Similarities and Differences in Stem Cells Between Cancer, Normal, and Injured Brain

Lei Huang and Peng Huang

Introduction

Mesenchymal stem cells (MSCs) have recently garnered tremendous interest within the field of neuroscience because MSCs communicate and interact with the nervous system during brain development, injuries, and even tumor formation. Also, MSCs are easily isolated, cultured, and manipulated. Furthermore, MSCs have several unique characteristics, like immunomodulation, homing to sites of injury and secreting trophic factors. All of these make MSCs as a promising candidate to treat neurological diseases. In this chapter, we are trying to answer several questions involving the relationship between MSC, brain development, and pathology based on an increasing amount of experimental evidences. For example, is MSC-initiated neuronal transdifferentiation possible? Where are MSCs located in the brain? How and why can MSCs be successfully used to treat brain injuries? What are the relationships between MSCs and brain tumors?

L. Huang, Ph.D. (✉)
Research Imaging Institute, University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA
e-mail: huangl@uthscsa.edu

P. Huang, M.D., Ph.D.
Department of Laboratory Medicine and Pathology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, USA
e-mail: Huang.Peng@Mayo.edu

C.J. Malemud, E. Alsberg (eds.), *Mesenchymal Stem Cells and Immunomodulation*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-3-319-46733-7_5

MSC and the Brain

MSC Transdifferentiate into Neural Cells

Transdifferentiation is a process where different stem cells are capable of crossing the germ layer boundary to form cell types of alternative layers. The transdifferentiation concept has changed the notion that multipotent stem cells are restricted in their potency to form the cell types of a derived germ layer. For example, numerous studies show that mesodermal MSCs could transdifferentiate into ectodermal cells like neurons and astrocytes in vivo and in vitro [1–9, 20, 21]. It is a promising concept as this will make MSCs a good candidate for treatment of neurodegenerative disease, aiming to replace damaged or lost cells. However, to fully prove the possibility is still challenging.

Last decade, Woodbury et al. [1] and Sanchez-Ramos et al. [2] demonstrated for the first time the concept of MSCs participating in vitro in neuronal transdifferentiation. Their studies reported that neurons can be obtained from MSCs treated with chemicals or a cocktail of trophic factors [1, 2]. However, subsequent studies challenged both methods [3, 4] and raised the question as to whether MSCs neuronal differentiation was an artifact.

Until now, four major approaches have been proposed in order to transdifferentiate MSCs into neurons or glial cells in vitro:

1. Chemical induction (chemical compounds): For example, Woodbury et al. [1] previously treated MSC with beta-mercaptoethanol, followed by dimethyl sulfoxide (DMSO) and butylated hydroxyanisole (BHA); Deng et al. [5] used dibutyryl cyclic AMP(dbcAMP) and isobutylmethylxanthine (IBMX) for 3 days to induce MSC transdifferentiation; Francesco et al. [6] modified a neuronal induction medium by adding forskolin and valproic acid, but left out BHA. After induction, some of the cells had neuronal-like morphology and expressed neural markers such as neuron-specific nuclear protein (NeuN) and neuron-specific enolase (NSE). However, some follow-up studies questioned the conclusion derived from these protocols. Studies showed the formation of neuronal morphologies did not only take place in MSCs but also in human embryonic kidney (HEK)-293 cells and pheochromocytoma cell (PC)-12 cells after chemical induction [3, 7], which was probably due to the consequences of cell shrinkage and cytoskeleton alterations. Also, some neural proteins were spontaneously expressed on MSCs under standard culture conditions [4]. More importantly, these studies lacked functional electrophysiological evidence that shows excitatory properties of typical neuron.
2. Trophic factors: Brain-derived neurotrophic factor (BDNF) [2] or basic fibroblast growth factor (bFGF) [8, 9] combined with/without retinoic acid (RA) has been shown to induce neural differentiation. After induction, cells showed neuronal morphology and expressed neural marker-NeuN, microtubule-associated protein 2 (MAP2), or glial marker–glial fibrillary acidic protein (GFAP). An electrophysiological study demonstrated K^+ current and K^+ channels on the MSCs exposed to trophic factors FGF and EGF [10]. Furthermore, Cho et al. [11] confirmed that

MSCs treated with RA had spontaneous electrical activity and postsynaptic current, which is a unique characteristic of neuronal cells. Although this trophic factor induction appears to be promising, the function of neural-like MSC-derived cells still needs to be tested before translating this method to clinical usage, especially function on synaptic transmission and neurotransmitter regulation. Also, the host microenvironment may affect the characteristic of neural-like MSC after transplantation, and maintenance of the neuronal property after trophic factor induction needs to be further evaluated.

