells" often refer to proliferative cells that have a limited self-renewal capacity and are usually unipotent or oligopotent,[66,67] or have already been "pushed" into committing themselves to a particular target cell type.

Not only can NSCs be found during development, they can also be found in the adult CNS. Studies of NSCs or neural progenitor cells (NPCs) can be dated back to several decades ago. In contrary to the dogma of Santiago Ramón y Cajal (whom many consider as the father of modern neuroscience), it was discovered in the early 1960s that cells in the adult brain were capable of division and differentiation with the application of a labelling technique using tritiated thymidine. The incorporation of the substance into DNA allowed Smart and Leblond[68] to observe glial cell division throughout the parenchyma in the mouse brain. Using the same technique, it was later reported that cell division was observed in the subventricular zone (SVZ) of rodent brains.[69] Atman and Das provided the early evidence of neurogenesis in the adult rat brain by demonstrating that SVZ-originated cells matured into neurons and migrated to the olfactory bulb.[70]

In addition to clear evidence of the possibility and existence of neurogenesis in the adult brain, the development and establishment of techniques to isolate, culture and expand NSCs or NPCs marked another important milestone in neuroscience research in the 1990s. Reynolds and Weiss[71] reported the first successful culture of NSCs in the form of adult mouse brain SVZ-derived neurospheres. Early *in vitro* studies explored the requirements for proliferation and differentiation of stem/ progenitor cells isolated from the CNS of animals in cell cultures, including the extrinsic growth factors required for such *in vitro* cultures.[71,72] Similarly, NSCs were also isolated from the adult mammalian spinal cord and cultured using a neurosphere assay *in vitro*.[73]

Years later, not only have NSCs been successfully cultured *in vitro* studies have also reported the successful transplantation of these cells in the adult brain. For example, human NSCs were demonstrated to encourage functional recovery in a mouse intracerebral haemorrhage stroke model after brain transplantation.[74] Transplanted human NSCs were also reported to restore cognition in a rodent model of traumatic brain injury.[75] Besides, research has also shown successful NSC engraftment and myelination in the human brain.[76] Decades of research has given much insight into neurogenesis and the behaviour and properties of NSCs/ NPCs, and today it is generally accepted that neural stem/progenitor cells reside in the adult brain, contributing to lifelong brain plasticity.

Within the adult brain, there exist two neurogenic niches in which NSCs/NPCs can be found. The first is the SVZ which lines the lateral ventricles and the second, the subgranular zone (SGZ) in the dentate gyrus of the hippocampus of the human brain.[77] Some scientists also consider the periventricular region of the central canal of

the spinal cord as the third well-defined niche of NSCs.[78] Research has shown that NSCs in the SVZ have an embryonic origin, which have become quiescent in early development until they get reactivated postnatally.[79] In the adult rat brain, a marked increase in cell proliferation was observed in the SVZ in the event of a stroke due to middle cerebral artery occlusion. New neurons generated as a result of the stroke were shown to migrate to the stroke-induced damaged area and they resembled the phenotype of most of the neurons destroyed by ischaemia, suggesting that the adult brain has self-repair capacity after pathological neuronal death.[80] Such evidence of brain plasticity raises the hope that NSCs may be a potential therapeutic option in diseases that involve neuronal loss, including AD.

3. Neural stem/progenitor cells as a potential therapeutic option in Alzheimer's disease

Numerous studies have explored the use of NSCs/NPCs as a potential therapeutic option in AD (Fig. 1). Thus far, these studies have used various types of animal models to investigate the role of NSCs in the Alzheimer's brain. These models may be natural-, lesioned- and transgenic AD animal models. There are generally two approaches in these studies: a) endogenous NSC activation or modulation and b) exogenous NSC transplantation. Some researchers have also explored the effects of endogenous NSC modulation by transplanted NSCs.

