REVIEW

Adult human neural stem cell therapeutics: Current developmental status and prospect

Hyun Nam, Kee-Hang Lee, Do-Hyun Nam, Kyeung Min Joo

Hyun Nam, Kee-Hang Lee, Do-Hyun Nam, Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, South Korea

Kee-Hang Lee, Do-Hyun Nam, Kyeung Min Joo, Samsung Advanced Institute for Health Sciences and Technology, Samsung Medical Center, Sungkyunkwan University, Seoul 135-710, South Korea

Kyeung Min Joo, Department of Anatomy and Cell Biology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, South Korea

Correspondence to: Kyeung Min Joo, MD, PhD, Department of Anatomy and Cell Biology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-Dong, Gangnam-gu, Seoul 135-710, South Korea. kmjoo@skku.edu
Telephone: +82-2-21489779
Fax: +82-2-21489829

Abstract

Over the past two decades, regenerative therapies using stem cell technologies have been developed for various neurological diseases. Although stem cell therapy is an attractive option to reverse neural tissue damage and to recover neurological deficits, it is still under development so as not to show significant treatment effects in clinical settings. In this review, we discuss the scientific and clinical basics of adult neural stem cells (aNSCs), and their current developmental status as cell therapeutics for neurological disease. Compared with other types of stem cells, aNSCs have clinical advantages, such as limited proliferation, inborn differentiation potential into functional neural cells, and no ethical issues. In spite of the merits of aNSCs, difficulties in the isolation from the normal brain, and in the *in vitro* expansion, have blocked preclinical and clinical study using aNSCs. However, several groups have recently developed novel techniques to isolate and expand aNSCs from normal adult brains, and showed successful applications of aNSCs to neurological diseases. With new technologies for aNSCs and their clinical strengths, previous hurdles in stem cell therapies for neurological diseases could be overcome, to realize clinically efficacious regenerative stem cell therapeutics.

Key words: Adult neural stem cell; Neurological diseases; Stem cell therapy; Preclinical trial; Clinical trial

Core tip: In this review, we compare advantages and disadvantages of various types of stem cells for regenerative therapy in neurological disease, and discuss the preclinical and clinical developmental hurdles of stem cell technologies. While at present, adult neural stem cells (aNSCs) have clinical advantages, technical issues in the isolation and expansion of aNSCs prevent active preclinical and clinical applications of aNSCs. However, several papers have recently reported scientific breakthroughs, on the basis of which broad application trials using aNSCs could be performed. In this review, we also summarize the current status of preclinical and clinical applications of aNSCs for various neurological diseases.

STEM CELL THERAPY FOR NEUROLOGICAL DISEASES

Neurological diseases are derived from the loss of functional neurons in the central nervous system (CNS). Although acute localized neurodegeneration could result from a temporal localized injury, such as stroke and trauma, chronic neurodegeneration usually develops over a long period of time, and has unclear multifactorial causes. Functional neurological deficits in chronic neurological diseases originate from either loss of a specific neuronal subtype, or universal brain damage. Alzheimer's and Huntington's diseases result in non-specific death of neurons in the brain, whereas Parkinson's disease is characterized by the specific and localized damage of dopaminergic neurons located in the substantia nigra. In the brain and spinal cord, amyotrophic lateral sclerosis (ALS) and traumatic spinal cord injury induce diffuse motor neuronal loss and localized nonspecific neural tissue damage, respectively. Although these neurodegenerative conditions have unique morphological pathologies, the molecular mechanisms for the neuronal death are complicated and ambiguous, making the development of mechanism-based therapeutic modalities elusive. Since functional loss of neural cells is the common final pathway of various neurological diseases, regardless of specific etiologies, regenerative treatment using stem cells that could repair damaged neural tissue is a viable and non-specific therapeutic option.

