Regenerative Medicine for Stroke – Are We There Yet?

Brigitte Onteniente^a Jérôme Polentes^b

^aINSERM UMR861/UEVE and ^bCECS, I-STEM, Evry, France

Key Words

Brain plasticity • Neural progenitors • Pluripotent stem cell •
Regenerative medicine • Stroke

Abstract

With the development of stem cell (SC) biology, cell-based therapy has become a highly challenging field for experimental and clinical research. Among neurological disorders, stroke has pioneered the clinical application of SC. Safety concerns have prevailed for pilot clinical studies and important preclinical work is ongoing to help SC therapy reach the level of generalization. Stroke is classically divided into an acute, a subacute and a chronic phase. Each phase is defined by a complex array of events with overlapping and distinct kinetics that lead to both rapid tissue degeneration and long-lasting remodeling. Each SC type possesses intrinsic properties – transposed via cell-autonomous and non-cell-autonomous signaling – that would more specifically address some of these events. A better definition of what is expected from SC therapy in stroke might help assign SC sources to the acute or chronic phases and possibly optimize their use in the clinic.

Copyright © 2011 S. Karger AG, Basel

Regenerative Therapy for Stroke: Lessons from Brain Plasticity Studies

A major scientific breakthrough of the XIXth century has been the identification of the amazing capacity of cerebral structures to remodel their molecular and structural characteristics in response to injury. Called brain plasticity after the term adopted by S. Ramón y Cajal from Ioan Minea [1], this capacity compensates for the limited possibilities of regeneration in the adult central nervous system (CNS). Postlesional plasticity is particularly demonstrative in stroke, where conspicuous functional recovery is observed in both animal models [2–4] and patients [5], and the anatomical and chemical substrates of these mechanisms are actively being deciphered. As could be expected, they include axonal sprouting, increased synaptic efficacy, angiogenesis and neurogenesis [3, 6–8].

The close relationship between brain plasticity and cell therapy was highlighted in the 1980s by intracerebral transplantation studies. Over a period of 20 years, experimental evidence has accumulated to show the ability of damaged CNS structures to adopt exogenous immature neurons and – although to a limited extent – to incorporate these neurons into the rewiring process after a brain

lesion, including stroke [9]. These results ultimately led to clinical application in Parkinson and Huntington's diseases and in spinal disorders [10]. Experiments are on their way to specify how the postischemic brain takes advantage of transplanted or injected immature cells to improve its innate adaptive capacities. For instance, neural cells genetically engineered to release NGF ameliorate the survival of host striatal projection neurons [11], confirming that release of neurotrophic factors is among the array of mechanisms that link cell therapy to poststroke plasticity.

Regenerative Therapy for Stroke: Decades of Evidence

Epidemiology data indicate a 4-min interval between each stroke-related death in the USA [12]. In Europe, cerebrovascular diseases are estimated to constitute 17% of the burden of all diseases. Worldwide, 15 million people suffer a stroke each year, with dramatic consequences in underprivileged populations with increased risk factors and poor medical insurance. Despite decades of academic and industrial research, stroke continues to thwart the attempts of developing effective treatments [13]. The major advance, thrombolysis, keeps aside a great majority of patients who fall outside of the clinical time window for effective treatment. New devices for thrombolectomy are being developed, and neuroprotective therapies that include the use of magnesium, statins, erythropoietin [14], induced hypothermia [15] or strategies to limit the deleterious effects of thrombolytics [16] are being explored.

The proof-of-principle of cell therapy in stroke had been provided at the preclinical level many years before the term 'stem cell' boomed in neurobiology. The potential of intracerebral transplantation was first demonstrated using fetal brain tissue from rats [17–20] and, more recently, from humans [21]. The development of SC biology and discovery – sometimes rediscovery – of the impressive number of SC types present in the developing and adult organism has led to the current enthusiasm and exponentially expanding applications in regenerative therapy paradigms, including stroke [22–24]. If stroke was not part of the initial wave of transplantation studies in humans using fetal tissue, the development of the SC field has rapidly brought cell therapy interventions in stroke to the forefront (table 1) [25, 26]. Experimental data teach that SC therapy can limit neuronal degeneration, help plasticity and improve the functional outcome.

