Review

In vitro and *in vivo* neurogenic potential of mesenchymal stem cells isolated from different sources

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Regenerative medicine is an evolving interdisciplinary topic of research involving numerous technological methods that utilize stem cells to repair damaged tissues. Particularly, mesenchymal stem cells (MSCs) are a great tool in regenerative medicine because of their lack of tumorogenicity, immunogenicity and ability to perform immunomodulatory as well as anti-inflammatory functions. Numerous studies have investigated the role of MSCs in tissue repair and modulation of allogeneic immune responses. MSCs derived from different sources hold unique regenerative potential as they are self-renewing and can differentiate into chondrocytes, osteoblasts, adipocytes, cardiomyocytes, hepatocytes, endothelial and neuronal cells, among which neuronal-like cells have gained special interest. MSCs also have the ability to secrete multiple bioactive molecules capable of stimulating recovery of injured cells and inhibiting inflammation. In this review we focus on neural differentiation potential of MSCs isolated from different sources and how certain growth factors/small molecules can be used to derive neuronal phenotypes from MSCs. We also discuss the efficacy of MSCs when transplanted *in vivo* and how they can generate certain neurons and lead to relief or recovery of the diseased condition. Furthermore, we have tried to evaluate the appropriate merits of different sources of MSCs with respect to their propensity towards neurological differentiation as well as their effectiveness in preclinical studies.

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Abbreviations used: AD, Alzheimer's disease; AF, amniotic fluid; AM, amniotic membrane; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; BM, bone marrow; BME, β-mercaptoethanol; ChAT, choline acetyltransferase; CTNF, ciliaryneurotrophic factor; DA, dopaminergic; EGF, epithelium growth factor; FSL, Flinders sensitive line; GDNF, glial-derived neurotrophic factor; HCNP, hippocampal cholinergic neurostimulating peptide; IBMX, isobutylmethylxanthine; MSC, mesenchymal stem cells; NGF, nerve growth factor; NGF, nerve growth factor; NIM, neuronal induction medium; NIM, neuronal induction medium; RA, retinoic acid; rDHE, rat denervated hippocampal extract; SHED, stem cells from human exfoliated deciduous teeth; SLC, Schwann like cells; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; UPDRS, Unified Parkinson's Disease Rating Scale; WJ, Wharton's jelly

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1. Introduction

The study of neural development draws on both neuroscience and developmental biology to describe the cellular and molecular mechanisms by which complex nervous systems emerge during embryonic development and throughout life. Some of the landmarks of the neural development include the birth and differentiation of neurons from stem cell precursors, their migration, protrusions of axons and dendrites, and finally generation of synapses between these axons. The survival of these neurons is regulated by certain survival factors called the trophic factors. Various factors have been identified but the most important that have been found to regulate the neurons are nerve growth factor (NGF), ciliaryneurotrophic factor (CTNF) and glial-derived neurotrophic factor (GDNF).

Neurodegeneration refers to progressive loss of structure or function of neurons including their apoptosis. The process of neurodegeneration causes diseases such as Parkinson's disease, Alzheimer's disease and many others. There are several reasons behind neurodegeneration, including genetic mutations (Thompson 2008), accumulation of intracellular toxic proteins, e.g. Amyloid-β in Alzheimer's disease (Rubinsztein 2006), or mitochondrial dysfunction resulting in cell death (DiMauro and Schon 2008). Apart from these causes, aging process is the greatest risk factor for neurodegenerative diseases. Neurons gradually lose their functions with age. In addition to neurodegeneration, ischaemia is another condition that can lead to rapid loss of brain function. Ischaemia can result from a blockage (thrombosis) or a haemorrhage (leaking of blood) arising in a blood vessel (Sims and Muyderman 2010). A stroke can lead to permanent neurological damage resulting in paralysis, cognitive dysfunction and blindness, among other disabilities. Present methods of management of these conditions leave much to be desired and there is a huge unmet clinical need that needs to be filled. Researchers and clinicians are hoping that stem cells could emerge as a suitable therapy for these degenerative diseases. However, they should qualify or satisfy certain criteria before reaching clinics (figure 1).

What are mesenchymal stem cells (MSCs)? MSCs are characterized by the set of criteria such as they must be plastic-adherent; express CD105, CD73 and CD90; lack expression of CD45, CD34, CD14 or CD11b, CD79 alpha or CD19 and HLA-DR surface molecules; and differentiate to osteoblasts, adipocytes and chondroblasts *in vitro* in response to specific stimuli (Dominici *et al.* 2006). MSCs have also found to differentiate to other cell types such as neuronal, cardiac and pancreatic lineages. The trans-differentiation of MSCs into neural lineage can be achieved by culturing them in neural induction media. This media generally consists of a cocktail of growth factors or small molecules that drive these cells towards neuronal lineage. The degree to which the culture will differentiate varies among individuals and how differentiation is induced. It is not clear whether this variation is due to a different amount of 'true' progenitor cells in the culture or variable differentiation capacities of individuals' progenitors (Engler *et al.* 2006).