3. Genetic manipulation: A study showed that upregulating BDNF gene induced neuronal transdifferentiation of MSC following RA induction, which also increased the survival rate of MSCs compared to trophic factor induction alone [12]. A high ratio of neurons also can be obtained by Notch intracellular domain (NICD) transfection of MSCs followed with treatment with three trophic factors, bFGF, forskolin, and ciliary neurotrophic factor [13, 14]. Na^+, K^+ current and action potentials, as well as expression of a neural marker, were found on these cells. Meanwhile, another study has confirmed that MSCs formed neurospheres and successfully differentiated into neurons, also by NICD transfection [13]. Most importantly, these cells improved functional recovery of "stroke" rats after transplantation and showed extended long neurites [13]. Upregulating expression of neurogenin1 (Ngn1) was also sufficient to induce MSCs differentiate into neurons [15], with expression of neuron-specific proteins and voltage-gated Ca^{2+} and Na^+ channels. Not only upregulating proneural gene expression could achieve neuronal transdifferentiation of MSCs, but also knocking down neuronal-related gene has shown the possibility of inducing MSCs into neurons which involved downregulating gene RE-1 silencing factor (REST) using siRNA [16]. Taken together the results of these studies indicated gene manipulation plus trophic factor induction as a better strategy for MSC transdifferentiation with long-term maintenance of neuronal characteristics and better electrophysiological function compared to trophic factor alone. However, more risk exists with viral gene transfection for clinical usage. Therefore, the long-term effects of gene manipulation of such cells need to be further evaluated in vivo and in vitro.
4. Coculture of MSCs with neural cell types: A few studies showed that coculture with several neuronal types of cells, like cerebellar granule neuron [17], or astrocyte [18], can induce MSCs to differentiate into neurons with morphologic and molecular evidence. However, the effect may be due to trophic factors secreted by cocultured cells thus making it hard to tell the role of direct cell–cell interaction in this process.

Although tremendous progress has been made in MSCs neural transdifferentiation studies in vitro, to completely fulfill the 'neuron' definitions on single neural-like MSC is still challenging, like whether synaptic transmission of neuron induced from MSC can be regulated by neurotransmitters.

Evidence from in vitro studies indicated that the neuronal microenvironment could be important factors for MSCs neural induction. Indeed, this MSCs neural transdifferentiation phenomenon has been demonstrated from in vivo studies. Pioneer studies from Azizi et al. [19] and Kopen et al. [20] transplanted human

MSCs into adult brain or lateral ventricle of neonatal mice in which the engrafted MSCs survived and migrated throughout the brain. Some MSCs expressed neural or glial marker (GFAP). Furthermore, rat MSCs were infused into embryonic rat brain to evaluate their survival and phenotypic expression [21]. After infusion, engrafted MSCs migrated along the radial glial process and expressed neural marker NeuN. Although these results were exciting, unfortunately, in vivo studies cannot totally avoid the concern that neural transdifferentiation of MSC may be caused by spontaneous cell fusion, even though it happens at an extremely low frequency, as it has been shown that MSCs can fuse with neural cell types spontaneously [22]. So in vivo studies need to better separate engrafted MSCs and host cells using various methods and demonstrate the function of neuron-like MSCs in the future.

Brain Pericytes

MSCs were initially isolated from the bone marrow of an adult organism. However, subsequent studies demonstrated MSCs can also be obtained from nonmarrow tissues, such as adult muscle [23], adipose tissue [24], even brain [25]. Using the same culture method for bone marrow-derived MSCs, MSCs were successfully isolated from mouse brain with expression of mouse MSCs marker as well as their ability to undergo mesodermal differentiation [26]. Similarly, a group of cells, isolated from human brain ventricular wall and neocortex, expressed MSCs marker and have true multilineage potential toward a mesodermal and neuroectodermal phenotype [27].

Although MSCs can be isolated retrospectively from different tissues, the native distribution of MSCs has long been a mystery. Two landmark studies [28, 29] published in 2008 partly unveiled the reason for this mystery. Thus, Crisan et al. [28] identified a subset of pericytes from multiple adult tissues, which expressed CD146, neural/glial antigen 2 (NG2), and platelet-derived growth factor (PDGF)-Rβ, and exhibited the same osteogenic, chondrogenic, adipogenic, and myogenic potential as MSCs. This indicated that the pericyte may be integral to the origin of the elusive MSCs [28]. Since then, the characteristics and function of pericyte have been reexplored and recognized, as this type of cells was first found 140 years ago [30]. Pericytes are perivascular cells, which form an incomplete layer on the abluminal surface of capillary endothelial cells. In addition, the known functions of pericytes include vascular support, participating in angiogenesis, matrix protein synthesis, vessel stabilization, and regulation of vascular tone [31]. Most importantly, recent studies showed that pericytes have been regarded as a potential reservoir of stem cells for adult tissue repair.

In the central nervous system, the pericyte is an important part of the neurovascular unit (NVU), which consists of neural cells and vascular cells. The pericyte is involved in the regulation of angiogenesis, vascular tone, and blood–brain barrier function. They are mainly distributed around cerebral capillaries and cover more than 30 % surface of capillaries [30]. Paul et al. [27] indicated that adult brain pericytes have all the features of MSCs, such as expressing MSCs immunological markers, $CD105^+$, $CD90^+$, $CD73^+$, as well as mesenchymal differentiation potential [27].