TYPES OF STEM CELLS AND THEIR APPLICATIONS TO NEUROLOGICAL DISEASES

Stem cells have two important characteristics: proliferation capacity, and differentiation potential into multiple cellular lineages. According to the source, stem cells can be classified into embryonic, fetal, and adult stem cells (ESCs, FSCs, and ASCs, respectively). Pluripotent ESCs are obtained from the blastocyst of fertilized egg[1]. ESCs proliferate robustly and have multi-potent differentiation potentials into three germ layer cells, which consist of the whole body[2]. However, ethical concerns[3-5], and risks of adverse effects, such as immune rejection and tumor formation[6] prevent their clinical applications. Recently, it was reported that somatic cells could be reprogrammed into pluripotent state, by overexpression of Oct4, Sox2, Klf4, and c-Myc[7,8]. Although these induced pluripotent stem cells (iPSCs) maintain the merits of ESCs, iPSCs still have limitations, such as low efficient generation, and the formation of teratomas or tumors *in vivo*. These critical limitations provoke hesitation in the use of ESCs and iPSCs as clinical therapies. As other sources of stem cells, fetal organs that contain FSCs have been suggested. In spite of the advantages of FSCs, including proliferation capacity, limited differentiation potential, and lack of teratoma formation[9], ethical problems of using fetal tissues still remain.

ASCs are classically defined as multi-potent cells that originate from various tissues within the adult body, including the bone marrow, skeletal muscle, central nervous system, and adipose tissue[10,11]. The important benefit of ASCs is possible autologous transplantation, in which stem cells can be primarily cultured from and applied to the same patient. This benefit bypasses the ethical problems that ESCs and FSCs harbour. However, in spite of these advantages of ASCs, their limited differentiation and proliferation ability interrupts their widespread use.

Therefore, in the current status, technical and ethical considerations indicate that compared with other stem cells, ASCs are the most clinically applicable.

ADULT NEURAL STEM CELLS FOR NEUROLOGICAL DISEASES

Among various ASCs, mesenchymal stem cells (MSCs) are the most widely used, and furthest progressed in preclinical and clinical trials[12]. The strengths of MSCs are relatively simple isolation, and *in vitro* expansion techniques. However, there are concerns about the clinical applications of MSCs[13]. First of all, in the culture methods of MSCs, bovine serum should be used. Because the dangers of bovine serum have not been well characterized, potential risks in clinical applications still exist[14]. Although xeno-free culture methods for MSCs have been developed, their quality needs to further study. Moreover, many previous studies suggested that the beneficial effects of MSCs for neurological diseases might originate from their paracrine effects involving immune modulation and/or secretory growth factors, and not from direct neuroregenerative effects producing functional neural cells[15-17].

Compared to MSCs, NSCs are cultivated and expanded in media containing low, or no bovine serum[18-22]. Many preclinical studies using NSCs suggest that NSCs not only have beneficial paracrine effects in the regeneration and repair of neural tissue, but also direct differentiation potential into diverse neuronal lineages, to form networks with surrounding neuronal cells[23-25]. Since the ultimate goal of regenerative treatment for neurodegenerative diseases is the functional repair of damaged neural tissues, NSCs seem to be a more optimal choice for neurological diseases.

Adult NSCs are tissue-resident multi-potent neural progenitor cells that have self-renewal capacity, so long as they

can be maintained undifferentiated. NSCs have the potential, under appropriate culture conditions, to differentiate into multiple neural cells, such as neurons, astrocytes, and oligodendrocytes. NSCs are observed in the developmental stage and mature CNS of mammalian species[26-29], specifically in the subventricular (SVZ) and subgranular zones (SGZ)[30-32]. The neurogenic niche surrounding SVZ and SGZ represents a unique microenvironment that regulates the survival and differentiation of NSCs[28,33].

TECHNICAL HURDLES AND RECENT BREAKTHROUGHS IN THE USE OF ADULT NEURAL STEM CELLS FOR NEUROLOGICAL DISEASES

Depending on the types of neurological diseases, undifferentiated NSCs themselves, or differentiated neural cells, have been applied to verify their efficacy in preclinical animal models. However, *in vitro* expansion of differentiated neural cells to acquire the necessary amount of cells for transplantation is very difficult, because differentiated cells cannot proliferate well. Therefore, regardless of transplantation cell types, aNSCs first need to be properly isolated, and effectively expanded *in vitro*. Compared with other stem cells, such as ESCs, fetal NSCs, and MSCs, aNSCs reside in restricted areas of the adult CNS[31,32], and have limited capacity to proliferate[34,35]. Therefore, difficulties in the primary isolation and stable *in vitro* expansion of aNSCs are major technical obstacles to be resolved, for the utilization of aNSCs.

