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Therapeutic Potential of Human Adipose-Derived Stem Cells in Neurological Disorders

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Abstract. Stem cell therapy has been noted as a novel strategy to various diseases including neurological disorders such as Alzheimer's disease, Parkinson's disease, stroke, amyotrophic lateral sclerosis, and Huntington's disease that have no effective treatment available to date. The adipose-derived stem cells (ASCs), mesenchymal stem cells (MSCs) isolated from adipose tissue, are well known for their pluripotency with the ability to differentiate into various types of cells and immuno-modulatory property. These biological features make ASCs a promising source for regenerative cell therapy in neurological disorders. Here we discuss the recent progress of regenerative therapies in various neurological disorders utilizing ASCs.

Keywords: neurological disorder, adipose-derived stem cell (ASC), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD)

1. Introduction

Stem cell therapy for central nervous system (CNS) repair has great clinical potential (1). In contrast to the controversy over utilization of embryonic stem cells which are theoretically highly beneficial due to their pluripotent ability, mesenchymal stem cells (MSCs) are recognized to be a relatively effective, ethically safe, and acceptable source for cell therapy to treat various neurological diseases such as stroke (2).

Up to now, large amount of evidences accumulated on MSC behavior demonstrated that under appropriate conditions, MSCs selectively differentiate not only into mesenchymal lineages but also into endodermal and ectodermal cell lineages in vitro (3 – 7). MSCs are present in adult tissues including bone marrow and adipose tissue. However, the clinical use of bone marrow–derived stem cells (BSCs) has presented problems including pain, morbidity, and low numbers of harvested cells. In contrast, adipose-derived stem cells (ASCs), which are derived from adipose tissue, are one of the most advantageous resources due to easier access to adipose tissue and abundance with proliferation and differentiation potential (8). ASCs have a high proliferation capacity in vitro and differentiate into cells with several neuronal and glial characteristics, including expression of neuronal and glial proteins (9, 10). Implantation of human ASCs leads to no adverse side effects such as tumorigenicity, chromosomal abnormalities, or immune rejection (11).

Although the stem nature of ASCs could be doubted, previous studies have suggested that BM-MSCs and ADSCs exhibit a virtually identical transcriptional profile for stemness-related genes (12, 13) and furthermore, MSC derived neuronal cells have been shown to be electrically excitable (7). In addition, some authors have found that few cells that were capable of engrafting into the nervous tissue fused with endogenous cells and thereby acquired the phenotype of the partner host cell (14 – 16).

Therefore, ASCs have recently received attention as a promising source of cells for cell therapy, and the

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Invited article

Table 1. Applications of adipose-derived stem cells (ASCs) in the treatment of neurological diseases

Disease	Animal model	Potential roles of ASCs	Ref.
Alzheimer's disease	Tg2576 mouse	Restoring memory deficit and neuropathology	(6)
	APP/PS1 double Tg mouse	Reducing Aβ deposition and restoring the learning/memory function	(7)
Amyotrophic lateral sclerosis	SOD1-G93A Tg mouse	Improving motor performance	(8)
		Delaying disease progression and prolonging life span	(9)
Huntington's disease	Quinolinic acid (QA)-induced rat, R6/2 Tg mouse	Slowing striatal degeneration and behavioral deterioration	(10, 12, 42)
	YAC128 Tg mouse	Reducing striatal atrophy	(11)
Parkinson's disease	MPTP-lesioned hemi-parkinsonian rhesus monkeys	Improving behavioral symptom replacing lost neuron	(13)
	Rotenone-injected rat	Immune-modulating and increasing neurotrophic factor, brain dopamine, and brain TH gene expression	(14)
Cerebral infarction (stroke)	Permanent middle cerebral artery occlusion (MCAO)-induced ischemic stroke SD rat	Improving functional recovery with reduced cell death and increased cell proliferation	(18, 23)
	Transient middle cerebral artery occlusion (MCAO)-induced ischemic stroke SD rat	Limiting ischemic volume and brain infaction area and improving functional recovery in sensory motor activity with neurogenesis	(17, 19, 23)
	Transient middle cerebral artery occlusion (MCAO)-induced ddY- or ischemic stroke C57BL/6 mouse	Showing neuroprotective and ameliorative effects with increased proliferative activity and decreased infarction size	(20, 25)
	Collagenase-induced intra-cerebral hemorrhagic (ICH) stoke SD rat	Decreasing neurological, behavioral deficit and severity	(21 – 24)
	Transient common carotid artery occlusion-induced hypoxia-ischemic (HI) neonatal SD rat	Attenuating brain damages and deficit in spatial learning and memory	(28)
Spinal cord injury (SCI)	SCI adult female SD rat	Forming a PNS-type myelin sheath with Ranvier node-like structures.	(31)
	Traumatic SCI adult female Wistar rat	Preventing secondary pathological events and improving neurologic function	(32)
Multiple sclerosis	EAE C57BL/6 mouse	Attenuating EAE symptoms	(34, 36)
	Cuprizone mouse	Remyelination of corpus callosum axons	(35)
	EAE C57BL/6 mouse	Providing neuroprotection, immunomodulation, and/or remyelination	(37)
Niemann–Pick disease	NP-C GsbsGFP/+ mouse	Rescuing imperiled Purkinje neurons and alleviating the inflammatory response	(43)
Neuropathy	Sciatic nerve chronic constriction injury mouse	Reverting nociceptive hypersensitivity	(44)