However, little is yet known on the components of the dialogue that establishes between the graft and the host to sustain these effects.

The quality of the host-graft dialogue, hence the extent of functional benefit, are closely related to the SC type. Each SC comes with specificities linked to its origin. These specificities determine the potential of the defined SC to interfere with one or more of the many aspects of stroke-induced degeneration and plasticity, and all SC sources might not display a similar ability to improve brain remodeling or to modulate fatal inflammation. Analysis of SC characteristics in the light of poststroke parenchymal events might help to better define their modalities of clinical application in terms of SC choice and timing of administration (fig. 1).

Engraftment versus Nonengraftment

Examples of engraftment and differentiation into neural cells have been produced for nonneural SC types mainly after intracerebral injection [27, 28]. Despite a robust tropism for injury sites, most studies describe the loss of intravenously injected cells after a rather short period of time. Consensus has been reached to consider that the reported improvement of stroke outcome after systemic injection of nonneural SC relies on their ability to release relevant factors, i.e. on non-cell-autonomous properties, rather than on engraftment into the lesioned area. These properties have mainly been investigated for SC derived from the bone marrow stroma, the BMSC. BMSC release a wide array of cytokines with trophic factor activity, promote synaptogenesis and neurogenesis, and limit the apoptosis of newborn cells [24, 27–29]. BMSC promote angiogenesis, through production of VEGF [30], and improve the regional cerebral blood flow when transplanted intracerebrally [31]. In addition, BMSC possess immunomodulatory properties [32–34], they can limit the local inflammation response by decreasing microglial and macrophagic activation [33] and impair T lymphocyte maturation [35]. This wide panel of actions contributes, in still unknown proportions, to the observed protection and limitation of functional deficits induced by stroke, and shows that definitive integration of exogenous cells is not a prerequisite for efficient interference with temporally limited events. Presence over a period of days might be sufficient to afford permanent tissue protection and permanent limitation of functional impairments. A precise correlation between each of the BMSC properties and each of the pathological events that

Table 1. Clinical trials on stem cell therapy in stroke**a** Results in the literature

Cell type	Phase	Administration route	Post-AVC delay	Enrollment	Reference	Results
Neural stem cells completed						
LBS-neuron Layton Biosci.	I	IC	0.5–4.5 years	12	[26]	Safety Improvement on some ESS scores
LBS-neuron Layton Biosci.	II randomized	IC	1–6 years	18	[26]	Safety Some improvements
Fetal porcine tissue	I	IC	1.5–10 years	5	[70]	Terminated
Fetal human tissue	Case series	Subarachnoidal	12 months	10	[71]	Side effects
Fetal NSC	Case series	IC/subarachnoidal	4 years	26	[72]	Transient side effects
Nonneural stem cells completed						
MSC autologous	I/II placebo-controlled	Intravenous	5 and 7 weeks	5/25 controls	[25]	Safety Bathel Index and MRS transiently improved
hUCBSC	Case series	Intravenous	23 months	10	[73]	Safety, no effects
MNC	Case series	Intra-arterial	4 days	1	[74]	Safety
Stimulation of endogenous SC						
G-CSF	II	Subcutaneous	48 h	20	NCT00901381 (Belkin)	No effects
NTx TM -265	II randomized placebo-controlled	Subcutaneous/ intravenous	24–48 h	134	NCT00663416 stem cell therapeutics	Terminated Analysis ongoing