MSCs and Brain Injury

Therapeutic Roles of MSC for Brain Injury

MSC transplantation in human patients began in 1995, aimed at promoting the survival of engrafted hematopoietic stem cell. Based on the safety of MSC transplantation and multiple potentials of MSCs, subsequent studies have been performed to investigate the therapeutic role in numerous diseases and disorders, including brain injury.

Stroke and traumatic brain injury (TBI) are the leading causes of adult disability worldwide, arising from the loss of neurons and impairment of neurological function. Unfortunately, there is limited treatment for these diseases. Preclinical studies, using MSCs transplantation to treat stroke and TBI, began early this century. Li et al. [32] transplanted bone marrow-derived MSCs into "stroke" mouse brain. They found that the engrafted MSCs survived and improved functional recovery. From that, numerous follow-up studies tried to figure out the optimized source of MSCs, delivery routes, time window, and dosage for MSCs transplantation for stroke and TBI.

Delivery Routes

Three major routes have been investigated for stroke treatment: intracerebral [33–36], intracarotid [37, 38], and intravenous [39–41]. A growing number of studies showed MSCs administration decreased infarct size and improved neurological outcome in "stroke" animals through all three routes. However, it remains unclear which route is more efficient based on existing experimental evidence, as these studies lacked a direct comparison with different delivery routes of MSCs. One meta-analysis, based on preclinical studies of MSCs for ischemic stroke, showed that the effect size of intracerebral administration was larger than with the intravenous one [42]. This indicated that direct transplant of MSCs into brain may be more efficient, but it is invasive and needs complex neurosurgery. Furthermore, intracerebral [43, 44] and intravenous [43] MSCs transplantation have also been evaluated for TBI treatment. Both routes of MSCs administration improved functional recovery after TBI. However, which route is ideal remains unclear.

Cell Resources

MSCs, derived from various resources, have been investigated for stroke and TBI treatment, including bone marrow [45, 46], placenta [47], peripheral blood [48], adipose [45, 46], and umbilical cord blood [49, 50]. All of these cells have been shown beneficial impact on neural injury after transplantation. However, few studies have compared the efficacy of different MSCs. There was one study that indicated adipose-derived MSCs maybe a preferable source than bone marrow-derived MSCs for stroke therapy because of higher proliferative activity, more vascular endothelial growth factor (VEGF) secretion, and easier access [45].

Timing

Time of MSCs delivery after stroke varied from 1 h to 1 month [42]. Several studies published recently indicated 24 h after stroke is optimized for MSCs intraarterial or intravenous transplantation with improved behavior and more cell migration to infarcts [51–53]. Also 24 h is clinically reasonable, when patients tend to be stabilized. For TBI, 24 h following TBI were typically used for MSC transplantation, based on known study results [54]. However, the optimized time (i.e., window) for transplantation remains unclear, since it is hard to decide based on current available information.

Dosage

The MSCs dosage used for stroke preclinical studies ranges from 4×10^5 to 1.2×10^8 cells/kg [54]. Chen et al. [41] evaluated the relationship between cell dose and efficacy. High dosage (3×10^6) was more efficient than low dosage (1×10^6) for MSCs intravenous transplantation on the cerebral ischemic rat with better behavioral recovery. Also MSCs transplantation dose dependently restored cerebral blood flow (CBF) and blood–brain barrier (BBB) function [55]. However, various quantity and presentation of cell dosage make it harder to compare the efficacy among different preclinical studies and to directly guide clinical studies. Thus, dosage used for TBI studies varied from 6×10^6 to 3.2×10^8 cells/kg depending on the administration route [54]. However, optimized dosage for stroke and TBI therapy still needs to be explored.

Mechanisms of MSCs Cell Therapy on Brain Injury

Immunomodulation

MSCs undergo crosstalk with the innate and adaptive immune system. Their immunomodulatory functions depend on the microenvironment, through cell contact and independent mechanisms (reviewed by Blanc et al. [56]). Stroke and TBI induce a strong inflammatory response that leads to subsequent recruitment of leukocytes to the infarct zone. MSCs transplantation significantly reduced inflammation and subsequent cell death. Ohtaki et al. [57] used microarray to detect gene changes after MSCs transplantation on global cerebral ischemic mice. Over 10 % of proinflammation genes were downregulated after human bone marrow-derived MSCs transplantation and three neuroprotective genes were upregulated [57]. Similarly, engrafted MSCs reduced brain inflammation and suppressed microglia and macrophage activity after TBI [44, 58]. The resolution of the postinjury inflammatory milieu will also ameliorate brain self-repair, as evidence has showed that MSCs reduced glial scar formation after stroke or TBI [59].

