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Review Article

Alzheimer's Disease and Stem Cell Therapy

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The loss of neuronal cells in the central nervous system may occur in many neurodegenerative diseases. Alzheimer's disease is a common senile disease in people over 65 years, and it causes impairment characterized by the decline of mental function, including memory loss and cognitive impairment, and affects the quality of life of patients. However, the current therapeutic strategies against AD are only to relieve symptoms, but not to cure it. Because there are only a few therapeutic strategies against Alzheimer's disease, we need to understand the pathogenesis of this disease. Cell therapy may be a powerful tool for the treatment of Alzheimer's disease. This review will discuss the characteristics of Alzheimer's disease and various available therapeutic strategies.

Key words: Alzheimer's disease, stem cell-gene therapy, transplantation

PATHOGENESIS OF ALZHEIMER'S DISEASE

The exact causes of dementia are not yet known. By type, Alzheimer's disease (AD), which is also known as (senile) dementia of the Alzheimer type (DAT), is caused by the aggregation of toxic proteins in the brain, and stroke-induced cerebrovascular dementia accounts for 80~90% of all dementia cases, with hydrocephalus or infectious diseases accounting for the rest. Dementia, which is a degenerative disease that is characterized by the general impairment of cognitive functions caused by temporary or lasting brain damage, is a serious "21st-Century disease."

In the United States, as of 2012, 1 out of 8 senior citizens (13%) is suffering from AD, making it the sixth most common cause of death. Over 5.4 million Alzheimer's patients are currently receiving medical care in the USA, and they incur care costs that are as high as $200 billion a year [1]. AD is a disease that is commonly characterized by a gradual decline of memory, language, and cognitive ability. It was first identified in 1907 by Alois Alzheimer, a German psychiatrist and neuropathologist, in his case report describing the pathological structure of senile plaques and neurofibrillary tangles in the brain of a 55-year-old woman who showed severe dementia symptoms with pathological features, such as a reduction of total brain volume, thinning of the cortical grey matter, ventricular enlargement, *and deposits of amyloid, tau, and cerebrovascular amyloid proteins* [2-5].

Senile plaques and neurofibrillary tangles are the hallmark pathological features that are observed in the brain of an Alzheimer's patient. Senile plaques are deposits of a distinct protein fragment called beta-amyloid (Aβ), which induces neuronal cytotoxicity, and neurofibrillary tangles are abnormal structures that are formed by changes in the tau protein inside nerve cell bodies. The nerve cells in the brains of Alzheimer's patients progressively shrink and die. Such neuronal cell death occurs first in the brain regions that are responsible for memory and language, but it ultimately spreads to the entire brain. The neural

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networks of Alzheimer's patients are impaired by the decreased brain concentrations of acetylcholine, which is a neurotransmitter that is involved in intercellular signaling, and deficiencies in the production of other neurotransmitters, such as somatostatin, serotonin, and norepinephrine [6]. Familial Alzheimer's disease (FAD) is caused by gene mutations, and the aggregation of Aβ is observed in FAD as a result of a gene mutation of the Aβ precursor protein, which is the main component of senile plaques, one of the hallmark pathological features of AD. Such excessive Aβ aggregation destroys neurons. Furthermore, there have been reports on the possible link between the *apolipoprotein E* (*APOE*) gene and the incidence of AD. There are three types of APOE, of which E4 is associated with AD, and E2 and E3 are known to serve the function of providing protection against AD. Everyone carries APOE gene, and APOE epsilon 4 is the susceptible gene. About 40% of AD patients are associated with APOE epsilon 4 (e4), whereas the remaining 50% or more are known to be not associated with APOE genotype. There are three types of APOE, of which E4 is associated with AD, and E2 and E3 are known to serve the function of providing protection against AD [7].

Once AD develops due to the various causes described above, cholinergic neurons and synapses become affected and gradually degenerate or die. Many brain regions then display amyloid plaques and neurofibrillary tangles. Distribution of amyloid plaques can be classified into three stages (stage A, B, C). It is known that they form relatively constant patterns [8]. Neurofibrillary tangles show a regular pattern of aggregation [9]. The disease starts in the transentorhinal cortex and progressively spreads to the entorhinal cortex, the hippocampus, and the cerebral cortex. With the clear manifestation of neuronal cell death, memory and cognitive functions gradually decline along with the progression of dementia, while accelerating the patient's death [10-12].