b Identifiers and locations

Cell type	Phase	Administration route	Post-AVC delay	Enrollment	Identifier (PI)	Location
BMSC (+ GSCF)	I (O) pediatrics	Intrathecal	No limits	10	NCT01019733 (Mancias-Guerra)	Monterrey, Mexico
MSC autologous	II (O)	Intravenous 2 doses	6 weeks	30	NCT00875654 (Detante)	Grenoble, France
CTX0E03 (hESC-derived line)	I (O)	IC	6–24 months	12	NCT01151124 (ReNeuron, Muir)	Glasgow, UK
MNC autologous	I (R)	Intravenous	<72 hours	10	NCT00859014 (Savitz)	Houston, USA
PBSC autologous	II (R)	IC	6–20 months	30	NCT00950521 (Lin)	University of China, Taiwan
PBSC fMRI study	Observational: remyelination of the corticospinal tract, focal perfusion; cortical activity			30	NCT01249287 No contacts	University of China
Unprecised SC type	+ GCSF (R) observational: changes in cortical excitability		6 months to 5 years	36	NCT01239602 No contacts	University of China
BMSC autologous	I (R)	Intra-MCA/ Intravenous	3–90 days	15	NCT00473057 (André)	Rio de Janeiro, Brasil
BMSC autologous	I-II (R)	Intra-MCA	<7 days	10	NCT00535197 (Chataway)	London, UK
BMSC autologous	I-II (R)	Intra-MCA	5–9 days	20	NCT00761982 (Hernández)	Oviedo, Spain
MSC allogenic	I-II (P) controlled	Intravenous	<10 days	78	NCT01091701 Stempeutics Res (Das)	Kuala Lumpur, Malaysia
MNC/BMSC autologous	I (P) randomized controlled	Intravenous	<72 h	33	NCT00908856 (Cramer)	Irvine, USA

LBS-neurons: NT2D1; immortalized NSC from the NT2 human teratocarcinoma cell line; IC: stereotactic in situ; hUCBSC = human umbilical cord blood SC; MSC = mesenchymal SC; MNC = mononuclear cells; PBSC = peripheral blood hematopoietic stem cells; fMRI = functional MRI; O = ongoing; R = recruiting; P = planned. From <http://www.clinicaltrials.gov>.

Fig. 1. Temporal correlations between the pathophysiological processes that lead to tissue degeneration in stroke, potential therapeutic approaches and 3 types of SC source. Neural SC and nonneural heterologous SC can be prepared and banked for immediate use and might target both the acute and chronic phases of stroke-induced tissue changes. Autologous SC requiring a preparation time that might delay their application are better suited for an application in the neurorestoration phase.

lead to tissue loss after stroke is now needed to fully classify nonneural SC effects.

In contrast to nonneural SC, neural SC are prone to engraft into the host brain. Survival, migration and neuronal differentiation have been demonstrated for all neural SC sources, i.e. fetal brain tissue, neural cell lines, and neural stem cells (NSC) derived from the adult brain, from embryonic stem cells (ESC), or from induced pluripotent stem cells (iPSC) [35–41]. However, most studies have used intracerebral transplantation as a delivery route for NSC, and their ability to engraft into the brain from the blood stream is less documented [42, 43].

Regarding integration, an important variable among SC sources is the neuron-to-glia ratio. Cell lines and neural progenitors derived from ESC and iPSC have a higher ability to form neurons after intracerebral transplantation than adult or fetal neural SC, for reasons likely linked

to their initial multipotency status. Astrocytes play important roles in neuronal physiology and are needed, together with blood vessels, for brain tissues. However, the damaged brain rapidly provides the graft with astrocytes and blood vessels, ensuring blood supply and the construction of a favorable environment for developing cells [40]. The emphasis can be put on generating discrete neuronal populations.