More interestingly, engrafted MSC-induced immunomodulation is not limited to injured brain and it affects peripheral immune organs as well. Thus, a recent study showed a dramatic spleen distribution of MSCs after intravenous administration to rat after induction of stroke [60]. Engrafted MSCs not only had a remote anti-inflammation role on brain but also reduced TNF-α expression in spleen [60].

Trophic Factors

Although numerous studies have confirmed the neural transdifferentiation potential of MSCs in vitro, solid evidences that indicate a therapeutic role for MSCs on stroke and TBI is due to cell replacement is still lacking. On the other hand, bystander effects of MSCs transplantation play a more important role in brain recovery, especially involving secreted trophic factors by engrafts. In vitro studies showed cocultured with stroke and TBI brain extracts upregulated MSCs synthesis and expression of trophic factors, BDNF, NGF, VEGF, and HGF in vitro [61]. Meanwhile, MSCs transplantation increased trophic factors expression not only in engrafted cells but also in host brain tissue after stroke [62]. Also, the expression of host NGF and BDNF genes was significantly increased after intravenous administration of MSCs for TBI [63]. Furthermore, compared to MSC alone, BDNF gene-modified human MSCs resulted in increased BDNF expression and enhanced the therapeutic effect of cell therapy on stroke [64]. As Li and Chopp et al. [65] described, transplanted MSCs work as 'small molecular factories' by continually secreting trophic factors for brain repair. Maybe that's why cell therapy is more efficient than single molecular therapy.

Angiogenesis

Angiogenesis is an important event related to the long-term repair and restoration process of the brain after brain injury. Cultured MSCs continually secrete angiogenic cytokines including, VEGF, bFGF, and placental growth factor (PLGF) [66–69]. Thus, MSC transplantation promoted VEGF secretion, VEGF receptor 2 (VEGFR2) expression, and angiogenesis in the ischemic boundary zone (IBZ) after stroke [70, 71]. A recent study also indicated that only exosomes derived from cultured MSCs were able to enhance angiogenesis in animals following stroke [72]. Furthermore, effect of brain angiogenesis after stroke was greater after transplantation of PLGF gene-modified MSCs, compared to nonmodified MSCs [73].

In addition to secreting angiogenic factors, MSCs also have the potential to differentiate into an endothelial lineage [74]. This unique property could be beneficial for vascular repair after brain injury. Indeed, Liao et al. [50] observed a subset of engrafted cells that differentiated into endothelial cells after intracerebral transplanted human umbilical-derived MSCs (UC-MSCs) in a rat model of stroke. Also, the UC-MSCs treatment increased vascular density and VEGF expression in ipsilateral hemisphere [50].

MSC and Cancer

Cancer Stem Cells (CSCs)

A tumor or cancer can be viewed as an aberrant organ initiated by a tumorigenic cancer cell that acquired the capacity for indefinite proliferation through accumulated mutation [75]. Two hypothetical models, stochastic and hierarchal, have been proposed to explain tumor initiation and development [76]. Cancer-stem-cell theory derived from the hierarchal model asserts that only a rare subset of cells within the tumor have the ability to generate new tumors [75]. In 1997, the confirmatory experimental evidence for this theory was demonstrated by Bonnet and Dick [77]. Since then, numerous studies have verified the existence of cancer stem cells in various kinds of cancer, for example, breast cancer [78], brain tumor [79, 80]. Compared to normal stem cells, cancer stem cells have similar properties of self-renewal and differentiation, but cancer stem cells usually have genomic or karyotypic mutation and aberrant differentiation [80]. The concept of cancer stem cell has propelled researchers in a direction to better understand the oncogenesis and to rethink the strategy for cancer therapy.

MSC and Brain Tumor

Glioblastoma multiforme is an aggressive and invasive neoplasm characterized by extensive neovascularization [76]. Several groups demonstrated tropism of MSC to gliomas by implanting MSCs into gliomas of animals and tracking the migration of MSCs [81, 82]. This tumor-specific migratory pattern makes MSCs a promising cellular vehicle for delivery of therapeutic agents, although whether tumor cells recruit endogenous MSCs remain to be clarified. Meanwhile, glioblastoma stem cells (GSC) are able to transdifferentiate into pericytes or MSC-like cells [83], contributing to the maintenance of microvasculature itself [84, 85]. In addition, the selective elimination of GSC-derived pericytes disrupted the neovasculature and potently inhibited tumor growth [84].

The effect of native MSCs on tumor growth is still controversial. On the one hand, MSCs have been shown to suppress tumor growth of glioma [81, 86], through suppressed tumor angiogenesis [86]. On the other hand, others have reported MSC implantation can promote tumor growth [87], partly by supporting tumor vasculature [82, 88], and by reducing tumor cell apoptosis [87].