Owing to their multipotent capacity, BM-MSCs have been investigated since their discovery as promising candidates for use in new cell-based regenerative therapies (Pereira *et al.* 2011). These MSCs have reached clinical trials in a short span of time in view of their safety and efficacy. However, it is necessary to consider alternative cellular sources for isolating MSCs because of the highly invasive method needed to obtain bone marrow. With respect to the field of cell therapy, it may be particularly crucial to determine the most suitable cell sources according to the target disease. In this article we have emphasized neuronal differentiation of MSCs derived from major sources in search of a better MSC source for neurodegenerative diseases.

2. MSCs derived from various human tissues in alphabetical order

1. Adipose-derived MSCs (Wu *et al.* 2012)
2. Amniotic fluid (AF) and amniotic membrane (AM)–derived MSCs (Roubelakis *et al.* 2012)
3. Bone marrow (BM) MSCs (Pittenger *et al.* 1999; Mamidi *et al.* 2012)
4. Dental pulp MSCs (Tirino *et al.* 2012)
5. Endometrial MSCs (Schuring *et al.* 2011)
6. Limb-bud-derived MSCs (Jiao *et al.* 2012)
7. Menstrual-blood-derived MSCs (Allickson *et al.* 2011)
8. Muscle- and periosteum-derived MSCs (Kisiel *et al.*, 2012)
9. Peripheral-blood-derived MSCs (Lyahyai *et al.* 2012)
10. Placenta- and fetal-membrane-derived MSCs (Raynaud *et al.* 2012)
11. Salivary-gland-derived MSCs (Rotter *et al.* 2008)
12. Skin- and foreskin-derived MSCs (Mamidi *et al.* 2011; Vishnubalaji *et al.* 2012)
13. Sub-amniotic human umbilical cord lining membrane–derived MSCs (Kita *et al.* 2010)
14. Synovial fluid MSCs (Morito *et al.* 2008)
15. Wharton's jelly (WJ)–derived MSCs (Witkowska-Zimny and Wrobel 2011)

Although MSCs have been derived from various human tissues, only a few have been studied for neurological investigations.