therapeutic efficacy of ASCs has been assessed in various animal models with specific neurological disorders (Table 1).

2. Therapeutic potential of ASCs for Alzheimer's disease (AD)

AD is the most common neurodegenerative disorder characterized by the accumulation of amyloid plaques and neurofibrillary tangles accompanied by cognitive dysfunction. There are no drug treatments available that can provide a cure for AD. However, medicines have been developed that can improve symptoms or temporarily slow down their progression in some people. There are two main types of medication used to treat AD: cholinesterase inhibitors and NMDA-receptor antagonists. Cholinesterase inhibitors include donepezil hydrochloride, rivastigmine, and galantamine. The NMDA-receptor antagonist is memantine (17 – 19).

Therapeutic potential of intracerebral (i.c.) or intravenous (i.v.) injection of human ASCs was previously reported in an AD mouse model, Tg2576 transgenic (Tg) mice, by our group. Our study showed that intravenously transplanted ASCs passed through the blood brain barrier (BBB) and migrated into the brains of Tg mice (20). I.v. or i.c. injected ASCs significantly improved learning and memory and restored neuropathology including amyloid beta deposition in Tg mice (20). In addition, elevating endogenous neurogenesis and synaptic and dendritic stability were shown in ASCs-transplanted Tg mouse brains (20). However, interleukin-10 (IL-10) and vascular endothelial growth factor (VEGF) were up-

regulated in ASCs-transplanted Tg mouse brains (6).

The therapeutic effects of i.c. ASCs transplantation were also evaluated in an APP/PS1 double Tg AD mouse model (21). The transplantation of ASCs reduced amyloid beta (Aβ) deposition and restored the learning and memory function in APP/PS1 double Tg mice (21).

These studies suggested that activated microglia might be involved in the mechanisms to ameliorate the neuropathological deficits in AD (20, 21). More activated microglia were detected in both regions of the hippocampus and cortex after ASCs transplantation, exhibiting decreased expression levels of pro-inflammatory factors and elevated expression levels of Aβ-degrading enzymes as well as neprilysin (20, 21). The mechanisms behind the role of ASC in microglial activation are largely unknown. However, activation of microglia by ASC transplantation may act as a natural defense mechanism to prevent Aβ accumulation or reduce Aβ deposits. Another study has also shown a similar set of results and the BM-MSCs were able to reduce the inflammatory response and also restore defective microglial function (22). This study suggested that activation of microglia with conversion to an alternative phenotype results in a feed-forward loop whereby significantly increased levels of microglial cells correlate with enhanced Aβ-clearance following BM-MSC transplantation.

3. Therapeutic potential of ASCs for amyotrophic lateral sclerosis (ALS)

ALS is a neurodegenerative disease that selectively affects motor neurons in the cortex, brain stem, and spinal cord. The precise pathogenic mechanism remains unknown, and there is no effective therapy.

The efficacy of the systemic administration of ASCs was assessed in an ALS mouse model, superoxide-dismutase 1 mutant [SOD1-G93A] Tg mice (23). Clinical and neurophysiological tests showed that the administration of ASCs to Tg mice at the clinical onset significantly delayed motor deterioration for several weeks (23). In ASCs-treated Tg mice, the number of lumbar motor neurons and levels of glial-derived neurotrophic factor (GDNF) and basic fibroblast growth factor (bFGF) were increased (23).