Even though the relationship between brain tumor/CSCs and MSCs is controversial, several studies indicated MSCs could be regarded as vector to deliver therapeutic molecules [89, 90], based on the homing property of MSCs to tumor. Sasportas et al. [89] gene-modified MSC to secrete cytokine tumor necrosis factor apoptosis ligand (TRAIL). In vitro and in vivo studies showed TRAIL-MSCs successfully inhibited growth of glioma through inducing tumor cell apoptosis [89]. Similar

results have been verified by another group [90]. Moreover, the results of this study demonstrated that antitumor effect of TRAIL-MSC was better than TRAIL alone using adenovirus-mediated delivery [90]. Other antitumor molecules, such as HSV-tk [91], IL-17 [92], and IFN-α [93], have also been investigated and have shown reduced tumor development. All of these studies indicated using MSCs as a vehicle to target tumor is a promising strategy for future tumor therapy.

References

1. Woodbury D, Schwarz EJ, Prockop DJ, Black IB (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 61:364–370
2. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR (2000) Adult bone marrow stromal cells differentiate into neural cells *in vitro*. Exp Neurol 164:247–256
3. Lu P, Blesch A, Tuszynski MH (2004) Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? J Neurosci Res 77:174–191
4. Deng J, Petersen BE, Steindler DA, Jorgensen ML, Laywell ED (2006) Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. Stem Cells 24:1054–1064
5. Deng W, Obrocka M, Fischer I, Prockop DJ (2001) In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. Biochem Biophys Res Commun 282:148–152
6. Jori FP, Napolitano MA, Melone MA, Cipollaro M, Cascino A, Altucci L, Peluso G, Giordano A, Galderisi U (2005) Molecular pathways involved in neural *in vitro* differentiation of marrow stromal stem cells. J Cell Biochem 94:645–655
7. Neuhuber B, Gallo G, Howard L, Kostura L, Mackay A, Fischer I (2004) Reevaluation of in vitro differentiation protocols for bone marrow stromal cells: disruption of actin cytoskeleton induces rapid morphological changes and mimics neuronal phenotype. J Neurosci Res 77:192–204
8. Trzaska KA, Kuzhikandathil EV, Rameshwar P (2007) Specification of a dopaminergic phenotype from adult human mesenchymal stem cells. Stem Cells 25:2797–2808
9. Barzilay R, Kan I, Ben-Zur T, Bulvik S, Melamed E, Offen D (2008) Induction of human mesenchymal stem cells into dopamine-producing cells with different differentiation protocols. Stem Cells Dev 17:547–554
10. Mareschi K, Novara M, Rustichelli D, Ferrero I, Guido D, Carbone E, Medico E, Madon E, Vercelli A, Fagioli F (2006) Neural differentiation of human mesenchymal stem cells: evidence for expression of neural markers and eag K+ channel types. Exp Hematol 34:1563–1572
11. Cho KJ, Trzaska KA, Greco SJ, McArdle J, Wang FS, Ye JH, Rameshwar P (2005) Neurons derived from human mesenchymal stem cells show synaptic transmission and can be induced to produce the neurotransmitter substance P by interleukin-1 alpha. Stem Cells 23:383–391
12. Zhao LX, Zhang J, Cao F, Meng L, Wang DM, Li YH, Nan X, Jiao WC, Zheng M, Xu XH, Pei XT (2004) Modification of the brain-derived neurotrophic factor gene: a portal to transform mesenchymal stem cells into advantageous engineering cells for neuroregeneration and neuroprotection. Exp Neurol 190:396–406
13. Hayase M, Kitada M, Wakao S, Itokazu Y, Nozaki K, Hashimoto N, Takagi Y, Dezawa M (2009) Committed neural progenitor cells derived from genetically modified bone marrow stromal cells ameliorate deficits in a rat model of stroke. J Cereb Blood Flow Metab 29:1409–1420
14. Dezawa M, Kanno H, Hoshino M, Cho H, Matsumoto N, Itokazu Y, Tajima N, Yamada H, Sawada H, Ishikawa H, Mimura T, Kitada M, Suzuki Y, Ide C (2004) Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest 113:1701–1710