Up to now, several research teams have addressed these difficulties, using various scientific and technical approaches. Surgical samples from the adult CNS are usually very small (1-2 mL). As the number of resident aNSCs within the tissue is also very small, isolation techniques have been optimized to increase the success rate of the primary isolation of aNSCs. To acquire aNSCs, CNS tissues are physically minced, and enzymatically digested into single cells. Among them, the enzymatic digestion is a critical step, because it directly affects the survival of aNSCs. The compositions of dissociating enzymes and incubation times are various among investigators. Papain, trypsin, and collagenase have usually been used, and in some reports, papain dissociation was suggested to be most optimal for the primary isolation of aNSCs[36,37].

After the mechanical and enzymatic dissociation of CNS tissues, the resulting single cells have been cultured by two alternative methods: the neurosphere, and adherent culture methods. Conventionally, the neurosphere culture method has been used for *in vitro* culture of NSCs[38-47]. This method was first used in the primary isolation of NSCs from murine brains. The neurosphere culture method was also applied to maintain aNSCs from human brains. However, difficulties in the stable *in vitro* expansion of aNSCs using suspension culture methods resulted in the need for another culture method to be developed.

Moreover, a single neurosphere may not be derived from a single NSC[48]. The possible heterogenic origin of neurospheres could not guarantee the homogeneity of *in vitro* expanded aNSCs in the suspension culture conditions[49-51].

To overcome the weak points of the neurosphere culture method, others, as well as ourselves, developed alternative adherent culture methods for NSCs[18-21,44,52-54]. Each group used their own coating plates to attach NSCs to the plates, and various culture medium compositions. Laminin and poly-L-ornithine (PLO) have frequently been used to coat plates, which increase the adherent efficiency of NSCs. To maintain stemness and proliferation of NSCs, the amount of EGF and basic FGF have been optimized[55]. For example, we expanded aNSCs from temporal lobectomy samples of epilepsy patients without any neoplasmic diseases, on PLO-coated plates in a DMEM/F12 media supplemented with 1% B27, 1% penicillin/streptomycin, EGF (50 ng/mL), bFGF (50 ng/mL), and 0.5% fetal bovine serum (Table 1)[18]. Using the adherent culture method, aNSCs were expanded *in vitro* from 10^4 to 10^{12} cells within 8 subcultures for 2 mo. Moreover, the expression of Nestin and Sox2 as NSC markers was stably maintained[18]. If the number of aNSCs required for transplantation is 10^7 per patient, at least one hundred thousand patients could be treated with a primary culture of aNSCs.

Table 1 summarizes various primary culture and *in vitro* expansion techniques for aNSCs. As indicated in Table 1, major obstacles in the primary isolation and stable *in vitro* expansion of aNSCs have been, or are being resolved, which would increase their clinical applicability.

CURRENT THERAPEUTIC STATUS OF ADULT NEURAL STEM CELLS FOR NEUROLOGICAL DISEASES

Since technical breakthroughs for the preclinical and clinical utilization of aNSCs were introduced relatively recently, scientific results showing treatment effects of aNSCs against neurological diseases are, at present, limited[18,56-59]. However, many previous reports have indicated that NSCs, compared with other stem cell types, are optimal for neurological diseases, since neural functional recovery requires direct neural cell supplementation, besides indirect paracrine effects. With brief presentation of the current developmental status of stem cell therapeutics for individual neurological disease, Tables 2 and 3 summarize preclinical and clinical results, respectively, of aNSCs against various neurological diseases.

Ischemic stroke

Various human stem cells and their derivatives can differentiate into neurons restoring functional losses in the rodent stroke model[60,61]. In particular, human ESC-derived NSCs, injected into the ischemic penumbra region in rat brains with ischemic stroke, have been reported to