A recent study from Kim et al. suggest that transplantation of ASCs in [SOD1-G93A] Tg mice delays disease progression and prolongs life span of Tg mice through neuroprotective effects by production of cytokines/growth factors (24). Human ASCs were intravenously (i.v.) and intracerebroventricularly (i.c.v.) transplanted into Tg mice before the appearance of clinical symptoms (24). In Tg mice transplanted with ASCs via the i.c.v. route, delayed clinical symptoms were shown by behavior assessments such as the rotarod test, paw grip endurance, and reflex index; and the survival of animals was extended (24). In cultured neuronal cells and in the ALS spinal cord, ASCs secreted high levels of neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotropic factor (BDNF), insulin-like growth factor 1 (IGF-1), and VEGF and reduced the cytotoxicity by these factors (24).

4. Therapeutic potential of ASCs for Huntington's disease (HD)

HD is a late-onset neurodegenerative disease caused by abnormal expansion of CAG repeats in the huntingtin gene, which is characterized by a progressive loss of medium spiny neurons in the basal ganglia. Several studies have examined the potential of MSCs including ASCs for transplantation into HD models as neuroprotective treatment or cell replacement therapies.

The effects of human ASCs on HD pathology was investigated by Lee et al. (25) in cell culture experiments and in two different rodent HD models, QA model and R6/2 Tg mice. In vitro study showed that human ASCs secreted multiple growth factors, including epidermal growth factor (EGF), BDNF, NGF, IGF-1, and ciliary neurotrophic factor (CNTF) (25). Transplantation of ASCs into the QA model revealed significant improvement in the apomorphine-induced rotation test over 4 weeks, reduced lesion volume, and a lower number of apoptotic striatal cells compared to control animals (25). In R6/2 Tg mice, transplantation of ASCs improved rotarod performance and limb clasping, increased survival, attenuated the loss of striatal neurons, and reduced the huntingtin aggregates (25).

In the same group, transplantation of human ASCs was compared with normal human ASCs in another HD animal model, YAC128 Tg mice, which showed slow disease progression for 12 months (26). An in vitro study showed that cultured HD ASCs express multiple growth factors (BDNF, HGF, IGF, LIF, NGF, and VEGF) with the same cell surface markers (CD13, CD29, CD31, CD34, and CD44) as those of normal ASCs (26). There was no significant difference in rotarod performance and body weight in Tg mice transplanted with either normal ASCs or HD ASCs compared with Tg control mice (26). Normal ASC transplantation reduced the striatal atrophy, while HD ASCs failed to prevent it (26). Transplantation of normal ASCs after onset of disease phenotype maintained the rotarod performance for 4 weeks, but no significant difference in striatal atrophy was observed. In the same case, transplantations of HD ASCs were not effective (26).

To examine the potential of ASC transplantation in the

HD model and to further assess the possibility of the paracrine effects of human ASCs via secreted multiple growth factors, the same researchers investigated effects of cell-free extracts of ASCs (ASCs-E) on the R6/2 HD mouse model (27). Injection of ASCs-E improved the performance in the rotarod test, ameliorated the striatal atrophy and reduced mutant huntingtin aggregation in the striatum in the R6/2 HD mouse model (27). Therefore, ASCs-E might slow disease progression in an animal model of HD and could be a potential resource for treatment of HD (27).

5. Therapeutic potential of ASCs for Parkinson's disease (PD)

PD is the second most common neurodegenerative disorder characterized by the loss of neurons in the substantia nigra pars compacta (SNpc) in middle-aged and elderly people.

A recent study investigated the therapeutic effects of the combined transplantation of neuronal-primed ASCs derived from rhesus monkey and adenovirus containing Neurturin (NTN) and tyrosine hydroxylase (TH) (Ad-NTN-TH) into methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned hemi-parkinsonin rhesus monkeys (28). An in vitro study with the use of LIM homeobox transcription factor 1, alpha (LMX1A) and NTN showed that NTN-conditioned medium protected dopaminergic neurons against 1-methyl-4-phenylpyridinium (MPP+) toxicity and the LMX1A- and NTN-infected ASCs showed a dopaminergic differentiation with secreting the dopamine (28). ASCs combined with Ad-NTN-TH were implanted into the striatum and SNpc of the rhesus monkey PD model (28). Transplantation of ASCs improved parkinsonian behavior as such in tremor recovery and motility in combination-transplanted monkeys (28). Single-photon emission computed tomography analysis showed that the transplantation of combined ASCs with Ad-NTN-TH increased dopamine transporter uptake at the striatum, but not the neuronal-primed ASC-transplantation (28). In postmortem analysis, neuronal-primed ASCs could replace lost neurons and reconstruct the nigrostriatal pathway in the brain (28). The grafting of ASCs combined with Ad-NTN-TH had more neuroprotective effects compared with Ad-NTN-TH or ASCs alone (28).