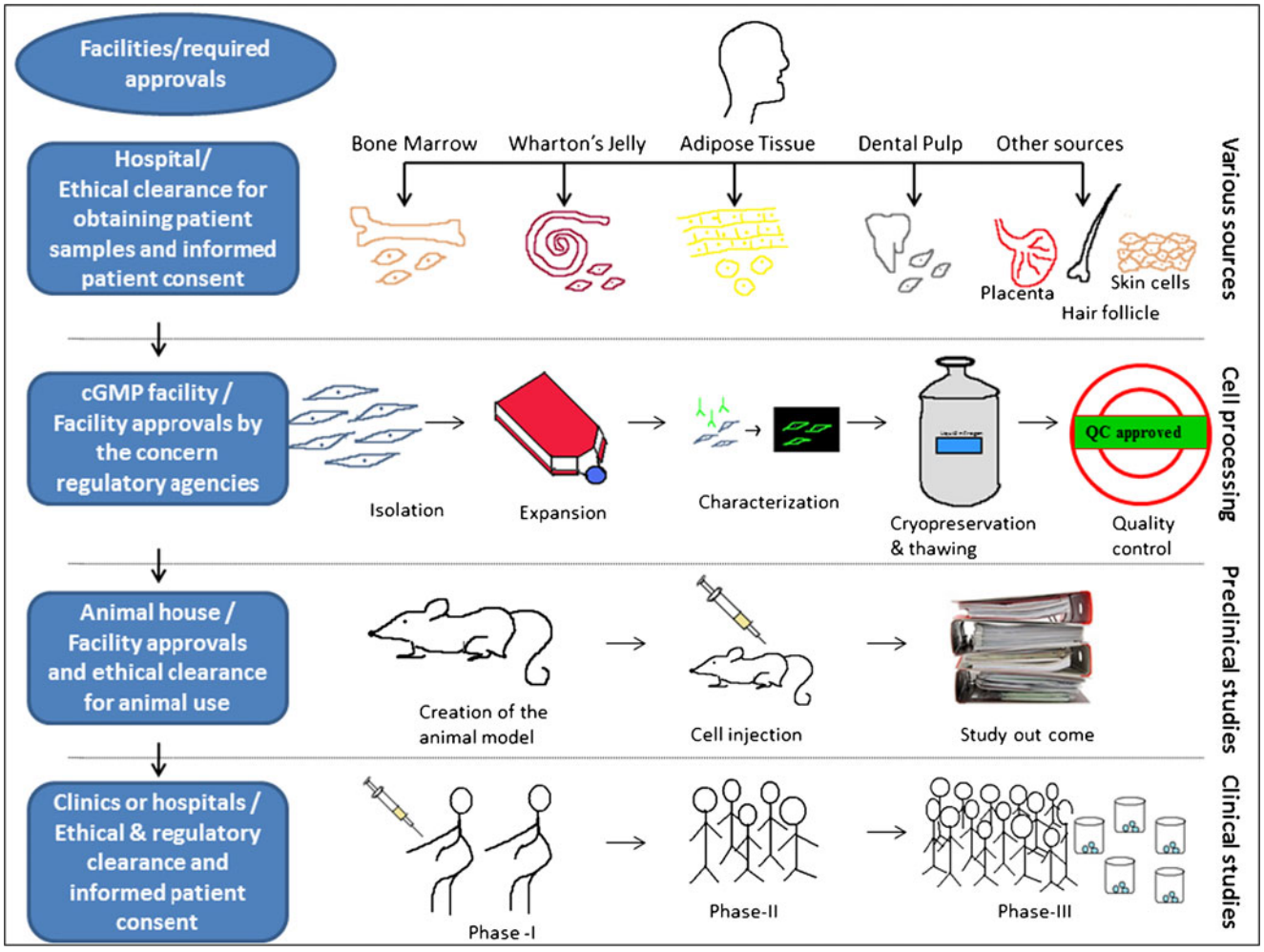

Figure 1. Steps to qualify for translation of cell-based medicine from bench to bedside.

3. Neuronal differentiation potential of MSCs isolated from different sources

3.1 *Neuronal phenotypes derived from BM-MSCs*

Various strategies have been employed to achieve BM-MSCs-derived neural phenotypes from different sources. Researchers used several factors such as EGF, HGF and FGF in different combinations to obtain neuronal, astrocytes and certain neural-specific phenotypes such as oligodendrocytes, dopaminergic and cholinergic neurons (Philippe *et al.* 2006; Fu *et al.* 2008; Naghdi *et al.* 2009; Bae *et al.* 2011; Datta *et al.* 2011). Apart from these studies, transcription factor such as Neurogenin-1 was effective in converting MSCs into neuron-specific protein-expressing cells (Barzilay *et al.* 2009a, b). The lentiviral delivery of transcription factor LMX1a enhances dopaminergic phenotype in differentiated human BM-MSCs (Barzilay *et al.* 2009a, b). These neurons were able to synthesize a higher level of the enzyme tyrosine hydroxylase (TH). It has been shown that the neuron-restrictive silence factors promote neural differentiation with enhanced electrophysiological properties (Trazaska *et al.* 2008; Yang *et al.* 2008). Researchers observe that the second messenger cAMP along with forskolin enhanced the neuronal differentiation process of MSCs (Rooney *et al.* 2009). Further, in serum-free conditions cAMP forms a neural-like morphology and elevates neuronal-specific markers. β-mercaptoethanol (BME) and nerve growth factor (NGF) were shown to enhance the trans-differentiation of MSCs, and it was further demonstrated that the cholinergic neurons are essential for treating spinal cord injuries (Naghdi *et al.* 2009). This treatment with BME led to generation of NF-68-positive neuroblasts, and in combination with NGF they could generate up to 80% cholinergic neurons from the MSCs.

3.2 *Neuronal phenotypes derived from ADSCs: Adipose derived stem cells*

The effect of substances known as inducers of neuronal differentiation on cultured ADSCs has been studied. Studies have shown factors like bFGF, EGF, insulin, retinoic acid, valporic acid and hydroxycortisone can support neuronal differentiation of AD-MSCs (Safford *et al.* 2002; Schaffler and Buchler 2007; Anghileri *et al.* 2008; Lim *et al.* 2010). Further forskolin has also been found to generate neuron-like morphology when cultured in serum-free conditions. AD-MSCs expressed sodium current on treatment with bFGF and forskolin by increasing the intracellular cAMP levels, which was found to be useful in neural induction (Kim *et al.* 2005; Rooney *et al.* 2009). A recent study has shown neuronal induction of ADSCs using BME, glial cell line–derived neurotrophic factor (GNDF), brain-derived neurotrophic factor (BDNF), retinoic acid (RA), 5-azacytidine, as well as their combinations (Pavlova *et al.* 2012). Researchers have explored the neuronal differentiation potential of ADSCs *in vitro* using epithelium growth factor (EGF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), isobutylmethylxanthine (IBMX), indomethacin and insulin (Ying *et al.* 2012). This study suggested that the administration of BDNF in the pre-induction medium may provide a new way to modify the culture method for getting more neuron-like cells *in vitro*. In another study researchers showed that both BM-MSCs and ADSCs differentiate into Schwann like cells (SLC) which have the potential to myelinate neuronal cells during regeneration (Mantovani *et al.* 2010).