Table 1 Isolation and *in vitro* culture methods for adult neural stem cells

Culture methods	Cell source	Dissociating method	Media composition	Plate coating	Maximal *in vitro* culture	Ref.
Adherent culture method	Temporal lobe	Physical Mincing and enzymatic digestion with papain	DMEM/F12 supplemented with 10 ng/mL bFGF, 20 ng/mL TGFα, 2.5 μg/mL heparin, 2% B27 (without retinoic acid), 10 mmol/L hepes, and 1% FBS			[54]
	Temporal lobe	Mechanical trituration and enzymatic dissociation using papain and DNase Ⅰ	DMEM/F12 supplemented with 1% B27, 50 ng/mL EGF, 50 ng/mL bFGF, and 0.5% FBS	Poly-L-ornithine	18 passages	[18]
Neurosphere culture method	Hippocampal and lateral ventricle wall tissue	Mechanical dissociation and enzyme digestion using hyaluronic acid, kynurenic acid, and trypsin	DMEM/F12 supplemented with 10 ng/mL EGF, 20 ng/mL EGF, B27, and 2 mmol/L glutamine			[39]
	Temporal lobe	Enzymic digestion with trypsin	N2 medium supplemented with 5% FBS	Poly-2-hydroxyethyl methacrylate		[40]
	Hippocampus Amygdala Frontal cortex Temporal cortex	Enzymic digestion with hyaluronidase, kynurenic acid, and trypsin	DMEM/F12 supplemented with 0.6% glucose, 2 mmol/L glutamine, 3 mmol/L sodium bicarbonate, 5 mmol/L HEPES buffer, 25 mg/mL insulin, 10 mg/mL heparan sulfate, 100 mg/mL transferrin, 20 nmol/L progesterone, 60 mmol/L putrescine, 30 nmol/L selenium chloride, 20 ng/mL EGF, and 20 ng/mL bFGF-2			[41]
	Temporal lobe from 11-wk-old postnatal male Hippocampus, ventricular zone, motor cortex and corpus callosum from and a 27-year-old male	Enzymic digestion with papain, DNase Ⅰ, and neutral protease	Initially, DMEM/F-12 supplemented with glutamine and 10% FBS After 24 h, DMEM/F12 supplemented with BIT-9500 (bovine serum albumin, transferrin, insulin, 20 ng/mL bFGF, 20 ng/mL EGF, and 20 ng/mL PDGF-AB) and 25% conditioned medium from rat stem cells that produces secretory bFGF and glycosylated form of cystatin C	Fibronectin	More than 70 population doublings in the 11-wk-old postnatal male More than 30 population doublings in the 27-year-old male	[42]
	Temporal lobe	Mechanical dissociation and enzymic digestion with DNase Ⅰ and trypsin	DMEM/F12 supplemented with 1 mol/L HEPES, 2% B27, 0.1% EGF, and 0.1% bFGF		11 mo	[43]
	Temporal lobe	Enzymic digestion with Papain and DNase Ⅰ	DMEM/F12 supplemented with bFGF and EGF		3–6 wk	[44,114]
	Lateral ventricular roof	Mechanical dissociation and enzyme digestion with DNase Ⅰ and trypsin	DMEM/F12 supplemented with bFGF, EGF, and B27		3-7 wk	[45]
	Hippocampus containing hilus, temporal cortex, and subventricular zone including anterior horn and segmented lateral ventricle	Physical mincing and enzyme digestion with trypsin	DMEM/F12 supplemented with N2, 35 μg/mL bovine pituitary extract, 5% fetal calf serum, 40 ng/mL EGF, and 20 ng/mL bFGF		> 60 population doublings	[46]
	Biopsies from filum terminale below conus medullaris	Physical mincing and enzyme digestion with trypsin	DMEM/F12 supplemented with B27, 10 ng/mL LIF, 10 ng/mL bFGF, and 20 ng/mL EGF	Ultra-low attachment dish		[47]

TGFα: Transforming growth factor alpha; DMEM/F12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; FBS: Fetal bovine serum; bFGF: Basic fibroblast growth factor; EGF: Epidermal growth factor.

move to the lesions, and improve motor performances[62]. Moreover, human adult temporal lobe-derived NSCs, grafted into the contralateral ventricle of the rat brains with focal cerebral stroke, significantly reduced the infarction area, and showed recovery of motor function[18]. When human fetal NSCs were transplanted into ischemic lesions of rodent brains, they migrated toward the injured regions and differentiated into neurons[63,64].

Table 2 Preclinical results of adult neural stem cells against neurodegenerative diseases					
Targeted disease animal model	Cell source	Injection method	Result	Animal species	Ref.
Demyelinated spinal cord injury	Frontal cortex, temporal cortex, hippocampus, and subventricular/ subependymal zone of frontal lobe	The midline of the dorsal columns of the spinal cord at three longitudinal sites	The cells elicited extensive remyelination with a peripheral myelin pattern similar to Schwanncell myelination The remyelinated axons conducted impulses at near normal conduction velocities	Rat	[56]
Multiple sclerosis (lysolecithin-demyelinated brain)	Temporal lobe	Local injection to demyelinated brain regions	Transplanted cells migrated to lesions without extending into normal white matter. Implanted progenitors matured as oligodendrocytes, and developed myelin-associated antigens	Rat	[57]
Global brain ischemia	Temporal lobe	The posterior periventricular region above the hippocampus	Adult human NPCs survived, migrated into ischemic regions, and differentiated into functional neural cells No information about therapeutic effects	Rat	[58]
Global brain ischemia	Temporal lobe	Left hippocampus After *in vitro* differentiation	Injected cells migrated preferentially into an ischemic lesion, which was mediated by SDF-1α and CXCR4 signaling pathways No information about therapeutic effects	Rat	[59]
Focal ischemic stroke	Temporal lobe	Contralateral lateral ventricle	Transplanted cells reduced infarction volumes and enhanced motor activity	Rat	[18]