Another group performed a biomarker study in a rotenone-induced PD rat model treated with ASCs (29). ASCs treatment restored the increased serum transforming growth factor β (TGF-β) and monocyte chemoattractant protein 1 (MCP-1) levels and increased serum BDNF, brain dopamine, and brain TH gene expression levels in a PD rat model (29). Their study suggests that ASCs infusion might be attributed to their immunomodulatory, anti-inflammatory, and neurotrophic effects.

6. Therapeutic potential of ASCs for stroke

Stroke is known to be the third most frequent cause of mortality in industrial countries (30) with ischemic stroke being the most prevalent. Considering the complications of thrombolytic therapy in acute ischemic stroke, development of an alternative therapeutic intervention is necessary and utilization of various kinds of stem cells including MSCs have been explored to treat ischemic stroke patients such as in cytotherapy. Among the MSCs, ASC is one of the most advantageous resources (31). Thus, a number of studies have demonstrated its positive effects on cerebrovascular insults such as hemorrhagic and mostly ischemic stroke (32 – 44). Although none has been proven practically efficient in human stroke patients to this point, numbers of experimental trials in vitro or in vivo animal models regarding ASC-associated therapy have been introduced as a promising therapeutic option against stroke.

6.1. Clinical potential for transplantation of ASCs against stroke

Intravenously transplanted autologous ASCs in transient or permanent middle cerebral artery occlusion (MCAO)-induced ischemic stroke adult Sprague-Dawley (SD) rats demonstrated positive effects in infarct size, apoptosis, oxidative stress, and inflammatory response with enhanced signatures of neurogenesis, oligodendrogenesis, angiogenesis, or synaptogenesis in addition to neurological functional recovery against experimental ischemic stroke (32 – 34), while no reduction in infarct volume or any migration/implantation of cells into the lesion were observed in certain cases (33, 34). Similarly, i.v. administration of allogenic ASCs into age-matched ischemic stroke C57BL/6J mice generated by transient MCAO attenuated ischemic damage such as infarct size and brain swelling and enhanced motor functional recovery with expression of some growth factors such as hepatocyte growth factor (HGF) and angiopoietin-1 in ischemic brain tissue, although ASCs were not fully incorporated into the infarct area (35). Despite ischemia is accountable for most cases of stroke, the therapeutic potential of ASCs in hemorrhagic stroke has been also demonstrated. Intraventricular (i.v.) injection of either human or murine ASCs as well as intraperitoneal (i.p.) administration of cell-free extract of human ASCs into rats inflicted with experimental intracerebral hemorrhage (ICH) resulted in the induction of neuronal differentiation and functional improvement represented by a considerable reduction in neurological or behavioral

deficit and severity in addition to a decrease in brain water content, hemorrhagic volume, and parenchymal atrophy with reduced apoptosis and cerebral inflammation (36 – 39).

However, due to the low survival rate and potential risk for tumorigenicity elicited by the implantation of stem cells, utilization of conditioned medium of ASCs (ASCs-CM) might provide an alternative way to overcome these limitations, and its application has also been attempted as ASC therapy.