3.3 *Neuronal phenotypes derived from WJ/UC-MSCs*

It has been shown that the MSCs isolated from human umbilical cord Wharton's Jelly were capable of differentiating into nerve-like cells using salvia miltiorrhiza or BME and these induced MSCs were shown to express neuron-related genes and neuronal cell markers (Ma *et al.* 2005). Dopaminergic (DA) neurons have been formed from both Wharton's jelly and placental tissue using a cocktail of growth factors and small molecules such as bFGF, FGF8, sonic hedgehog, IBMX and retinoic acid (Chen *et al.* 2009; Datta *et al.* 2011). It has been shown that the microRNAs play an important role in the neuronal differentiation of WJ-MSCs (Shing-Jyh *et al.* 2011). These small RNAs are of 18–24 nucleotides in length, were involved in regulation of gene expression and found to enhance motility and oxidative phosphorylation in neural cells derived from the WJ-MSCs. Researchers showed that the human UC-MSCs obtained from WJ, when treated with neuronal induction medium (NIM) consisting of brain-derived neurotrophic factor (BDNF), low-serum media and supplemented with hippocampal cholinergic neurostimulating peptide (HCNP) or rat denervated hippocampal extract (rDHE) or in combination promotes enhanced action of choline acetyltransferase (ChAT) (Zhang *et al.* 2012). These *in vitro* generated functional ChAT-positive cells may be useful candidates for cell transplantation to treat Alzheimer's patients.

3.4 *Neuronal phenotypes derived from DPSCs*

Human third molar tooth isolated DPSCs were differentiated into functionally active neurons and further shown to enhance expression of nestin and GFAP under neural inductive conditions (Arthur *et al.* 2008). DPSCs from human exfoliated deciduous teeth (SHED) were able to differentiate into neural cells by using growth factors such as Shh, FGF8, GDNF and forskolin. This was a two-step induction process – (a) to form neurosphere-like aggregates and (b) differentiation towards dopaminergic phenotype. Its therapeutic efficacy has been investigated in a Parkinsonian model. An increase of dopamine content in the striatum was observed along with behavioural recovery (Wand *et al.* 2010). Neural cells could also be isolated directly from the dental pulp and found to be effective in cerebral stroke models (Nanette *et al.* 2010). It has been reported that adult rat dental pulp cells have the ability to form neurospheres when cultured in serum-free culture medium on super-hydrophilic plates (Sasaki *et al.* 2008). Another group showed the neuronal differentiation of DPSCs by using three-step protocol involving epigenetic reprogramming, followed by simultaneous PKC/PKA activation and then by incubation with neurotrophic medium resulting in robust neuronal differentiation (Kadar *et al.* 2009).

3.5 *Neuronal phenotypes derived from other sources of MSCs*

A recent study has shown that nor-epinephrine, a neurotransmitter, when added *in vitro* can generate oligodendrocytes from the umbilical-cord-derived multipotent progenitor cells in a three-dimensional environment (Hedvika *et al.* 2011). Conversion of human nestin-positive AF-MSCs into Schwann-like cells using glia growth factors (Jiang *et al.* 2010) has been demonstrated; these cells may have the potential for future cellular therapy for peripheral neurological disorders. Further, the neuronal differentiation potential of fetal lung-derived MSCs are also known (Fan *et al.* 2005). Apart from these studies, various other strategies have been employed to obtain neural cells types from different sources of MSCs, as listed in tables 1 and 2.

Table 1. Role of growth factors/small molecules during MSC differentiation to neuronal lineage