CHOLINERGIC HYPOTHESIS

In the latter half of the 1970s, neurochemical studies of post-mortem tissue specimens reported damage to the cholinergic system, resulting in decreased acetylcholine-producing choline acetyltransferase (ChAT) activity, decreased choline absorption, and decreased acetylcholine release [13-15], as well as decreased cortical acetylcholinesterase activity [16, 17]. Cholinergic basal forebrain nuclei (ChBF) are the major neural pathways over which cholinergic neurons enter the hippocampus and cerebral cortex, and these nuclei are crucial for memory, concentration, and other cognitive procedures [12, 18]. In many animal experiments, the removal of cholinergic neurons or treatment with cholinergic

antagonists, such as scopolamine or hyoscine, has been shown to elicit impairments of memory and other cognitive functions [19-23].

The hypofunction of cholinergic neurons in the ChBF and cerebral cortex impairs Alzheimer's patients' cognitive functions [11]. The cholinesterase inhibitors (CEI) - rivastigmine, donepezil, and galantamine- suppress the acetylcholinesterase activity of decomposing acetylcholine, reducing cholinergic damage and leading to some improvements in behavior, concentration, and social involvement, as well as cognitive functions. However, they have the drawback of side effect and drug resistance [24] for long-term use. However, a glutamatergic *N*-methyl-D-aspartate receptor antagonist memantine, can also prevent amyloid-induced cholinergic neuron loss, and it is expected to bring about good results if used in combination with a CEI [25].

AMYLOID AND TAU HYPOTHESES

The suppression or removal of the formation of amyloid or neurofibrillary tangles is also crucial in the treatment of AD. Aβ is generated in normal people as well, however, unlike in Alzheimer's patients, amyloid precursor protein (APP) undergoes a sequential cleavage first by α-secretase and then by γ-secretase, generating a water-soluble and nonpoisonous peptide different from Aβ [26]. In contrast, amyloid or Aβ in Alzheimer's patients is a insoluble 4-kDa peptide that is generated when APP is cleaved by β- and γ-secretases [27]. γ-secretase is a multiprotein complex comprising presenilin (PSEN) 1 and PSEN 2, which generates Aβ by cleaving the transmembrane domain of APP after its cleavage by β-secretase [28, 29]. For the most part, Aβ generates $Aβ_{40}$, which consists of 40 amino acids, but, due to a large number of cleavage sites, it occasionally generates a small amount of $Aβ_{42}$, which is more likely to form fibrils more resistant to decomposition, making it more toxic to neurons compared to $Aβ_{40}$.

While late-onset AD occurs in people aged 65 or older, FAD develops earlier because FAD is triggered by gene mutations of APP (chromosome 21), PSEN1 (chromosome 14), or PSEN2 (chromosome 1), thereby eliciting Aβ aggregation in earlier years [30, 31]. Of these 3 types, the PSEN1 mutation has a relatively high proportion of $Aβ_{42}$. As $Aβ_{42}$ is more toxic than $Aβ_{40}$, FAD progresses more rapidly in this case, and the time of onset can come as early as 20~30 years of age.

The brain has a small quantity of antioxidant enzymes despite its high amount of oxygen consumption, which makes it susceptible to reactive oxygen species. Aβ causes damage to mitochondrial membranes and hence increases the amount of intracellular H_2O_2, thus affecting the genes downstream by interacting with numerous

receptors and damaging neurons, ultimately accelerating cell death [32].

Tau is a neuronal microtubule-associated protein that stabilizes axonal microtubules by binding with them. If tau proteins get phosphorylated, they are separated from microtubules, and they form paired helical filaments in the neuronal cytoplasm [33]. Neurofibrillary tangles are abnormal intracellular aggregates of bundles of 12-kDa protein consisting of the residual microtubule-binding sites of tau protein after the truncation of the N- and C-terminal domains. Although it is not clearly known whether Aβ plays a role in the formation of neurofibrillary tangles, a study has reported [34] that the injection of $A\beta_{42}$ into the brain of a tau-transgenic mouse resulted in a 5-fold increase in the formation of neurofibrillary tangles that was elicited by an increase of tau phosphorylation.

Whereas Aβ triggers the formation of neurofibrillary tangles, it is the formation of neurofibrillary tangles rather than Aβ itself that aggravates Alzheimer's symptoms. In fact, in the case of FTDP-17, which is a chromosome 17-type dementia involving mutations in the tau gene, the symptoms of dementia are manifested without any Aβ aggregation [35].

Stem cell therapy for AD

Stem cells have therapeutic effects using regeneration and substitution of cells and tissues themselves. The therapeutic strategy of stem cell has two directions. One is to induce the activation of endogenous stem cell and the other is to regenerate injured cell or tissues through stem cell transplantation (Table 1).