6.2. Clinical potential for administration of ASCs-CM against stroke

The neuroprotective effects of human and murine ASCs-CM were observed in vitro. Pretreatment with ASCs-CM collected from the cultures of ASCs originally isolated from human subcutaneous adipose tissue or the fat pad of female C57BL/6-Tg mice significantly reduced glutamate-induced excitotoxicity in the SH-SY5Y or PC12 cells (41, 43). Related to the glutamate-induced excitotoxicity, rat cortical neurons were also co-cultured with human ASCs-CM or ASCs that were separated from neurons with porous membrane to see the effects of ASCs-CM. The neuroprotective effects were verified against glutamate excitotoxicity supported by the data showing inhibition of neuronal cell damage, apoptosis, and glutamate-induced energy depletion as well as the promotion of nerve regeneration and repair (42). A range of in vivo models employing ASCs-CM have also been introduced. Pretreatment of murine or human ASCs-CM by i.c.v. administration before MCAO demonstrated significant reduction in infarct area and volume. In addition, rapid treatment of ASCs-CM immediately after MCAO was also effective, although administration 2 h after MCAO was not (41). I.v. administration of concentrated ASCs-CM collected from SD rat into 7-day-old neonatal SD rat either 1 h before or 24 h after induction of hypoxia-ischemia significantly protected hippocampal and cortical volume loss and demonstrated significant behavioral and learning functionality (44). Although treatment of the initial phase of the stroke is essential, increased intracerebral pressure, which could worsen the consequences of the stroke and increase lethality, is likely due to the infusion of ASCs-CM at the acute phase of the stroke. Continuous infusion of ASCs-CM collected from human ASCs in a three-dimensional spheroid form was administered in the subchronic phase of the SD rat stroke model induced by standard MCAO, and significant therapeutic effects against stroke were also observed (40). Accordingly, utilization of ASCs-CM appears to exert similar ameliorative effects on both in vitro and in vivo stroke models. More discussions in depth for clinical feasibility and plausible approaches of ASC-associated therapy to treat chronic stroke were also proposed (45).

7. Therapeutic potential of ASCs for other neuronal disorders

7.1. Spinal cord injury (SCI)

Therapeutic promise for SCI has also been assessed using ASCs. SCI typically results from sustained trauma to the spinal cord, resulting in loss of neurologic function at the level of the injury. When the induced ASCs were engrafted to SCI lesions, they formed a peripheral nervous system (PNS)-type myelin sheath on CNS axons (46). In in vitro study, ASCs expressed Schwann cells (SCs) markers such as A2B5, GFAP, O4, p75, S100, Sox10, Krox-20, and L1, indicating the possibility to differentiate into SCs (46). In addition, the ASCs induced into SCI expressed several neurotrophic factors in repaired tissue (46). Especially, the secondary damage following SCI exacerbates the injury and retards repair mechanisms. ASCs have a strong, long-lasting immunosuppressive capacity via soluble factors. Direct injection of ASC extracts (ASC-E) mixed with matrigel into the spinal cord immediately after SCI also resulted in reduced apoptotic cell death, astrogliosis, and hypomyelination but did not reduce the extent of microglia infiltration (47). In addition, ASC-E injected animal showed significant functional improvement of hind limbs (47).

7.2. Multiple sclerosis

Multiple sclerosis (MS), also known as disseminated sclerosis or encephalomyelitis disseminate, is an inflammatory disease (48). Previous studies have demonstrated the potential of stem cell–based MS therapy in autoimmune and cuprizone models of MS (49 – 51). I.v. administration of ASCs before disease onset significantly reduces the severity of experimental autoimmune encephalomyelitis (EAE) by immune modulation and decreases spinal cord inflammation and demyelination in MS animal model (49). Combined therapy of i.v. transplantation of ASCs with 17β-estradiol (E2) showed the enhanced efficacy on remyelination of corpus callosum axons in a cuprizone mouse model of MS compared with ASCs monotherapy (50). A more recent study demonstrated that therapeutic efficacy of human ASCs from older donors was reduced compared with that of cells from younger donors for disease prevention. ASCs from older donors failed to ameliorate the neurodegeneration associated with EAE and increased CNS inflammation and demyelination (51). Therapeutic effects of ASCs were compared in two independent routes of injection, i.p. and i.v. (52). ASCs transplantation in the

i.p. or i.v. route showed immunomodulative and neuroprotective effects; however, i.p. injection of ASCs has an enhanced efficacy in maintaining the splenic CD4 + CD25 + FOXP3 + T cell population and increase of IL-4 secretion (52).

8. Conclusion

To explain mechanisms underlying the ameliorative and neuroprotective effects of ASC therapy against neurological disorders, researchers introduced stacks of mediators mostly involved in angiogenesis, synaptogenesis, gliogenesis, and/or neurogenesis. The mediators expressed or secreted from ASCs include various growth factors containing neurotrophic factors such as NGF, BDNF, GDNF, VEGF, HGF, IGF-1, and others such as chemotactic factors (SDF-1 and CXCR4) or a synaptic vesicle protein, synaptophysin, along with diverse neuronal and glial cell proteins and MSC markers (32, 33, 35, 36, 43, 53).