15. Kim SS, Yoo SW, Park TS, Ahn SC, Jeong HS, Kim JW, Chang DY, Cho KG, Kim SU, Huh Y, Lee JE, Lee SY, Lee YD, Suh-Kim H (2008) Neural induction with neurogenin1 increases the therapeutic effects of mesenchymal stem cells in the ischemic brain. Stem Cells 26:2217–2228
16. Trzaska KA, Reddy BY, Munoz JL, Li KY, Ye JH, Rameshwar P (2008) Loss of RE-1 silencing factor in mesenchymal stem cell-derived dopamine progenitors induces functional maturity. Mol Cell Neurosci 39:285–290
17. Wislet-Gendebien S, Hans G, Leprince P, Rigo JM, Moonen G, Rogister B (2005) Plasticity of cultured mesenchymal stem cells: switch from nestin-positive to excitable neuron-like phenotype. Stem Cells 23:392–402
18. Bossolasco P, Cova L, Calzarossa C, Rimoldi SG, Borsotti C, Deliliers GL, Silani V, Soligo D, Polli E (2005) Neuro-glial differentiation of human bone marrow stem cells in vitro. Exp Neurol 193:312–325
19. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ (1998) Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats--similarities to astrocyte grafts. Proc Natl Acad Sci U S A 95:3908–3913
20. Kopen GC, Prockop DJ, Phinney DG (1999) Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 96:10711–10716
21. Munoz-Elias G, Marcus AJ, Coyne TM, Woodbury D, Black IB (2004) Adult bone marrow stromal cells in the embryonic brain: engraftment, migration, differentiation, and long-term survival. J Neurosci 24:4585–4595
22. Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM (2003) Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. Proc Natl Acad Sci U S A 100:2088–2093
23. Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM (2002) Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp Hematol 30:896–904
24. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279–4295
25. da Silva Meirelles L, Chagastelles PC, Nardi NB (2006) Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 119:2204–2213
26. Kang SG, Shinojima N, Hossain A, Gumin J, Yong RL, Colman H, Marini F, Andreeff M, Lang FF (2010) Isolation and perivascular localization of mesenchymal stem cells from mouse brain. Neurosurgery 67:711–720
27. Paul G, Ozen I, Christophersen NS, Reinbothe T, Bengzon J, Visse E, Jansson K, Dannaeus K, Henriques-Oliveira C, Roybon L, Anisimov SV, Renstrom E, Svensson M, Haegerstrand A, Brundin P (2012) The adult human brain harbors multipotent perivascular mesenchymal stem cells. PLoS One 7:e35577
28. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhring HJ, Giacobino JP, Lazzari L, Huard J, Peault B (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3:301–313
29. Covas DT, Panepucci RA, Fontes AM, Silva WA Jr, Orellana MD, Freitas MC, Neder L, Santos AR, Peres LC, Jamur MC, Zago MA (2008) Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. Exp Hematol 36:642–654
30. Lange S, Trost A, Tempfer H, Bauer HC, Bauer H, Rohde E, Reitsamer HA, Franklin RJ, Aigner L, Rivera FJ (2013) Brain pericyte plasticity as a potential drug target in CNS repair. Drug Discov Today 18:456–463
31. Winkler EA, Bell RD, Zlokovic BV (2011) Central nervous system pericytes in health and disease. Nat Neurosci 14:1398–1405

32. Li Y, Chopp M, Chen J, Wang L, Gautam SC, Xu YX, Zhang Z (2000) Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. J Cereb Blood Flow Metab 20:1311–1319
33. Shichinohe H, Kuroda S, Yano S, Ohnishi T, Tamagami H, Hida K, Iwasaki Y (2006) Improved expression of gamma-aminobutyric acid receptor in mice with cerebral infarct and transplanted bone marrow stromal cells: an autoradiographic and histologic analysis. J Nucl Med 47:486–491
34. Ikeda N, Nonoguchi N, Zhao MZ, Watanabe T, Kajimoto Y, Furutama D, Kimura F, Dezawa M, Coffin RS, Otsuki Y, Kuroiwa T, Miyatake S (2005) Bone marrow stromal cells that enhanced fibroblast growth factor-2 secretion by herpes simplex virus vector improve neurological outcome after transient focal cerebral ischemia in rats. Stroke 36:2725–2730
35. Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M (2001) Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J Neurol Sci 189:49–57
36. Zhao MZ, Nonoguchi N, Ikeda N, Watanabe T, Furutama D, Miyazawa D, Funakoshi H, Kajimoto Y, Nakamura T, Dezawa M, Shibata MA, Otsuki Y, Coffin RS, Liu WD, Kuroiwa T, Miyatake S (2006) Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF gene transfer with HSV-1 vector. J Cereb Blood Flow Metab 26:1176–1188
37. Shen LH, Li Y, Chen J, Zhang J, Vanguri P, Borneman J, Chopp M (2006) Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. Neuroscience 137:393–399
38. Li Y, Chen J, Wang L, Lu M, Chopp M (2001) Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology 56:1666–1672
39. Cui X, Chen J, Zacharek A, Li Y, Roberts C, Kapke A, Savant-Bhonsale S, Chopp M (2007) Nitric oxide donor upregulation of stromal cell-derived factor-1/chemokine (CXC motif) receptor 4 enhances bone marrow stromal cell migration into ischemic brain after stroke. Stem Cells 25:2777–2785
40. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M (2003) Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 73:778–786
41. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M (2001) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 32:1005–1011
42. Vu Q, Xie K, Eckert M, Zhao W, Cramer SC (2014) Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. Neurology 82:1277–1286
43. Mahmood A, Lu D, Chopp M (2004) Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. Neurosurgery 55:1185–1193
44. Zanier ER, Montinaro M, Vigano M, Villa P, Fumagalli S, Pischiutta F, Longhi L, Leoni ML, Rebulla P, Stocchetti N, Lazzari L, De Simoni MG (2011) Human umbilical cord blood mesenchymal stem cells protect mice brain after trauma. Crit Care Med 39:2501–2510
45. Ikegame Y, Yamashita K, Hayashi S, Mizuno H, Tawada M, You F, Yamada K, Tanaka Y, Egashira Y, Nakashima S, Yoshimura S, Iwama T (2011) Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy 13:675–685
46. Gutierrez-Fernandez M, Rodriguez-Frutos B, Ramos-Cejudo J, Teresa Vallejo-Cremades M, Fuentes B, Cerdan S, Diez-Tejedor E (2013) Effects of intravenous administration of allogenic bone marrow- and adipose tissue-derived mesenchymal stem cells on functional recovery and brain repair markers in experimental ischemic stroke. Stem Cell Res Ther 4:11
47. Kranz A, Wagner DC, Kamprad M, Scholz M, Schmidt UR, Nitzsche F, Aberman Z, Emmrich F, Riegelsberger UM, Boltze J (2010) Transplantation of placenta-derived mesenchymal stromal cells upon experimental stroke in rats. Brain Res 1315:128–136
48. Ukai R, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD (2007) Mesenchymal stem cells derived from peripheral blood protects against ischemia. J Neurotrauma 24:508–520