MSC source	Growth factor/small molecules	Derived cell types	Characterization markers	Reference
Bone marrow	EGF, b FGF	NSC	Nestin and musashi1	Fu *et al.* 2008
	EGF, HGF, FGF	Neuronal (neuro glial)	NSE. NeuN, GFAP, MAP2, Gal C (oligo)	Bae *et al.* 2011
	rhEP	Neuronal	Akt, GSK3β, ERK, CXCR 4, VEGFR2, MMP2	Koh *et al.* 2009
	LMX1a	Dopaminergic neurons	TH, Tuj 1, MAP2, Pitx3, VMAT2	Barzilay *et al.* 2009a, b
	RA	Neuronal, astrocyte	nestin, NSE, MAP-2, GFAP, GDNF	Bi et al. 2010
	bFGF and poly lysine	Neuronal	Nestin, NF-L, beta 2 tubulin	Philippe *et al.* 2006 Datta *et al.* 2011
	SH, FGF8, bFGF	Dopaminergic neurons	Nestin, musashi12, A2B5, TH, Nurr1, beta3tubulin, Map2ab	Lu *et al.* 2004
	BME/DMSO/BHA	Neuronal	NSE, Neu-N	Kaka *et al.* 2012
	BME/DMSO/BHA and RA, b FGF, PDGF, heregulin, triiodothryonine/forskolin	Oligodendrocyte	Nestin, NF-68, O4, O1, MBP, S100, GFAP	Levy *et al.* 2008
	EGF, DHA, BHA, AMP, IBMX and RA	Dopaminergic neurons	Tuj1, α-synuclein, MAP2, GFAP, Nurr 1, TH	Choong *et al.* 2007
	FGFβ, BDNF, EGF and neural growth factor	Neuronal	GAP-43, NF-H, Neu-N	Naghdi *et al.* 2009
	BME, nerve growth factor	Cholinergic neuronal cells	NF-68, 160, 200, Neuro-D, CbAT	Maria *et al.* 2004
	bFGF, forskolin, ciliaryneurotrophic factor, GDNF	Neuronal	Map-2ab, NF-M, GFAP, GalC, O4, TH, DAT	Rooney *et al.* 2009
	8-bromo-cAMP, forskolin	Neuronal	beta 3 tubulin, nestin, GFAP	
Wharton's jelly	miR-34a	Neuronal	Neu N	Shing-Jyh *et al.* 2011
	Norepinephrine, forskolin, K252a	Oligodendrocytes	Sox1, A2B5, PDGFR-α/β, O1, O4, MBP	Hedvika *et al.* 2011
	Human umbilical cord perivascular cells conditions media	Increase in viability and proliferation of glial cells	GFAP, MAP2, O4	Salgado *et al.* 2010
	bFGF, retinoic acid, ascorbic acid, IBMX	Dopaminergic neurons	Nestin, TH, Neu-N, DAT	Chen *et al.* 2009
	SH, FGF8, bFGF	Dopaminergic neurons	Nestin, musashi1, A2B5, TH, Nurr1, beta3tubulin, Map2ab	Datta *et al.* 2011
	hEGF, bFGF, N2	Neurotrophic factor producing cells, astrocytes	GFAP, GDNF, VEGF, BDNF, IGF-1	Yust-Katz *et al.* 2012
	hFGF, h EGF, NSF-1	Neural precursors	Nestin, NSE, Neu-N, MAP2, GFAP,	Mareschi *et al.* 2009
	bFGF, butylatedhydroxyanisole, dimethylsulfoxide	Neuronal, oligodendrocytes, astrocytes	beta3tubulin, NF-M, TH, GAP43, Tuj1	Mitchell *et al.* 2003
Adipose tissue	bFGF and forskolin	Neuronal	Nestin, Tuj1,MAP2, NFL, NFM, NFH, NSE, NeuN, GAP43, SNAP25 (synaptic markers), GFAP (astrocyte), CNPase (oligo)	Sujeong *et al.* 2010
	TRAIL	Astrocyte, oligodendrocyte	Tuj 1, GFAP, CNPase, adiponectin, sialoprotein	Choi *et al.* 2011
	BDNF and retinoic acid	Neuronal	MAP 2, Neu-N, nestin, GalC, S-100, GFAP, TH	Anghileri *et al.* 2008
	Valporic acid, insulin, hydroxyanisole, hydrocortisone, EGF, FGF	Neuronal	GFAP, Neu-N, nestin, IF-M	Schaffler and Buchler 2007; Safford *et al.* 2002

Table 1 (continued)

MSC source	Growth factor/small molecules	Derived cell types	Characterization markers	Reference
	bFGF and EGF	Neuronal	Nestin, Sox2, vimentin, A2B5, GFAP, tuj1	Lim *et al.* 2010
Dental pulp	Shh, fgf8, GDNF,forskolin	Neuronal, dopaminergic neurons	β3 tubulin, nestin, MAP2, TH	Wand *et al.* 2010
	Saline	NSC	Nestin, beta 3 tubulin	Nanette *et al.* 2010
	EGF, FGF, ITS,retinoic acid	Neuronal	Nestin, PSA-NCAM, NF-M,	Arthur *et al.* 2008

Recombinant human erythropoietin – rhEP, retinoic acid – RA, sonic hedgehog – SH.

4. *In vivo* MSC transplantation: Pre-clinical studies

Upon transplantation, MSCs promote endogenous neuronal growth and reduce apoptosis. They act through paracrine signalling, thus establishing connections with damaged neurons and secrete certain factors that lead to the recovery of these neurons (Nanette *et al.* 2010). The efficacy of MSCs has been checked in a wide variety of pre-clinical models. MSCs treated with stromal derived factor-1 (SDF-1) increased the release of dopamine and also helped in preserving the TH positive cells (Wang *et al.* 2010) in Parkinsonian rat model. In another study the MSCs were first induced into neural lineage with a cocktail of growth factors and then transplanted. These cells grafted well and also displayed dopaminergic traits (Levy *et al.* 2008). Untreated MSCs were able to generate a cellular response but this was not effective in improving the neuronal behaviour. The umbilical cord MSCs on the other hand could bring some improvement in the movement deficits in hemiparkinsonian animal models (Low *et al.* 2008). MPTP neurotoxin leads to degeneration of the dopaminergic neurons. Stem cells from human exfoliated deciduous teeth (SHED), transplanted post-neural induction with Shh, FGF8, GDNF and forskolin, differentiated into dopaminergic neurons, which elevated the dopamine content (Wand *et al.* 2010).

Alzheimer's disease (AD) is characterized by gradual memory loss which is mainly caused due to reduced synthesis of the neurotransmitter acetylcholine. Amyloid-beta deposits are a fundamental cause of this disease. BM-MSCs secreting GLP-1 have been transplanted in AD animal models. Transplanted cells were able to reduce the Aβ peptides while the number of reactive astrocytes in the hippocampus also subsequently decreased (Harmening *et al.* 2009). Neuro-ectodermally converted BM-MSCs led to decrease of Aβ peptides by regulation of two genes *F-spondin* and *neprilysin* (Habisch *et al.* 2010). Another study has also shown similar set of results and here the BM-MSCs were able to reduce the inflammatory response and also restore defective microglial function (Lee *et al.* 2010). Dental pulp stem cells upon transplantation in hippocampus of mice underwent proliferation and maturation, forming NPCs and neurons (Huang *et al.* 2008). This can be a suitable alternative in the treatment of AD.