Initial clinical trials with stem cells have been completed in stroke[61]. Unfortunately, no significant clinical outcomes were observed when autologous MSCs were injected intravenously into ischemic patients[65]. Although other clinical studies adopting intravenous or intra-arterial administration of autologous bone marrow-derived stem cells in stroke patients are in progress or planning[23], NSC-based regenerative treatment with both paracrine and neuronal supplementation effects would be more effective. Recently, a clinical trial for stroke with immortalized NSCs generated from human fetal cortex was planned in the United Kingdom[23], which would yield scientific data that might possibly demonstrate the superior regenerative and treatment activities of NSCs.

Although there are scientific data demonstrating the therapeutic effects of aNSCs on ischemic stroke, aNSCs have not applied to clinical trials for ischemic stroke yet. In contrast, clinical trials using MSCs for ischemic stroke are continuously planned and performed world widely. Compared with MSC clinical trials, the most different feature of NSC trials is the injection route; while MSCs are usually injected intravenously, NSCs are stereotactically transplanted in the brain. Since the penetration of MSCs across brain-blood barrier is still controversial, direct implantation of NSCs into the brain would potentiate the therapeutic effects against ischemic stroke.

Spinal cord injury
Human NSCs transplanted into a mouse model of spinal cord injury were observed to differentiate into neurons and oligodendrocytes to lead the recovery of locomotion[66]. Treatment mechanism study indicated that neurons derived from transplanted stem cells integrated into the host neuronal circuitry and mediated functional recovery[19,67]. On the other hand, the functional recovery after NSC transplantation into spinal cord injury models was proportional to the number of transplanted stem cell-derived oligodendrocytes and the amount of regenerated myelin[68]. Those preclinical results indicate that the supplementation of mature neural cells by implanted stem cells would also be important in clinical settings, for the functional recovery of spinal cord injury patients.

Highly refined oligodendrocyte progenitor cells (OPCs) generated *in vitro* from human ESCs differentiated into oligodendrocytes, and induced remyelination of the demyelinated spinal cord of mouse[69]. Based on these observations, a first phase I clinical trial using human ESC-derived OPCs is under planning by the United States company, Geron[23]. This first clinical trial has raised worries about the risk for tumorigenicity, which is difficult to determine in preclinical situations[70]. Since the results from animal models could not be directly translated into human, the possible risks need to be further validated. Moreover, utilization of aNSCs, instead of fetal origin stem cells, would reduce the possible tumorigenicity, due to their limited proliferation potential.

Parkinson's disease
Human embryonic mesencephalic tissue which contains many post-mitotic dopaminergic neuroblasts was tried clinically, which have showed proof of concept that regenerative approach could have therapeutic effects in Parkinson's disease (PD) patients[71]. Dopaminergic neuroblasts for preclinical animal models have been cultured from various different stem cell sources, including ESCs[72-79], fetal NSCs and precursors of embryonic ventral mesencephalon[80-83], adult NSCs from the SVZ[84], bone marrow stem cells[85], and fibroblast-derived iPSC cells[86].

Although a small portion of dopaminergic neurons derived from transplanted cells contain disease-specific Lewy bodies 11 to 16 years after transplantation[87,88], implanted cells remained viable[23,89]. However, definitive

Table 3 Current clinical trials of neural stem cells against neurodegenerative diseases