49. Koh SH, Kim KS, Choi MR, Jung KH, Park KS, Chai YG, Roh W, Hwang SJ, Ko HJ, Huh YM, Kim HT, Kim SH (2008) Implantation of human umbilical cord-derived mesenchymal stem cells as a neuroprotective therapy for ischemic stroke in rats. Brain Res 1229:233–248
50. Liao W, Xie J, Zhong J, Liu Y, Du L, Zhou B, Xu J, Liu P, Yang S, Wang J, Han Z, Han ZC (2009) Therapeutic effect of human umbilical cord multipotent mesenchymal stromal cells in a rat model of stroke. Transplantation 87:350–359
51. Lee SH, Jin KS, Bang OY, Kim BJ, Park SJ, Lee NH, Yoo KH, Koo HH, Sung KW (2015) Differential migration of mesenchymal stem cells to ischemic regions after middle cerebral artery occlusion in rats. PLoS One 10:e0134920
52. Yavagal DR, Lin B, Raval AP, Garza PS, Dong C, Zhao W, Rangel EB, McNiece I, Rundek T, Sacco RL, Perez-Pinzon M, Hare JM (2014) Efficacy and dose-dependent safety of intra-arterial delivery of mesenchymal stem cells in a rodent stroke model. PLoS One 9:e93735
53. Toyoshima A, Yasuhara T, Kameda M, Morimoto J, Takeuchi H, Wang F, Sasaki T, Sasada S, Shinko A, Wakamori T, Okazaki M, Kondo A, Agari T, Borlongan CV, Date I (2015) Intra-arterial transplantation of allogeneic mesenchymal stem cells mounts neuroprotective effects in a transient ischemic stroke model in rats: analyses of therapeutic time window and its mechanisms. PLoS One 10:e0127302
54. Monsel A, Zhu YG, Gennai S, Hao Q, Liu J, Lee JW (2014) Cell-based therapy for acute organ injury: preclinical evidence and ongoing clinical trials using mesenchymal stem cells. Anesthesiology 121:1099–1121
55. Borlongan CV, Lind JG, Dillon-Carter O, Yu G, Hadman M, Cheng C, Carroll J, Hess DC (2004) Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats. Brain Res 1010:108–116
56. Le Blanc K, Mougiakakos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 12:383–396
57. Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, Prockop DJ (2008) Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. Proc Natl Acad Sci U S A 105:14638–14643
58. Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, Chen FF, Jiang XD (2013) Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation 10:106
59. Li Y, Chen J, Zhang CL, Wang L, Lu D, Katakowski M, Gao Q, Shen LH, Zhang J, Lu M, Chopp M (2005) Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. Glia 49:407–417
60. Acosta SA, Tajiri N, Hoover J, Kaneko Y, Borlongan CV (2015) Intravenous bone marrow stem cell grafts preferentially migrate to spleen and abrogate chronic inflammation in stroke. Stroke 46:2616–2627
61. Chen X, Katakowski M, Li Y, Lu D, Wang L, Zhang L, Chen J, Xu Y, Gautam S, Mahmood A, Chopp M (2002) Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. J Neurosci Res 69:687–691
62. Wakabayashi K, Nagai A, Sheikh AM, Shiota Y, Narantuya D, Watanabe T, Masuda J, Kobayashi S, Kim SU, Yamaguchi S (2010) Transplantation of human mesenchymal stem cells promotes functional improvement and increased expression of neurotrophic factors in a rat focal cerebral ischemia model. J Neurosci Res 88:1017–1025
63. Mahmood A, Lu D, Chopp M (2004) Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. J Neurotrauma 21:33–39
64. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H (2004) BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. Mol Ther 9:189–197
65. Chopp M, Li Y (2002) Treatment of neural injury with marrow stromal cells. Lancet Neurol 1:92–100