Endogenous stem cells can be induced and show neuroprotective effects by Chemical compounds and factors stimulating stem cells such as allopregnanolone (Apα), fluoxetine, granulocyte colony stimulating factor (G-CSF), AMD3100 and stromal cell-derived factor-1a (SDF-1α). Apα induced endogenous neural precursor cells (NPCs) activation and promoted survival of newly generated cells showing significantly increasing BrdU+ cells as well as improvement of learning and momory in 3xTgAD mice [36]. Another research group used three factors to stimulate endogenous hematopoietic progenitor cells (HPC). GCSF and AMD3100, CXCR4 antagonist, and SDF-1α facilitated the mobilization and migration of bone marrow derived hematopoietic progenitor cell (BM-HPCs) into brain. AD model mice were improved memory as well as hippocampal neurogenesis in AD animal models after treatment of three factors, whereas Aβ deposition was not changed. These factors may act synergistically to migrate HPC and to produce a therapeutic effect [37]. Fluoxetine treatment was shown the neuronal differentiation and protective effects of NSCs against Aβ induced cell death [38].

It has recently been reported [39-45] that Alzheimer's symptoms could be alleviated by transplanting stem cells derived from human umbilical cord, ammniotic membrane-derived epithelial cells and mesenchyme into the brains of Alzheimer's transgenic animals. The treatment led to improve cognitive and memory performances and increased neuronal survival as a result of the decrease in Aβ, APP generation, and microglia activation. Another study [46] has reported a therapeutic effect of decreasing the size and number of Aβ as a result of differentiating peripheral mononuclear cells into microglia by injecting stromal cell-derived factor 1 into Alzheimer's transgenic animals.

Transplantation of stem cells has shown promise for improving functional recovery for Alzheimer's disease. MSCs could promote survival, increased the metabolic activity and help to rescue the AD cell model in vitro [47]. The coculture of human MSCs and BV-2, mouse microglia, increased neprilysine expression, the Aβ-degrading enzyme, under the exposure of Aβ [48]. The transplantations of human and mouse MSC derived stem cells were shown to reduce Aβ deposition, to improved memory and to alleviate the AD pathology in AD mouse models [49-51]. Mouse NSCs were colonized around amyloid plaques and modified to express metalloproteinase 9 (MMP9), a secreted protease reported to degrade aggregated Ab peptides, whereas these NSCs didn't migrate into other regions after transplantation in AD mouse brain [52]. ADSCs also improved AD pathology involving reduction of Aβ deposition and memory improvement due to decreasing of proinflammatory factors [53]. Human amniotic epithelial cells (HSECs) were observed their survivals and no any immune rejection for 8 weeks. HAEC transplantation significantly ameliorated spatial memory deficits in TG mice, as well as increased acetylcholine levels and the number of hippocampal cholinergic neuritis [39].

Stem cell itself has therapeutic effects however, further studies are needed to determine the appropriate conditions to improve the therapeutic effects for AD pathology.

Gene therapy for AD

For the development of new medical drugs, it is necessary to gain a deeper understanding of the genetic factors of AD, the roles of amyloid and tau protein, and the mechanisms involved in neuronal degeneration. The current therapeutic mechanism of Alzheimer's is to provide maximum support for the functions of the neurons remaining in the patient's brain. The latest research direction of DAT focuses on early diagnosis, given that the medication that is administered upon the initial manifestation of memory loss can help to maintain the quasi-normal state of cognitive functions longer.

Table 1. Stem cell therapy for AD

Cell	Additional Treatment	Model
Endogeneous bone marrow derived hematopoietic progenitor cell (BM-HPCs)	GCSF, AMD3100 and SDF-1α	APP/PS1 mice
Endogeneous NPCs	Subcutaneous inj. of Allopregnanolone (Apα)	3xTgAD mice
NSCs	Fluoxetine treatment	In vitro
MSCs	Coculture with AD model cells	Truncated tau (151-391) expressing neuroblastoma cells
HUMSCs	Coculture with BV2 and Aβ transplantation	APP/PS1 mice, Aβ exposure
Bone marrow derived mesenchymal stem cells (BM-MSCs)	Transplantation	AD mice
Human umbilical cord mesenchymal stem cell (HUMSCs)	Transplantation	APP/PS1 mice
Bone marrow derived monocytic cells (BMM)	Monocyte Differentiation	Irradiated mice
NSCs	Transplantation	APPswe/PS1dE9 Line 85 mice
Adipose derived stem cells (ADSCs)	Intracerebral transplantation	APP/PS1 mice
Human amniotic epithelial cells (HAECs)	Transplantation	APP/PS1 mice