Disease	NSC source	Brief title	Trial ID	Condition	Location	Period	Cell source	Route	Phase
ALS	Fetal NSCs	Human neural stem cell transplantation in amyotrophic lateral sclerosis	NCT01640067	Recruiting	Italy	2011-12 -2016-09	Fetal neural stem cells	Spinal cord	I
		Dose escalation and safety study of human spinal cord derived neural stem cell transplantation for the treatment of amyotrophic lateral sclerosis	NCT01730716	Ongoing	United States	2013-05 -2014-09	Spinal cord of a single fetus 8 wk of gestation	Spinal cord after laminectomy	II
		Human spinal cord derived neural stem cell transplantation for the treatment of amyotrophic lateral sclerosis	NCT01348451	Ongoing	United States	2009-01 -2015-12	Spinal cord of a single fetus 8 wk of gestation	Spinal cord after laminectomy	I
Stoke	Adult NSCs	Pilot investigation of stem cells in stroke	NCT01151124	Ongoing	United Kingdom	2010-06 -2015-03	CTX0E03 DP allogeneic neural stem cells	Putamen region of the brain	I
		Pilot Investigation of stem cells in stroke phase II efficacy	NCT02117635	Recruiting	United Kingdom	2014-06 -2015-12	CTX0E03 DP allogeneic neural stem cells	Intracranially *via* stereotaxic	II
SCI	Adult NSCs	Safety study of human spinal cord-derived neural stem cell transplantation for the treatment of chronic SCI	NCT01772810	Recruiting	United States	2014-08 -2016-02	Human spinal cord-derived neural stem cell	Direct injections into spinal parenchyma	I
		Study of human central nervous system stem cells in patients with thoracic spinal cord Injury	NCT01321333	Ongoing	Canada, Switzerland	2011-03 -2015-05	Human central nervous system stem cells	Thoracic spinal cord	I , II
		Study of human central nervous system stem cell transplantation in cervical spinal cord injury	NCT02163876	Recruiting	United States	2014-10 -2017-05	Human central nervous system stem cells	Cervical spine	II

NSCs: Neural stem cells; ALS: Amyotrophic lateral sclerosis; iPSCs: Induced pluripotent stem cells.

successful clinical trials have not yet been reported in the case of human stem cell-derived dopaminergic neurons. In contrast, a group of patients who had embryonic mesencephalic graft showed dyskinesia[90-92]. Those reports have provoked major concern about the possible side effects of transplanted ESC cell-derived dopaminergic neuroblasts[77], and the need for safer stem cell sources, such as aNSCs.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a lethal neuro-degenerative disease with premature degeneration of motor neurons in the CNS[23,93]. For the regeneration and/or supplementation of motor neurons, motor neurons were generated *in vitro* from ESCs[94-97], fetal NSCs[98-100], and iPSCs[101,102].

Recently, transplantation of fetal spinal cord- or iPSC-derived NSCs was reported to be effective in slowing down the disease progression of ALS animal models[103,104]. Based on the preclinical results, a phase I clinical trial of intra-spinal cord injection of fetal NSCs into ALS patients was attempted in the United States. Clinical outcomes from 6 to 18 mo after the transplantation showed that the intervention did not accelerate disease progress[105]. In contrast, their

efficacy could not be determined, although higher dose of injection showed better results in some evaluating factors. On-going and/or planned phase II and III clinical trials would further determine the optimal therapeutic dose, and their therapeutic efficacy against ALS[106].

Until now, there have been few preclinical and clinical trials using aNSCs for ALS. However, ALS could be a good treatment target of aNSCs, having regard to its fatality, and lack of proven therapeutic options for it.