Ischaemic cerebral stroke is another common disorder where previous studies have shown that MSCs can be used a treatment option. BM-MSCs induced with neurogenin 1 could improve motor function in animal stroke model post-implantation. A comparative study between BM-MSCs and adipose-tissue-derived stem cells revealed ADSCs to be more effective as they were highly proliferative and they showed an increased production of VEGF and HGF. They could also effectively differentiate into neural, glial and vascular endothelial cells (Ikegame *et al.* 2011). It has been shown that the AD-MSCs transfected with TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) showed therapeutic efficacy against brainstem gliomas and these MSCs were able to differentiate into neural cell types, thus reducing the tumour volume *in vivo* (Choi *et al.* 2011).

MSC-based therapies hold promise even in psychiatric conditions like schizophrenia and major depression, where the pathophysiology may not be as well-characterized as the neurological conditions mentioned above. Impaired neurogenesis in hippocampus and dentate gyrus has been linked to the pathophysiology of major depression (Gronli *et al.* 2006; Husum *et al.* 2006). Injection of adult BM-MSCs into the lateral ventricle of Flinders sensitive line (FSL) rats, an animal model of depression, resulted in engraftment mainly to hippocampus and dentate gyrus with subsequent neurogenesis, that correlated with improvement in behavioural performance (Tfilin *et al.* 2010). The success of these preclinical studies encouraged researches to exploit the clinical potential of MSCs for various neurological indications.

5. Clinical studies of MSC transplantation for neurological indications

There have been several studies using autologous mesenchymal stem cells for stroke. The first major study was published by a group from the Ajou University School of

Table 2. Transplantation of MSC/MSC derived neuronal cells for neurodegenerative diseases

Cell type	Model used (preclinical/clinical)	Dose of cells delivered	Route of delivery	Outcome of study	Reference
Bone marrow	Parkinsonian rats	2,00,000/4 μL	Intrastriatal	No specific data has been shown to understand the effect of MSC on the dying dopaminergic neurons	Camp *et al.* 2009
	Female SD parkinsonian rats	1,000,0000	Right striatum	Rats with MSC displayed significant preservation in the density TH + fibers in the striatum and substantianigra pars compacta	Wang *et al.* 2010
	Double transgenic mice mutant expressing APP and presenelin-1	—	Right ventricle	GLP-1 transfected hMSCSs showed a reduction in Aβ40/42 positively stained plaques and the number of reactive astrocytes measured in the dentate gyrus of the hippocampus also decreased.	Harmening *et al.* 2009
	APP/PSI mice	10000 cells/3 μL cell suspension	Intracerebral	Reduced amyloid beta-peptide deposition, restoration of defective microglial function and decreased inflammatory responses. Improvement in motor function	Lee *et al.* 2010
	Hydroxydopamine lesion rats	5,00,000/5 μL	Intrastriatal	Transplanted cells were found within the striatum	Levy *et al.* 2008
	Ischemic stroke mouse model	1,00,000 cells	Cerebral artery	Contribution towards reduction in ischemic damage	Ikegame *et al.* 2011
	ICH induced rats	5,000,000 cells	Intralesional	Significant improvement after 2 months resulted by reactivation of endogenous neurogenesis	Otero *et al.* 2011
	Patients with traumatic brain injury	Primary dose: 107–109 Second dose: 108–1011	Injured area Intravenously	No immediate or delayed toxicity observed, neurologic function was significantly improved at 6 months after cell therapy	Zhang *et al.* 2008
	Parkinson's disease model rats	1,00,000/8 μL	Ipsilateral striatum	Trophic factors released from the transplanted cells may contribute to the functional recovery of the rats	Maria *et al.* 2004
Wharton's jelly	MPTP induced rats	1,00,000/5 μL	Intracranial	Protection of dopaminergic neurons against the neurotoxic effects and motor deficits	Pereira *et al.* 2011
	CNS injury animal models	—	—	Reduction in lesion sizes, behavioural recover, relief in movement	Low *et al.* 2008
Adipose tissue	Brainstem glioma rats	1,60000/12 μL	Right brainstem	Short-term reduction of tumor volume and long-term therapeutic efficacy	Choi *et al.* 2011
	Ischemic stroke mouse model	1,00,000 cells	cerebral artery	Higher proliferative activity with greater production of VEGF, reduction in ischemic damage	Ikegame *et al.* 2011
Dental pulp	Hemiparkinsonian rats	200,000/μL	DA depleted striatum sites	TH positive cells in grafted striatum	Wand *et al.* 2010
	MCAO rats	1,00,000 – 2,00,000/3 μL saline	Right dorsolateral striatun	Rats regained a balanced head swing and recovered from neurologic dysfunction	Nanette *et al.* 2010
	Immune suppressed mice	25000 cells per microliter	Hippocampus	Cells underwent proliferation forming NPCs and neurons and also gave rise to astrocytes and microglia to a lesser extent	Huang *et al.* 2008