Table 1. Continued

Cell	Results	Ref.
Endogeneous bone marrow derived hematopoietic progenitor cell (BM-HPCs)	*Induction of endogeneous BM-HPCs *Improved memory	37
Endogeneous NPCs	*Apα promoted survival of newly generated cells and restored cognitive performance	36
NSCs	*Enhance neuronal differentiation *Protective effects against Aβ induced cell death	38
MSCs	*Promote survival *Increased the metabolic activity *Rescue the AD cell model	47
HUMSCs	*Increase neprilysin expression	48
Bone marrow derived mesenchymal stem cells (BM-MSCs)	*Reduction in Aβ deposits	49
Human umbilical cord mesenchymal stem cell (HUMSCs)	*Reduced Aβ deposition *Improved memory *Microglia activation *Increased antiinflammatory cytokine	50
Bone marrow derived monocytic cells (BMM)	*Reduce Aβ burden *Alleviating the AD pathology	51
NSCs	*Colonization in white matter tracts *High conc. Of MMP9 around amyloid plaques *No migration	52
Adipose derived stem cells (ADSCs)	*Reduced Aβ deposition *Improved memory *Decreased proinflammatory factors	53
Human amniotic epithelial cells (HAECs)	*Survival of HAECs for 8 weeks *Migration without immune rejection *Ameliorated memory *Increased acetylcholine levels	39

Table 2. Gene therapy for Alzheimer's disease (AD)

Cell	Gene	Model	Results	Ref.
Encapsulated cell (EC)	Nerve growth factor (NGF)	Human AD patient	Adverse effects in 1/3 of patients, No toxicity, Improved cognitive functions, Improved electroencephalography, Improved nicotinic receptor binding	64
hNSC, F3.ChAT	Choline acetyltransferase (ChAT)	Drug-induced AD Rat model	Improved learning and memory performance, Increase of acetylcholine, Cell migration in the entire brain, Differentiation into neurons and glial cells	66
hNSC F3.NGF	Human NGF	APP/PS1 TG mouse	Improved water maze performance, Increase of DeltaNp73 expression, Improved proliferation of transplanted cells, Decrease of senile plaques	67

There have been recent encouraging results in animal studies with administration of Aβ antibodies to PDAPP mice in order decrease Aβ. They showed the recovery of acetylcholine release and choline absorption in the hippocampus. The learning capacity was also improved [54], The results have led to related clinical trials in humans [55, 56]. Continuous reduction of Aβ might be another method of addressing Alzheimer's. This can be done with proteases such as neprilysin [57], insulin-degrading enzyme [58, 59], plasmin [60], and cathepsin B [61]. The intraventricular injection of the human neprilysin gene expressing viruses into amyloid transgenic mouse models has contributed to a decrease in Aβ aggregation and neuronal degradation in the frontal cortex and hippocampus [62]. According to a report [63], intraventricular injections of the human neprilysin gene expressing fibroblasts into Aβ-aggregation transgenic mouse models have resulted in a considerable decrease of amyloid plaques. These studies have provided the evidence that proteases can be used as Aβ-reducing therapeutic agents on account of their function of decomposing Aβ and paved the way for cure-oriented studies that focus on protease-expressing neural stem cells.

In an effort to overcome this drawback, the Tuszynski research team has recently conducted a study of ex vivo gene therapy with NGF (Table 2) [64]. Similarly, in clinical trials where human NGF genes were grafted into the fibroblasts harvested from each patient and that were transplanted back into the basal forebrain area of the patient, 6 patients were confirmed to show improvements of cognitive functions and increases of cerebral cortex metabolism in positron emission tomography after 22 months of the intervention without any side effects or toxicity, with 2 of them showing improvements in cognitive performances, electroencephalography, and nicotinic receptor binding [65].

ChAT-overexpressing human neural stem cells (HB1.F3.ChAT) were transplanted into Alzheimer's animal models [40, 66]. As a result, it was confirmed that learning and memory functions were restored, the volume of acetylcholine in the cerebrospinal fluid was increased, and the transplanted cells were successfully migrated to several brain regions [40, 66]. According to a report, it was observed that transplantation of nerve growth factor (NGF) expressing human NSCs into hippocampus region of ibotenic acid-injected mice (one of the cognitive dysfunction models) could be improved the learning and memory as well as differentiated into neuron and astrocytes. These NGF carrying hNSCs showed the further neuro-protective effects than parental hNSCs against cytotoxic agents [67].

Among the cells expressing foreign genes that are used for Alzheimer's therapy, HB1.F3.ChAT has proved its effectiveness. When these cells were applied to animal models with cognitive defects induced by AF64A and kainic acid, similarly safe and effective results were obtained [66]. Taken together, it can be expected that therapies with cells that simultaneously express neurotransmitters and growth factors could achieve better results.

CONCLUSION

Alzheimer's therapies so far have revolved around retarding the progression of the disease rather than restoring the damaged neurons. However, the recent trend is to focus on removing the causes of the disease with stem cell-based therapies. If the causes of AD are understood more deeply and safer cell therapies are developed, AD could be conquered in the not too distant future.

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