Alzheimer's disease

Alzheimer's disease (AD) is the most frequent neurological disease, which is characterized by the increased amyloid plaques and neurofibrillary tangles in the brain[107]. Amyloid plaques are extracellular aggregations consisting of amyloid-peptides. Neurofibrillary tangles are intracellular aggregations of hyperphosphorylated tau, a microtubule-associated protein within neuron[108]. The causative relationship between amyloid plaques/neurofibrillary tangles and AD is still under investigation[109]. Widespread non-specific neuronal death in the AD brain makes stem cell-based regeneration challenging. For effective cell therapy for AD, NSCs need to migrate to multiple regions
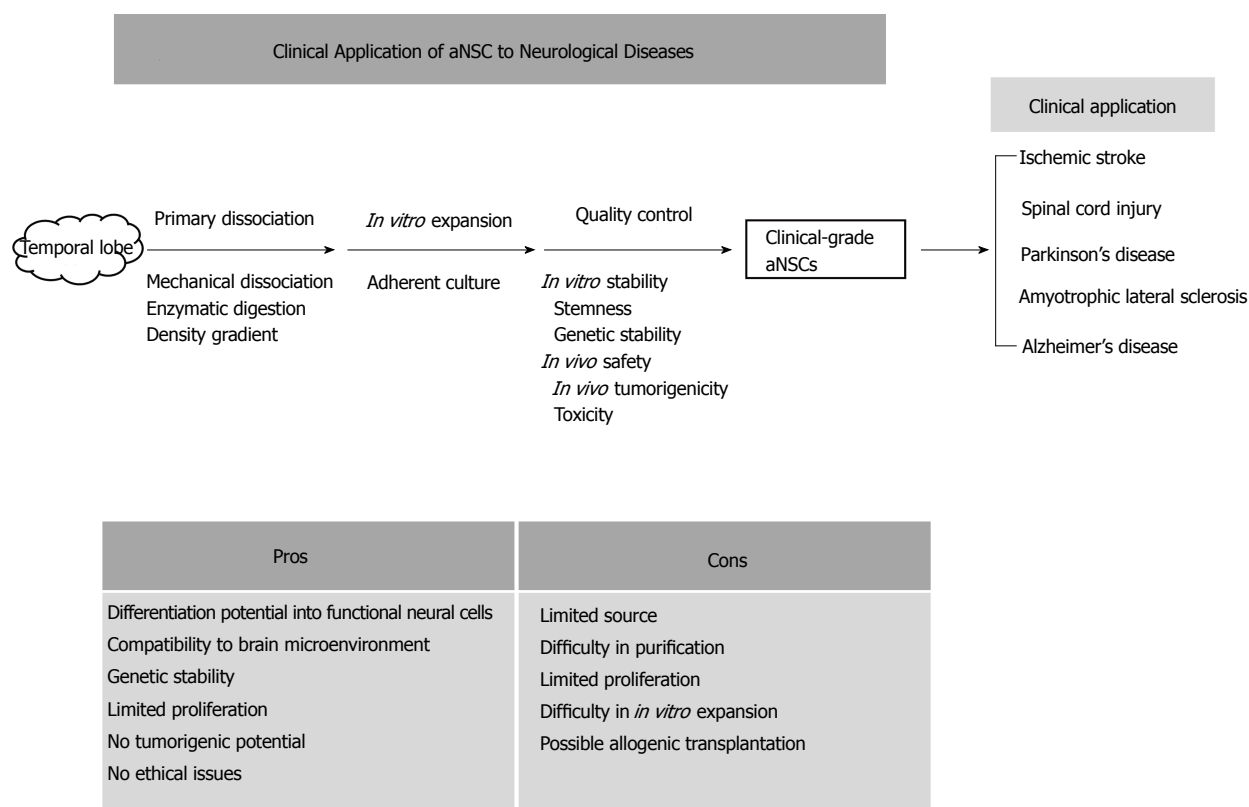
Figure 1 A diagrammatic summary of the approaches/strategies of adult neural stem cells and their pros/cons. aNSCs: Adult neural stem cells.

of the brain and then differentiate into numerous multiple subtype neural cells[110]. Moreover, the effect of amyloid plaques on the survival, migration, and differentiation of injected stem cells should be taken into consideration[111].

Human NSCs transplanted into the brains of AD animal models showed little neurogenesis, but unwanted gliosis around the plaque-like structures[112]. Therefore, stem cell-based regenerative therapies need to be further developed preclinically, before clinical applications to AD. The disappointing preclinical data have resulted in few clinical trials using NSCs against AD. However, MSC is in relative advanced clinical trial stages. For example, human umbilical cord blood-derived MSCs are currently in a phase I clinical trial. Most trials using MSCs hire one-time direct injection of MSCs into the patient's brain. As AD is a progressive disease, long term investigations are necessary, to examine the lasting effects, as well as the safety of transplanted stem cells[113].

PERSPECTIVES

Based on few scientifically proven treatment modalities for neurological diseases, and the regenerative potentials of stem cells, cell therapies using various stem cells have been preclinically and clinically applied to neurological diseases. There are many controversies about the therapeutic effects of stem cell treatments and their treatment mechanisms. The controversies could be derived from the diverse types of stem cells, and from their unique pros and cons.

Compared with other stem cell sources, aNSCs have several advantages, such as differentiation potential into functional neural cells, limited proliferation capacity, and few ethical problems (Figure 1). Although difficulties in the isolation and *in vitro* expansion of aNSCs prevent the active applications of aNSCs to neurological diseases, the technical obstacles have been continuously resolved. Therefore, at the current status, aNSCs can be attractive stem cell sources, to be introduced into the preclinical and clinical trials targeting various neurological diseases.

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