A second aspect of graft integration is the participation in brain rewiring. Here again, available data are sparse, probably due to technical difficulties. Reports show that grafted NSC promote axonal sprouting [44] and that synaptic contacts form between transplanted neurons and tissue targets [36, 45]. Participation in remodeling is also suggested by the formation of long projections by ESC-derived neural progenitors in rodents [46] and monkeys [47]. Host projection afferents display

a topographically correct identification of their relevant target neurons in grafts from hESC-derived NSC [40]. Patch-clamp recording on sections from grafted brains revealed spontaneous excitatory postsynaptic potential and epiplast SC in graft-derived neurons, indicating that they received synapses [36]. These data suggest that grafted cells are included into the global reorganization scheme implemented by the poststroke brain. In a clinical perspective, it must also be considered that human neurons require several months to fully acquire their terminal neurochemical phenotype after transplantation [40]. Recovery linked to fully functional integration should not be expected before.

The integration or nonintegration of SC progenies into the cerebral parenchyma, associated with cell-autonomous or non-cell-autonomous effects, conditions a third parameter for SC therapy application, their timing of administration for optimal benefit to the patient.

The Acute versus the Chronic Phase

Far from adding confusion to the application of SC therapy in stroke, the variety of available cell sources can be an asset to match the variety of clinical situations. Stroke is characterized by an acute phase of tissue destruction followed by a long-lasting phase of reorganization. The specific properties of SC sources might assign them to one or the other phase.

Intervention in the acute phase implies that grafts are available within days after stroke. This criterion selects SC sources amenable to large-scale amplification and banking, leaving aside sources that request several weeks of amplification after the onset of stroke. In addition, considering the frequent comorbidity associated with stroke, it can be foreseen that neurosurgeons will be reluctant to perform intracerebral surgery before patient stabilization and will prefer less invasive administration routes.

As active factories of factors able to interfere with cell death, BMSC are good candidates for application in the acute phase. They can be safely injected intravenously in humans [25] and several trials are in progress with bone-marrow-derived cells (table 1). Autologous BMSC, which avoid immunosuppression and associated side effects, require several weeks for *in vitro* amplification after taking of bone marrow. This delay can diminish their impact on deleterious inflammatory and immune reactions, and might explain the reversal of benefits observed in humans after several months [48]. The use of allogeneic

BMSC can considerably shorten the intervention delay and accurately address the early phases of poststroke brain damage. It is noteworthy that stroke is associated with T lymphopenia and reduction of interferon- γ production that might reduce the need for immunosuppression after allogeneic grafting. Mesenchymal SC (MSC) can also be derived from hESC [49], and be banked and thawed on demand to provide an immediately available transfusion product. Finally, MSC can be derived from hiPSC [50], which would allow grafting of HLA-compatible MSC from haplotyped banks, and definitely eliminate the burden of immunosuppression.

NSC derived from pluripotent cell lines (hESC or hiPSC) fulfill the bankability and amplification requirements for a large-scale use in clinical settings. Protocols that produce large amounts of NSC with great homogeneity have been established [51]. The final differentiation into therapeutic products can be achieved with GMP conditions in specialized centers linked to emergency neurovascular units. However, intravenous or intra-arterial administration would be preferred to take full advantage of NSC properties in the acute phase, and data on their distribution and engraftment properties after intravenous injection should be reinforced, as is the knowledge of their ability to produce factors able to counteract cell death [52–54].

Expectancies from SC therapy in the chronic phase mainly come from an action on brain remodeling, associated with long-term integration of grafted cells into the host brain circuitry. Stroke typically demonstrates the ability of the adult mammalian brain to reorganize its circuitry following an insult [55]. The possibility that grafted cells can take part in remodeling events has been shown mainly for locally implanted neural SC [38, 45–47], although Liu et al. [56] have reported the ability of BMSC injected intravenously to promote axonal sprouting in the host parenchyma. Studies are still expected to fully characterize the long-term benefits of these approaches.