Medicine in 2005 (Boncoraglio *et al.* 2010). Later they published 5 year data as well. In their initial study, 5 patients who received two doses of 50 million cells at 4–5 and 7–9 weeks showed improved Modified Rankin's scores and Barthel's index. As compared to controls, though, the improvement was not statistically significant. The MRI done after a year also showed improvements. Five years later the group reported 16 patients along with 36 controls (Lee *et al.* 2010); while the safety data was impressive, the efficacy data was not entirely convincing. In a more recent study, the authors injected autologous MSCs in 12 patients of ischaemic stroke 36–133 days after the event (Honmou *et al.* 2011). They were able to demonstrate excellent recovery both in clinical status as well as of the radiological lesion.

Parkinson's disease is another condition that has been treated with MSCs with promising results. Our group published the results of an uncontrolled trial using unilateral autologous BM-MSC transplantation in Parkinson's disease (Venkataramana *et al.* 2010) in which we showed in that autologous BM-MSCs injected into the sublateral ventricular zone of 7 patients with Parkinson's disease caused an improvement in the Unified Parkinson's Disease Rating Scale (UPDRS) in 3 patients out of 7. We were also able to demonstrate the safety of this invasive approach to the management of this disease. Other studies using similar approaches to the problem of traumatic brain injury have also shown promising results (Zhang *et al.* 2008). Degenerative diseases have also been the subject of trials. Lee *et al.* in 2008 transplanted the BM-MSC into 11 patients with multiple system atrophy through consecutively intra-arterial and intravenous injections and found improvements of their neurological scores (Lee *et al.* 2008). Another study showed that the injection of MSCs intrathecally and intravenously in patients with multiple sclerosis and amyotropic lateral sclerosis was safe except for minor transient effects and 6–25 month follow-up did not reveal any long-term adverse events (Karussis *et al.* 2010). The cells injected in this trail were labelled with superparamagnetic iron oxide [ferumoxides (Feridex)] in order to trace them subsequent to the injection; MRI images revealed that the cells were distributed from the lumbar site of the injection to the occipital horns, meninges, spinal cord and spinal roots. While there was no major improvement in the clinical condition, the inexorable deterioration was arrested in a majority of patients.

There are several unanswered questions which need to be addressed before such therapies become uniformly successful. One important question is the time of injection. Most animal studies on which clinical therapy is based are acute studies with the injection being done almost immediately after the creation of the model. In clinical practice, however, therapy is seldom started in the same time frame. This may be the reason why success in preclinical models has not been replicated in clinical practice. For instance, in stroke, the blood–brain barrier, which prevents entry of most molecules and cells to the brain, is disrupted in the first few days after the insult. Intravenous therapies therefore must target this window in order to be successful. A similar consideration holds good for spinal cord injuries. Several clinical studies and preclinical models have suggested that the best results can be obtained by therapy which is initiated almost immediately after the injury rather than later during the course of the illness (Das *et al.* 2011; Gopurappilly *et al.* 2011).

Other important unanswered questions include the dosages that need to be used, the necessity or otherwise of repeated doses, the mode of administration, among others (Mamidi *et al.* 2012). All these questions are now the subject of trials and more data can be expected over the next few years, which will clear the air in this regard. Until then, therapy for neurologic conditions with MSCs must be considered experimental and be conducted only under controlled conditions in clinical trials.

6. Mimicking the *in vivo* sign in calling cues

The purpose of this article is to understand the extent to which the MSCs from various sources can generate the different neural cell types *in vitro* which can mimic the process *in vivo*. According to our understanding of the *in vivo* system, ectodermal cells are responsible for neural differentiation by shutting down the mesodermal pathways. During neural induction, noggin and chordin are produced by the notochord, which inhibit the activity of BMP4. This inhibition of BMP4 causes cells to differentiate into neural cells. Apart from this, Notch, sonic hedgehog and wnt signalling pathways have been found to be important in the process of adult neurogenesis. Activation of the wnt/beta-catenin pathway increases the expression of Neuro D1, which is known to promote neuronal differentiation (Wexler *et al.* 2009). Sonic hedgehog on the other hand affects the level of primary cilia which is regulated by Kif3a (Han *et al.* 2008). However, the factors such as EGF, bFGF and HGF have been found effective in deriving neural phenotypes from mesenchymal stem cells. But their *in vivo* signalling mechanisms have not been clearly understood.

Several criteria have to be looked into before concluding which is the best possible source. Depending on the source, the MSC differentiation potential may likely vary (Musina *et al.* 2006). In terms of research, BM-MSCs have been most widely used. This can be due to the effective isolation and expansion protocols which can generate a large group of potential cells. However, umbilical-cord-derived MSCs are more primitive in origin. Thus, in terms of differentiation potential they should be more effective. On the other hand, stem cells from adipose tissue and dental pulp are easy to isolate as they do not require any donor most of the time and can be derived from the patient itself and is a less painful process as compared to bone marrow aspiration.