In addition, transplantation of ASCs ameliorates the increased levels of pro-inflammatory cytokine expression, such as TGF-β and IL-1β; secretes anti-inflammatory cytokine, IL-10; and is thought to participate in immunomodulation (54, 55) (Fig. 1). Furthermore, in line with the positive consequences obtained from ASCs-CM approaches, recent findings underline the importance of the "paracrine effect" of stem cell treatment (56). Although we discussed current clinical applications of ASCs in neurological disorders in this review, the potential relevance of ASCs or even other MSCs for the treatment of neuropsychiatric disorders such as schizophrenia, autism or depression is plausible. However, it seems to be too immature to discuss in this review since the evidences related to clinical applications utilizing ASCs are rare and even those are suggestive and currently in trial stage (57 – 61).

Altogether, ASC-associated therapy is very likely to

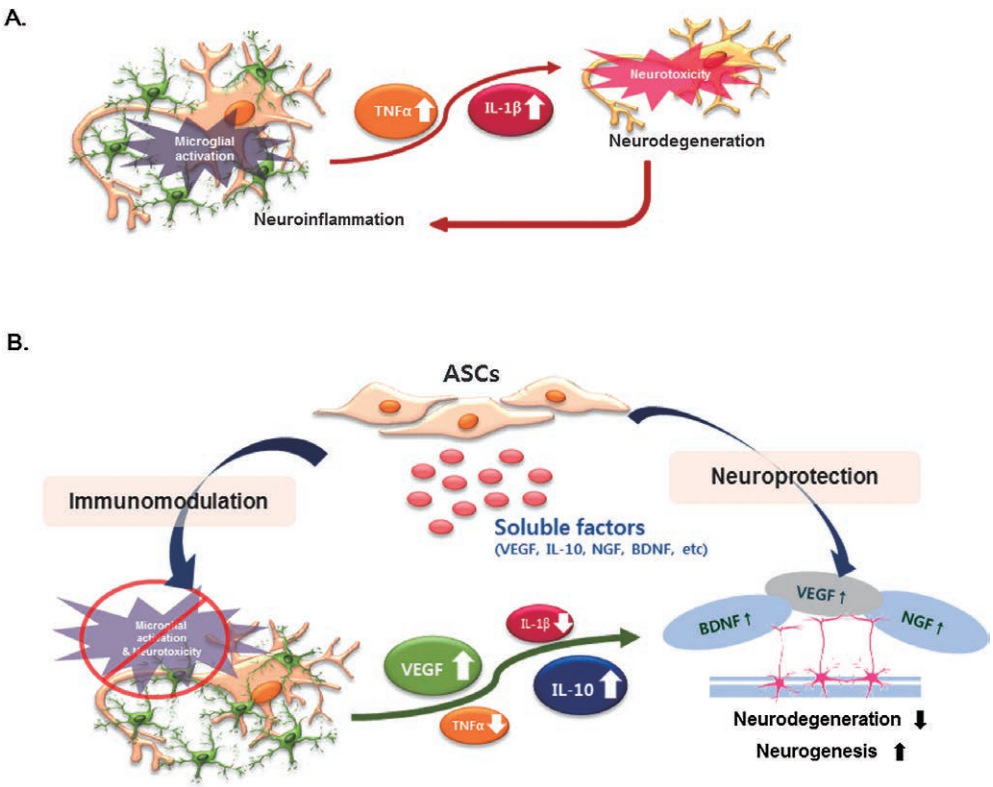

Fig. 1. Paracrine and immunomodulatory effects as possible mechanisms of roles of adipose-derive adult stem cells (ASCs) in the treatment of neurological diseases. A: Neurological diseases are associated with immune alterations and neuroinflammation including pro-inflammatory cytokines (i.e., IL-1β and TNFα) overproduction. B: Intracerebrally or intravenously injected ASCs are thought to modulate a hostile microenvironment of diseases through secretion of large amounts of several bioactive molecules (paracrine activity) such as VEGF and IL-10. In addition, transplanted hASCs might benefit the brain by inducing proliferation of endogenous early-stage neurons and surrounding cells through increasing neurotropic factors such as BDNF, NGF, and VEGF, suggesting the increase of neurogenesis and dendrite and synaptic stability. Therefore, these molecules derived from transplanted ASCs attribute to their immunomodulatory, anti-inflammatory, and neurotrophic effects; neurogenesis; or synaptogenesis.

be a promising clinical option for the treatment of diverse neurological disorder patients. To optimize and maximize the efficiency with safety and expediency in ASC therapy, future trials need to sharpen our current knowledge and perspectives for the clinical utilization of ASC or ASC-derived resources, including ASCs-CM.

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