3.1 Endogenous NSCs/NPCs

Several substances have been shown to activate or modulate endogenous neurogenesis (Table 1). For example, allopregnanolone, a metabolite of progesterone produced in the embryonic and adult CNS has been shown to enhance NPC proliferation and neurogenesis in the SGZ of transgenic mice. Wang *et al*. injected allopregnanolone into AD mouse model and showed that it significantly increased BrdU+ cells (proliferating cells) in the SGZ in these animals even in the absence of clear evidence of AD pathology. The increase in cell proliferation was reported to be dose-dependent, and the injection of allopregnanolone resulted in the reversal of cognitive deficits and restoration of learning and memory performance in the experimental animals, to the level comparable to that in the control group. A correlation between the survival of NPCs and memory performance induced by allopregnanolone was also observed in the same study.[81] Based on these findings, it was inferred that allopregnanolone may be useful in alleviating cognitive deficits in MCI or AD.

Fluoxetine or Prozac® is an FDA-approved selective serotonin reuptake inhibitor (SSRI) used in the treatment of depression. Studies have shown that the use of this drug enhances neurogenesis in animal models. In one study, chronic treatment with fluoxetine was shown to stimulate γ-aminobutyric acid (GABA)ergic interneuron neurogenesis in adult mice cortex. Interestingly, fluoxetine-treated mice also showed a significantly lower level of active caspase-3 (an apoptotic marker) expression in these GABAergic neurons following ischaemia.[82] In another study, the number of neurons in the hippocampal dentate gyrus in transgenic AD mouse model increased significantly after intraperitoneal injection of fluoxetine. A reduction in Aβ deposition, an increase in β-catenin level, inhibition of GSK-3β activity, as well as an improvement in the impaired spatial learning ability were also observed in the experimental animals. It was, therefore, suggested that fluoxetine may exert a protective effect on neuronal loss and may be beneficial in

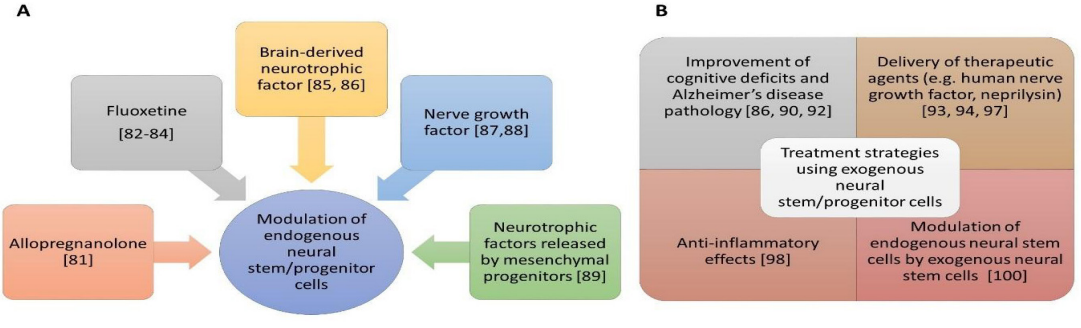

FIG. 1: Neural stem/progenitor cells as a potential therapeutic option in Alzheimer's disease. A) Modulation of endogenous neural stem/progenitor cells by various substances and B) treatment strategies using exogenous neural stem/progenitor cells.