Activation of neurogenesis is the most spectacular self-repair attempt in the poststroke adult brain. Adult neurogenesis is regulated by extracellular signals that include growth factors, neurotransmitters, hormones, extracellular matrix components and cytokines [57, 58]. Stroke typically modifies the expression and release of these regulatory agents [55] and concomitantly increases adult neurogenesis in the rat subventricular area and hippocampus [59, 60], and in stroke patients [61]. A causal relationship between the two events is suggested by the repopulation of hippocampal cell layers by direct infu-

sion of trophic factors after excitotoxic lesion [62]. Interestingly, these are factors used for SC culture. Neural and nonneural stem cells also release trophic factors and induce the expression of these factors in the surrounding parenchyma [63]. Therefore, paracrine effects are likely to account for a great part in the activation of adult neurogenesis observed after poststroke intravenous injection of BMSC [64–66]. Similar implications remain to be demonstrated for NSC. Besides the release of trophic factors, activation of adult neurogenesis by SC might result from the modulation of immune responses [67]. For instance, inflammation is detrimental to neurogenesis in the dentate gyrus [68], and immunodeficiency enhances poststroke cortical neurogenesis [69]. Stem cells with an-

ti-inflammatory properties might be instrumental in increasing the production, possibly survival [69], of newly generated neurons.

Conclusion

Transplantation studies have received a great deal of attention with the development of SC biology. In the case of stroke, grafted cells may address both neuroprotection and regenerative therapy. The constraints related to the kinetics of the degenerative and remodeling events might guide their choice and modalities of application to put all chances of success on the patient side.