66. Schinkothe T, Bloch W, Schmidt A (2008) *In vitro* secreting profile of human mesenchymal stem cells. Stem Cells Dev 17:199–206
67. Kagiwada H, Yashiki T, Ohshima A, Tadokoro M, Nagaya N, Ohgushi H (2008) Human mesenchymal stem cells as a stable source of VEGF-producing cells. J Tissue Eng Regen Med 2:184–189
68. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE (2004) Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms. Circ Res 94:678–685
69. Eckert MA, Vu Q, Xie K, Yu J, Liao W, Cramer SC, Zhao W (2013) Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke. J Cereb Blood Flow Metab 33:1322–1334
70. Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M (2003) Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res 92:692–699
71. Pavlichenko N, Sokolova I, Vijde S, Shvedova E, Alexandrov G, Krouglyakov P, Fedotova O, Gilerovich EG, Polyntsev DG, Otellin VA (2008) Mesenchymal stem cells transplantation could be beneficial for treatment of experimental ischemic stroke in rats. Brain Res 1233:203–213
72. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M (2013) Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J Cereb Blood Flow Metab 33:1711–1715
73. Liu H, Honmou O, Harada K, Nakamura K, Houkin K, Hamada H, Kocsis JD (2006) Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. Brain 129:2734–2745
74. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C (2004) Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 22:377–384
75. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414:105–111
76. Bexell D, Scheding S, Bengzon J (2010) Toward brain tumor gene therapy using multipotent mesenchymal stromal cell vectors. Mol Ther 18:1067–1075
77. Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737
78. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100:3983–3988
79. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA (2002) Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia 39:193–206
80. Vescovi AL, Galli R, Reynolds BA (2006) Brain tumour stem cells. Nat Rev Cancer 6:425–436
81. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, Bizen A, Honmou O, Niitsu Y, Hamada H (2004) Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. Gene Ther 11:1155–1164
82. Bexell D, Gunnarsson S, Tormin A, Darabi A, Gisselsson D, Roybon L, Scheding S, Bengzon J (2009) Bone marrow multipotent mesenchymal stroma cells act as pericyte-like migratory vehicles in experimental gliomas. Mol Ther 17:183–190
83. Tso CL, Shintaku P, Chen J, Liu Q, Liu J, Chen Z, Yoshimoto K, Mischel PS, Cloughesy TF, Liau LM, Nelson SF (2006) Primary glioblastomas express mesenchymal stem-like properties. Mol Cancer Res 4:607–619
84. Cheng L, Huang Z, Zhou W, Wu Q, Donnola S, Liu JK, Fang X, Sloan AE, Mao Y, Lathia JD, Min W, McLendon RE, Rich JN, Bao S (2013) Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell 153:139–152
85. Gilbertson RJ, Rich JN (2007) Making a tumour's bed: glioblastoma stem cells and the vascular niche. Nat Rev Cancer 7:733–736

86. Ho IA, Toh HC, Ng WH, Teo YL, Guo CM, Hui KM, Lam PY (2013) Human bone marrow-derived mesenchymal stem cells suppress human glioma growth through inhibition of angiogenesis. Stem Cells 31:146–155
87. Yu JM, Jun ES, Bae YC, Jung JS (2008) Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo. Stem Cells Dev 17:463–473
88. Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, Umezawa A, Kijima H, Fukuda S, Saijo Y (2011) Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med 17:579–587
89. Sasportas LS, Kasmieh R, Wakimoto H, Hingtgen S, van de Water JA, Mohapatra G, Figueiredo JL, Martuza RL, Weissleder R, Shah K (2009) Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. Proc Natl Acad Sci U S A 106:4822–4827
90. Kim SM, Lim JY, Park SI, Jeong CH, Oh JH, Jeong M, Oh W, Park SH, Sung YC, Jeun SS (2008) Gene therapy using TRAIL-secreting human umbilical cord blood-derived mesenchymal stem cells against intracranial glioma. Cancer Res 68:9614–9623
91. Miletic H, Fischer Y, Litwak S, Giroglou T, Waerzeggers Y, Winkeler A, Li H, Himmelreich U, Lange C, Stenzel W, Deckert M, Neumann H, Jacobs AH, von Laer D (2007) Bystander killing of malignant glioma by bone marrow-derived tumor-infiltrating progenitor cells expressing a suicide gene. Mol Ther 15:1373–1381
92. Gunnarsson S, Bexell D, Svensson A, Siesjo P, Darabi A, Bengzon J (2010) Intratumoral IL-7 delivery by mesenchymal stromal cells potentiates IFNgamma-transduced tumor cell immunotherapy of experimental glioma. J Neuroimmunol 218:140–144
93. Sato H, Kuwashima N, Sakaida T, Hatano M, Dusak JE, Fellows-Mayle WK, Papworth GD, Watkins SC, Gambotto A, Pollack IF, Okada H (2005) Epidermal growth factor receptor-transfected bone marrow stromal cells exhibit enhanced migratory response and therapeutic potential against murine brain tumors. Cancer Gene Ther 12:757–768