TABLE 1: Activation or modulation of endogenous NSCs/NPCs

Therapeutic strategy	Key findings	Reference
Allopregnanolone	• Significantly increased BrdU+ cells (proliferating cells) in the SGZ in the absence of clear evidence of AD pathology. • Increase in cell proliferation was dose-dependent. • Reversal of cognitive deficits and restoration of learning and memory performance. • A correlation between the survival of NPCs and memory performance induced by allopregnanolone was observed.	Wang *et al*., 2010 [81]
Fluoxetine	• Stimulation of γ-aminobutyric acid (GABA)ergic interneuron neurogenesis in adult mice cortex with chronic treatment of fluoxetine. • Fluoxetine-treated mice showed a significantly lower level of active caspase-3 expression in these GABAergic neurons following ischaemia.	Ohira *et al*., 2013 [82]
Fluoxetine	• Number of neurons in the hippocampal dentate gyrus in transgenic AD mouse model increased significantly after intraperitoneal injection of fluoxetine. • A reduction in Aβ deposition. • An increase in β-catenin level. • Inhibition of GSK-3β activity. • An improvement in the impaired spatial learning. • Findings suggest protective effects on neuronal loss.	Ma *et al*., 2017 [83]
Fluoxetine	• NSCs treated with fluoxetine showed enhanced proliferation and neural differentiation in a dose-dependent manner. • Fluoxetine exerted protective effects against Aβ(42) induced cytotoxicity and cell death in NSCs.	Change *et al*., 2012 [84]
Brain-derived neurotrophic factor (BDNF)	• Intrahippocampal infusion of BNDF in adult rats resulted in an increase in granule cell neurogenesis, ectopic granule cell migration and widespread effects on hippocampal neurogenesis, either due to altered activity or direct effects of the BDNF infusion.	Scharfman *et al*., 2005 [85]
Brain-derived neurotrophic factor (BDNF)	• In AD transgenic experiments, recombinant BDNF delivery alone resembled the effects of NSC transplantation. • A lack of NSC-derived BDNF showed failure of cognition improvement or hippocampal synaptic density restoration in transgenic AD mice.	Blurton-Jones *et al*., 2009 [86]
Nerve growth factor (NGF)	• Phase I clinical trial • Genetically modified fibroblasts that express human NGF (hNGF) were implanted into eight patients with mild AD. • Six patients were followed up for a mean duration of 22 months • No detectable long-term adverse effects. • Improved rate of cognitive decline. • One patient's brain autopsy revealed vigorous growth responses to NGF	Tuszynski *et al*., 2005 [87]

Therapeutic strategy	Key findings	Reference
Nerve growth factor (NGF)	• NGF gene therapy in 10 human subjects either via ex vivo or in vivo gene transfer • The patients were followed up for one to ten years. • At autopsy, all the subjects demonstrated a trophic response by means of axonal sprouting to the source of NGF • The expression of NGF was found in both neurons with or without tau pathology	Tuszynski *et al*., 2015 [88]
Neurotrophic factors released by mesenchymal progenitors	• Secretome (fibroblast growth factor-2 and nerve growth factor) of human umbilical cord perivascular cells (HUCPVCs) were capable of potentiating neuronal survival and differentiation *in vitro* and *in vivo*.	Teixeira *et al*., 2015 [89]

patients with AD.[83] On the other hand, Chang *et al*. demonstrated that NSCs treated with fluoxetine showed a dose-dependent enhanced proliferation and neural differentiation. The drug also exerted protective effects against Aβ(42) induced cytotoxicity and cell death in NSCs, suggesting the therapeutic potential of fluoxetine in AD.[84]

In addition, a neurotrophic growth factor called brain-derived neurotrophic factor (BDNF) has been studied extensively for its role in neuroprotection and synaptic plasticity. Intrahippocampal infusion of BNDF in adult rats led to increased granule cell neurogenesis, ectopic granule cell migration and widespread effects on hippocampal neurogenesis, either as a result of an altered activity or direct effects of the BDNF infusion.[85] Blurton-Jones *et al*. further showed that in transgenic experiments, the delivery of recombinant BDNF alone resembled the effects of NSC transplantation whereas a lack of NSC-derived BDNF showed a failure of cognition improvement or hippocampal synaptic density restoration in transgenic AD mice.[86]