References

- 1 Stahnisch FW, Nitsch R: Santiago Ramón y Cajal's concept of neuronal plasticity: the ambiguity lives on. *Trends Neurosci* 2002; 25:589–591.
- 2 Endres M, Engelhardt B, Koistinaho J, Lindvall O, Meairs S, Mohr JP, Planas A, Rothwell N, Schwabinger M, Schwab ME, Vivien D, Wieloch T, Dirnagl U: Improving outcome after stroke: overcoming the translational roadblock. *Cerebrovasc Dis* 2008;25:268–78.
- 3 Murphy TH, Corbett D: Plasticity during stroke recovery: from synapse to behaviour. *Nat Rev Neurosci* 2009;10:861–872.
- 4 Kreisel SH, Hennerici MG, Bazner H: Pathophysiology of stroke rehabilitation: The natural course of clinical recovery, use-dependent plasticity and rehabilitative outcome. *Cerebrovasc Dis* 2007;23:243–255.
- 5 Dancuse N, Barbay S, Frost SB, Plautz EJ, Chen D, Zoubina EV, Stowe AM, Nudo RJ: Extensive cortical rewiring after brain injury. *J Neurosci* 2005;25:10167–10179.
- 6 Winship IR, Murphy TH: In vivo calcium imaging reveals functional rewiring of single somatosensory neurons after stroke. *J Neurosci* 2008;28:6592–6606.
- 7 Xiong Y, Mahmood A, Chopp M: Angiogenesis, neurogenesis and brain recovery of function following injury. *Curr Opin Investig Drugs* 2010;11:298–308.
- 8 Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O: Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med* 2002;8:963–970.
- 9 Lindvall O, Kokaia Z: Recovery and rehabilitation in stroke: stem cells. *Stroke* 2004;35:2691–2694.
- 10 Döbrossy M, Busse M, Piroth T, Rosser A, Dunnett S, Nikkha G: Neurorehabilitation with neural transplantation. *Neurorehabil Neural Repair* 2010;24:692–701.
- 11 Andsberg G, Kokaia Z, Bjorklund A, Lindvall O, Martinez-Serrano A: Amelioration of ischaemia-induced neuronal death in the rat striatum by NGF-secreting neural stem cells. *Eur J Neurosci* 1998;10:2026–2036.
- 12 Daadi MM, Davis AS, Arac A, Li Z, Maag AL, Bhatnagar R, Jiang K, Sun G, Wu JC, Steinberg GK: Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. *Stroke* 2010;41:516–523.
- 13 Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenland K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Roger VL, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J: Heart disease and stroke statistics – 2010 update: a report from the American Heart Association. *Circulation* 2010;121:e46–e215.
- 14 Meairs S, Wahlgren N, Dirnagl U, Lindvall O, Rothwell P, Baron JC, Hossmann K, Engelhardt B, Ferro J, McCulloch J, Kaste M, Endres M, Koistinaho J, Planas A, Vivien D, Dijkhuizen R, Czlonkowska A, Hagen A, Evans A, De Libero G, Nagy Z, Rastenyte D, Reess J, Davalos A, Lenzi GL, Amarenco P, Hennerici M: Stroke research priorities for the next decade – A representative view of the European scientific community. *Cerebrovasc Dis* 2006;22:75–82.
- 15 Kennedy J, Buchan AM: C-EPO: ready for prime-time preconditioning? *Cerebrovasc Dis* 2005;19:272–273.
- 16 Van der Worp HB, Macleod MR, Kollmar R: Therapeutic hypothermia for acute ischemic stroke: ready to start large randomized trials? *J Cereb Blood Flow Metab* 2010;30:1079–1093.
- 17 Benchenane K, Lopez-Atalaya JP, Fernandez-Monreal M, Touzani O, Vivien D: Equivocal roles of tissue-type plasminogen activator in stroke-induced injury. *Trends Neurosci* 2004;27:155–160.
- 18 Aihara N, Mizukawa K, Koide K, Mabe H, Nishino H: Striatal grafts in infarct striatopallidum increase GABA release, reorganize GABA_A receptor and improve water-maze learning in the rat. *Brain Res Bull* 1994;33:483–488.
- 19 Borlongan CV, Koutouzis TK, Jorden JR, Martinez R, Rodriguez AI, Poulos SG, Freeman TB, McKeown P, Cahill DW, Nishino H, Sanberg PR: Neural transplantation as an experimental treatment modality for cerebral ischemia. *Neurosci Biobehav Rev* 1997;21:79–90.
- 20 Nishino H, Koide K, Aihara N, Kumazaki M, Sakurai T, Nagai H: Striatal grafts in the ischemic striatum improve pallidal GABA release and passive avoidance. *Brain Res Bull* 1993;32:517–520.
- 21 Grabowski M, Brundin P, Johansson BB: Functional integration of cortical grafts placed in brain infarcts of rats. *Ann Neurol* 1993;34:362–368.
- 22 Darsalia V, Kallur T, Kokaia Z: Survival, migration and neuronal differentiation of human fetal striatal and cortical neural stem cells grafted in stroke-damaged rat striatum. *Eur J Neurosci* 2007;26:605–614.
- 23 Burns TC, Verfaillie CM, Low WC: Stem cells for ischemic brain injury: a critical review. *J Comp Neurol* 2009;515:125–144.
- 24 Lindvall O, Kokaia Z: Stem cells in human neurodegenerative disorders – time for clinical translation? *J Clin Invest* 2010;120:29–40.
- 25 Zhang ZG, Chopp M: Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. *Lancet Neurol* 2009;8:491–500.