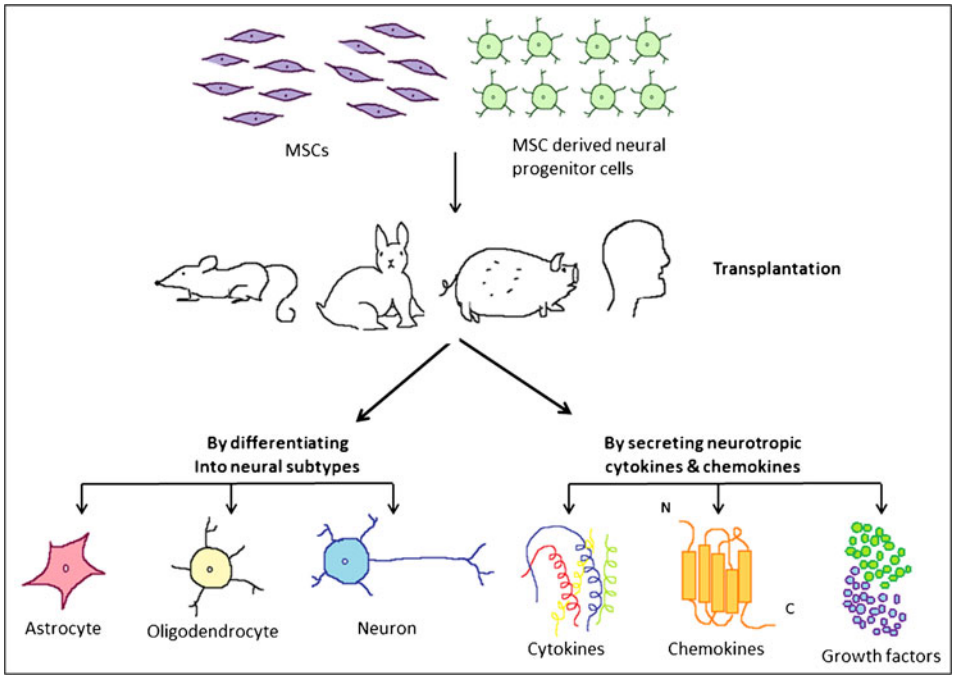

Figure 2. Hypothesized or proposed stem cell mechanism of action.

When it comes to neural induction, the cells from all these sources behave differently. Under certain induction conditions and with certain growth factors, all of these cells can be differentiated into the neural lineage. However, certain cells are able to generate more specific cell types which are useful while treating certain diseases. In comparison to BM-MSC a study showed that the ASCs were more effectively able to differentiate into neural, glial and vascular endothelial cell types. They also produced greater amounts of VEGF, HGF and angiopoeitin 1, which resulted in greater relief in an animal stroke model (Ikegame *et al.* 2011). In another study, the neuronal plasticity of WJ-MSC and BM-MSC towards dopaminergic cell type was compared. The marker analysis showed similar kind of results and the level of released dopamine from both the cell types was also found to be similar (Datta *et al.* 2011). In that case the WJ-MSCs would be more suitable due to easy availability. With a suitable cocktail of growth factors, the mesenchymal stem cells are induced into neural lineage. At the gene expression level, a study has shown comparison of transcriptome between MSCs of different sources. It was found that umbilical-cord-derived stem cells show high level of expression of LIF, ZIC1 and NTF3, which have known roles in neurogenesis. In contrast, BM-MSC express genes that are involved in endodermal and mesodermal development and ADSCs express high number of genes involved in immunity and inflammation (Jansen *et al.* 2010). However, *in vitro* BM-MSCs were able to generate more specified neural cell types.

In terms of transplantation, all the MSCs being non-immunogenic are suitable for treating the various brain-related neurodegenerative diseases and this allows the use of allogenic cell types. The MSCs will release certain trophic factors which results in functional recovery of the damaged neurons (figure 2). When we look at the preclinical trials, BM-MSCs have been more often used. These cells are able to graft within the injured site and bring about functional recovery. For effective transplantation, one of the requirements is a high number of potential cells. Although WJ and DP are good sources of MSCs, the total number of cells obtained is low, when compared to BM. On the other hand, generation of AD-MSCs are simpler than BM isolation process. Transcriptomic comparison studies of ADSC vs. BM-MSCs revealed over-expression of genes in BM-MSC which are involved in cell movement and migration, bone formation as well as cardiovascular and nervous system development, whereas ADSCs are more prone towards myogenic differentiation (Monaco *et al.* 2012).

7. Conclusion

MSCs have become good candidates for both autologous and allogenic stem cell transplantation. As discussed above, these cells can be isolated from a wide variety of

sources. The choice of the best source for treating neurodegenerative diseases still remains controversial. Some of these cells are easy to isolate whereas the others have a greater potential to differentiate toward the neural lineage. But most of the MSCs have been able to show positive effect and have proved their potential towards converting into neural phenotypes.

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