Nerve growth factor (NGF) is another neurotrophic factor that plays a crucial role in neuronal growth, as well as maintenance, proliferation and survival. In a phase I clinical trial, genetically modified fibroblasts that expressed human NGF (hNGF) were implanted into eight patients with mild AD. Six patients were followed up for a mean duration of 22 months with no detectable long-term adverse effects. The cognitive decline rate was improved and one patient's brain autopsy revealed vigorous growth responses to NGF.[87] Similarly, in a more recent study, NGF gene therapy in 10 human subjects either via *ex vivo* or *in vivo* gene transfer, has shown to activate neuronal responses in AD. The patients were followed up for one to ten years. At autopsy, all the subjects demonstrated a trophic response to the source of NGF by means of axonal sprouting and the expression of NGF was found in both neurons with or without tau pathology.[88]

It is worth mentioning that MSCs can activate or modulate endogenous NSCs/NPCs via their paracrine effects, as these cells are known to exert modulatory effects through the release of several substances including neurotrophic factors. In one study, *in vitro* and *in vivo* experiments have shown that the secretome of human umbilical cord perivascular cells (HUCPVCs) (a population of mesenchymal progenitor found in the Wharton Jelly of the umbilical cord) were capable of potentiating neuronal survival and differentiation. When human telencephalon neural precursor cells (htNPCs) were cultured with HUCPVCs-conditioned medium, a higher density of mature and immature neurons was observed. On the other hand, increased endogenous proliferation and an elevated number of newborn neurons were observed following the injection of HUPVCs and their conditioned medium into the dentate gyrus of adult rat hippocampus. The substances involved were mainly fibroblast growth factor-2 (FGF-2) and NGF, to a lesser extent.[89]

3.2 Exogenous NSCs/NPCs

Exogenous NSCs used in transplantation can be obtained in three ways. Firstly, they can be isolated from primary tissues both from animals such as mice and rats and also from humans. The isolation of NSCs from humans may involve obtaining the cells from cadaveric donors.[81,90] NSCs have been derived from sites such as the striatum, dentate gyrus, SVZ, periventricular

region, olfactory bulb and cerebellum in previous studies. A second way to obtain NSCs is by differentiation from pluripotent stems cells, which include ESCs and iPSCs. This method provides an attractive alternative of obtaining NSCs apart from isolating the cells from primary tissues. The third way of acquiring NSCs is by transdifferentiation, which involves converting one type of cell into another through lineage reprogramming (reviewed by Tang *et al*., 2017).[91]

3.2.1 Transplanted NSCs improved cognitive deficits and AD pathology

In the past, numerous studies have explored the effects of NSC transplantation in AD experimental animals. One way transplanted NSCs may help in the treatment of AD is by improving cognitive function and/or AD pathology in the host brain. In an earlier study, NSC transplantation was shown to improve cognitive functions in transgenic mice without any alterations in Aß or tau pathology.[86] A later study by Lee *et al*. transplanted human NSCs (hNSCs) derived from cadaveric foetal brain tissue in transgenic AD mice demonstrated engraftment, migration and differentiation *in vivo*. The study reported an improvement in spatial memory, reduced tau phosphorylation and Aβ42 levels in these animals and that Trk-dependent Akt/GSK3β signalling was involved in the alleviation of AD pathology. hNSC transplantation was also demonstrated to contribute to synaptic plasticity and anti-apoptotic effects.[90]

More recently, hNSC transplantation targeted in the fimbria fornix in a murine model of AD revealed cognitive improvement. Novel object recognition testing suggested enhancement of short-term non-associative memory in NSC transplanted animals, which was not observed in control animals. Moreover, NSCs engraftment was believed to be transient as they were not detectable after 17 weeks post-transplantation by immunohistochemical detection. Nevertheless, the Aβ plaque load in both the hippocampus and cortical brain regions significantly decreased, when compared to the control group of animals.[92]