- 26 Bang OY, Lee JS, Lee PH, Lee G: Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol* 2005;57:874–882.
- 27 Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, Decesare S, Jovin T, Zafonte R, Lebowitz J, Flickinger JC, Tong D, Marks MP, Jamieson C, Luu D, Bell-Stephens T, Teraoka J: Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. *J Neurosurg* 2005;103:38–45.
- 28 Liu Z, Li Y, Zhang ZG, Cui X, Cui Y, Lu M, Savant-Bhonsale S, Chopp M: Bone marrow stromal cells enhance inter- and intracortical axonal connections after ischemic stroke in adult rats. *J Cereb Blood Flow Metab* 2010;30:1288–1295.
- 29 Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC: Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp Neurol* 2002;174:11–20.
- 30 Bao X, Wei J, Feng M, Lu S, Li G, Dou W, Ma W, Ma S, An Y, Qin C, Zhao RC, Wang R: Transplantation of human bone marrow-derived mesenchymal stem cells promotes behavioral recovery and endogenous neurogenesis after cerebral ischemia in rats. *Brain Res* 2010;1367:103–113.
- 31 Deng YB, Ye WB, Hu ZZ, Yan Y, Wang Y, Takon BF, Zhou GQ, Zhou YF: Intravenously administered BMSCs reduce neuronal apoptosis and promote neuronal proliferation through the release of VEGF after stroke in rats. *Neurol Res* 2010;32:148–156.
- 32 Borlongan CV, Lind JG, Dillon-Carter O, Yu G, Hadman M, Cheng C, Carroll J, Hess DC: Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats. *Brain Res* 2004;1010:108–116.
- 33 Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, Prockop DJ: Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. *Proc Natl Acad Sci USA* 2008;105:14638–14643.
- 34 Aggarwal S, Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005;105:1815–1822.
- 35 Lee ST, Chu K, Jung KH, Kim SJ, Kim DH, Kang KM, Hong NH, Kim JH, Ban JJ, Park HK, Kim SU, Park CG, Lee SK, Kim M, Roh JK: Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. *Brain* 2008;131:616–629.
- 36 Yagi H, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, Kobayashi N, Yarmush ML: Mesenchymal stem cells: mechanisms of immunomodulation and homing. *Cell Transplant* 2010;19:667–679.
- 37 Bühnemann C, Scholz A, Bernreuther C, Malik CY, Braun H, Schachner M, Reymann KG, Dihne M: Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain* 2006;129:3238–3248.
- 38 Ishibashi S, Sakaguchi M, Kuroiwa T, Yamasaki M, Kanemura Y, Shizuko I, Shimazaki T, Onodera M, Okano H, Mizusawa H: Human neural stem/progenitor cells, expanded in long-term neurosphere culture, promote functional recovery after focal ischemia in Mongolian gerbils. *J Neurosci Res* 2004;78:215–223.
- 39 Jin K, Mao X, Xie L, Galvan V, Lai B, Wang Y, Gorostiza O, Wang X, Greenberg DA: Transplantation of human neural precursor cells in Matrigel scaffolding improves outcome from focal cerebral ischemia after delayed postischemic treatment in rats. *J Cereb Blood Flow Metab* 2010;30:534–544.
- 40 Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, Masel J, Yenari MA, Weissman IL, Uchida N, Palmer T, Steinberg GK: Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *Proc Natl Acad Sci USA* 2004;101:11839–11844.
- 41 Seminatore C, Polentes J, Ellman D, Kozubenko N, Itier V, Tine S, Tritschler L, Brenot M, Guidou E, Blondeau J, Lhuillier M, Bugi A, Aubry L, Jendelova P, Sykova E, Perrier AL, Finsen B, Onteniente B: The post-ischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. *Stroke* 2010;41:153–159.
- 42 Polentes J, Jendelova P, Cailleret M, Braun H, Tropel P, Seminatore C, Kozubenko N, Brenot M, Baldauf K, Turnovcova K, Côme J, Tournais T, Reymann K, Sykova E, Viville S, Onteniente B: Human-induced pluripotent stem cell therapy improves stroke outcome. In preparation.
- 43 Pendharkar AV, Chua JY, Andres RH, Wang N, Gaeta X, Wang H, De A, Choi R, Chen S, Rutt BK, Gambhir SS, Guzman R: Biodistribution of neural stem cells after intravascular therapy for hypoxic-ischemia. *Stroke* 2010;41:2064–2070.