3.2.2 Transplanted NSCs as a vehicle for delivery of therapeutic agents

In addition to directly improving AD pathology, one of the proposed functions of NSCs is that they may be used as a vehicle in delivering therapeutic agents in AD. In one study, NSCs were genetically modified with human nerve growth factor (hNGF, a protein that increases the survival and functioning of neurons) and enhanced green fluorescent protein (*eGFP*). It was shown that the resultant NSC-*hNGF-eGFP*, but not unmodified NSCs, integrated into the surrounding cells in the host brain and that the expression of hNGF was observed in different host brain regions. NSC-*hNGF-eGFP* was also reported to improve memory and learning in lesioned rat model treated with okadaic acid in the same study. The study proposed that hNGF played the role in NSC survival, which explained why the unmodified NSCs alone did not achieve the same effects.[93] In another study, Marei *et al*. demonstrated survival and proliferation of adult human olfactory bulb neural stem/progenitor cells expressing hNGF in rat hippocampus treated with ibotenic acid, as well as restoration of cognitive deficiencies in the AD model rats.[94]

Other than NGF, previous research also reported the use of neprilysin (NEP) delivered by genetically modified NSCs as a therapeutic strategy in AD. NEP is believed to play a key role in the degradation of Aβ as evidenced by the fact that NEP-deficient mice develop diseases resembling AD[95] and that its expression is lower in the AD brain.[96] Blurton-Jones *et al*. demonstrated in transgenic mice that NSC-mediated delivery of NEP was able to reduce Aβ pathology and enhance synaptic density. The reduction in Aβ loads was not only observed in the hippocampus but also several other regions in the brain, adjacent to where the NSCs engrafted.[97]

3.2.3 Other beneficial effects of transplanted NSCs

The role of NSCs/NPCs is not limited to cognitive and pathological improvement or delivery of therapeutic agents. Research has also shown that NPCs exert anti-inflammatory and neurogenesis-promoting effects in the host brain. In one study, transplanted NPCs were demonstrated to reduce microgliosis by 38% in the rat hippocampus and the pro-inflammatory cytokine, TNF-α was reduced by 40%, suggesting the anti-inflammatory role of transplanted NPCs in AD rats. Furthermore, a 45% neuronal loss and 26% neuronal recovery were also observed in NPC-transplanted animals.[98] Similarly, the anti-inflammatory effects of NPCs were also reported in a multiple sclerosis model. The observed therapeutic effects were thought to be related to immunomodulation rather than neuronal replacement.[99] On the other hand, transplanted NSCs were also reported to modulate endogenous

TABLE 2: Exogenous transplantation of NSCs/NPCs as a therapeutic strategy in AD

Therapeutic strategy	Key findings	Reference
Improvement of cognitive deficits	• NSC transplantation improved cognitive functions in transgenic mice without any alterations in Aß or tau pathology	Blurton-Jones *et al*., 2009 [86]
Improvement of cognitive deficits and AD pathology	• Transplanted human NSCs (hNSCs) derived from cadaveric foetal brain tissue in transgenic AD mice demonstrated engraftment, migration differentiation *in vivo*. • Improvement in spatial memory • Reduced tau phosphorylation and Aβ42 levels • Trk-dependent Akt/GSK3β signalling was involved in the alleviation of AD pathology. • hNSC transplantation contributed to synaptic plasticity and anti-apoptotic effects.	Lee *et al*., 2015 [90]
Improvement of cognitive deficits and AD pathology	• hNSC transplantation targeted in the fimbria fornix in murine model of AD revealed cognitive improvement. • Novel object recognition testing suggested enhancement of short-term non-associative memory. • NSCs engraftment was transient and detected after 17 weeks post-transplantation by immunohistochemical detection. • Aβ plaque load in both the hippocampus and cortical brain regions decreased significantly.	McGinley *et al*., 2018 [92]
Delivery of therapeutic agent	• NSCs genetically modified with human nerve growth factor (hNGF) and enhanced green fluorescent protein (eGFP). • Resultant NSC-hNGF-eGFP, but not unmodified NSCs, integrated into the surrounding cells in the host brain. • Expression of hNGF was observed in different host brain regions. • NSC-hNGF-eGFP improved memory and learning in lesioned rat model treated with okadaic acid. • Study proposed that hNGF played the role in NSC survival.	Wu *et al*., 2008 [93]
Delivery of therapeutic agent	• Adult human olfactory bulb neural stem/progenitor cells expressing hNGF survived and proliferated in rat hippocampus treated with ibotenic acid. • Restoration of cognitive deficiencies in the AD model rats.	Marei *et al*., 2015 [94]
Delivery of therapeutic agent	• In transgenic mice, NSC-mediated delivery of NEP reduced Aβ pathology and enhanced synaptic density. • Reduction in Aβ loads was observed in the hippocampus and several other regions in the brain, adjacent to where the NSCs engrafted.	Blurton-Jones *et al*., 2014 [97]
Anti-inflammatory effects	• Transplanted NPCs reduced microgliosis by 38% in the rat hippocampus. • The pro-inflammatory cytokine, TNF-α was reduced by 40%. • A 45% neuronal loss and 26% neuronal recovery were observed in NPC-transplanted animals.	Ryu *et al*., 2009 [98]
Modulation of endogenous NSCs	• Bilateral hNSC transplantation in the hippocampus improved spatial memory improvement in AD transgenic mice. • Accompanied by an increase in doublecortin-positive cells (indicating new neuron generation) in the dentate gyrus.	Lilja *et al*., 2015 [100]

NSCs. In AD transgenic mice receiving bilateral hNSC transplantation in the hippocampus, spatial memory improvement was accompanied by an increase in doublecortin-positive cells (indicating new neuron generation) in the dentate gyrus.[100] The therapeutic strategies of exogenous transplantation of NSCs/NPCs are summarised in Table 2.

CONCLUSIONS

Several concluding remarks can be made from this review. Firstly, AD is a leading cause of dementia and a major health concern worldwide due to its increasing economic burden on the global economy. Secondly, although the underlying pathology of AD has been well studied and supported by various hypotheses, there are still no definitive ways to diagnose the disease, especially in the early, preclinical stage. The use of biomarkers and imaging has helped in the early diagnosis of AD but the establishment of blood biomarkers remains challenging. Next, only a handful of FDA-approved drugs are available for the treatment of AD, which explains the growing body of research on non-pharmacological ways to combat the problem. Hence, numerous studies have explored the possibility and feasibility of using NSCs/NPCs as an alternative treatment option in AD.

There is vast research on the activation and modulation of endogenous NSCs/NPCs by various substances. However, this approach remains challenging because a reduction in NSC proliferation and neurogenesis is thought to give rise to cognitive impairment as well as a decreased plasticity during aging.[101] This means that ageing and neurodegeneration may not be in favour of endogenous NSC/NPC differentiation into new neurons in AD patients. In addition, even though there are some human studies[87,88] in the published literature, the number of patients involved in these studies was small. Therefore, findings from these studies are suggestive but not conclusive, and studies of a larger scale are needed. Furthermore, as NSC proliferation and neurogenesis decrease with age, endogenous stimulation may be limited and run into the risk of cellular exhaustion using chemical means. One possible way of alleviating this is perhaps, by using other stem cells, such as mesenchymal progenitors to exert their paracrine effects through the release of neurotrophic factors.[89]

Many studies have also explored the practicality of exogenous NSC/NPC transplantation. While the results are encouraging and promising, several obstacles still remain in the journey of conquering AD with stem cell-based therapy. Most of these studies have been conducted on lesioned- or transgenic animals and the AD pathology in these animals is not quite the same as that in human. One of the concerns is the long-term survival of the transplanted cells as engraftment may be transient[92] whereas research has shown that cognitive improvements may not always be accompanied with the alleviation of AD pathology.[86] More importantly, deeper exploration is warranted to uncover the underlying mechanisms of the beneficial effects of NSC transplantation. Last but not least, large-scale human studies are needed to investigate the safety and efficacy of NSC transplantation before they can be used as a treatment strategy in patients with AD.

Conflict of interest: The authors declare they have no conflict of interest.

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