- 44 Bacigaluppi M, Pluchino S, Peruzzotti Jammitti L, Kilic E, Kilic U, Salani G, Brambilla E, West MJ, Comi G, Martino G, Hermann DM: Delayed post-ischaemic neuroprotection following systemic neural stem cell transplantation involves multiple mechanisms. *Brain* 2009;132:2239–2251.
- 45 Daadi MM, Davis AS, Arac A, Li Z, Maag AL, Bhatnagar R, Jiang K, Sun G, Wu JC, Steinberg GK: Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. *Stroke* 2010;41:516–523.
- 46 Gaillard A, Prestoz L, Dumartin B, Cantereau A, Morel F, Roger M, Jaber M: Reestablishment of damaged adult motor pathways by grafted embryonic cortical neurons. *Nat Neurosci* 2007;10:1294–1299.
- 47 Aubry L, Bugi A, Lefort N, Rousseau F, Pechanski M, Perrier AL: Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. *Proc Natl Acad Sci USA* 2008;105:16707–16712.
- 48 Hayashi J, Takagi Y, Fukuda H, Imazato T, Nishimura M, Fujimoto M, Takahashi J, Hashimoto N, Nozaki K: Primate embryonic stem cell-derived neuronal progenitors transplanted into ischemic brain. *J Cereb Blood Flow Metab* 2006;26:906–914.
- 49 Lee JS, Hong JM, Shin KW, Bang OY; START-ING collaborators: A long-term follow-up study of autologous mesenchymal stem cell transplantation in patients with acute ischemic stroke. *Stem Cells* 2010;28:1099–1106.
- 50 Liu YP, Seckin H, Izci Y, Du ZW, Yan YP, Baskaya MK: Neuroprotective effects of mesenchymal stem cells derived from human embryonic stem cells in transient focal cerebral ischemia in rats. *J Cereb Blood Flow Metab* 2009;29:780–791.
- 51 Teramura T, Onodera Y, Mihara T, Hosoi Y, Hamanishi C, Fukuda K: Induction of mesenchymal progenitor cells with chondrogenic property from mouse-induced pluripotent stem cells. *Cell Reprogram* 2010;12:249–261.
- 52 Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L: Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* 2009;27:275–280.
- 53 Bacigaluppi M, Pluchino S, Peruzzotti Jammitti L, Kilic E, Kilic U, Salani G, Brambilla E, West MJ, Comi G, Martino G, Hermann DM: Delayed post-ischaemic neuroprotection following systemic neural stem cell transplantation involves multiple mechanisms. *Brain* 2009;132:2239–2251.
- 54 Lee JP, Jeyakumar M, Gonzalez R, Takahashi H, Lee PJ, Baek RC, Clark D, Rose H, Fu G, Clarke J, McKercher S, Meerloo J, Muller FJ, Park KI, Butters TD, Dwek RA, Schwartz P, Tong G, Wenger D, Lipton SA, Seyfried TN, Platt FM, Snyder EY: Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. *Nat Med* 2007;13:439–447.
- 55 Ourednik J, Ourednik V, Lynch WP, Schachner M, Snyder EY: Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. *Nat Biotechnol* 2002;20:1103–1110.
- 56 Rodríguez-González R, Hurtado O, Sobrino T, Castillo J: Neuroplasticity and cellular therapy in cerebral infarction. *Cerebrovasc Dis* 2007;24(suppl 1):167–180.

- 57 Liu Z, Li Y, Zhang ZG, Cui X, Cui Y, Lu M, Savant-Bhonsale S, Chopp M: Bone marrow stromal cells enhance inter- and intracortical axonal connections after ischemic stroke in adult rats. *J Cereb Blood Flow Metab* 2010; 30:1288–1295.
- 58 Moyse E, Segura S, Liard O, Mahaut S, Mechawar N: Microenvironmental determinants of adult neural stem cell proliferation and lineage commitment in the healthy and injured central nervous system. *Curr Stem Cell Res Ther* 2008;3:163–184.
- 59 Nicoleau C, Benzakour O, Agasse F, Thiriet N, Petit J, Prestoz L, Roger M, Jaber M, Coronas V: Endogenous hepatocyte growth factor is a niche signal for subventricular zone neural stem cell amplification and self-renewal. *Stem Cells* 2009;27:408–419.
- 60 Wiltout C, Lang B, Yan Y, Dempsey RJ, Vemuganti R: Repairing brain after stroke: a review on post-ischemic neurogenesis. *Neurochem Int* 2007;50:1028–1041.
- 61 Kokaia Z, Lindvall O: Neurogenesis after ischaemic brain insults. *Curr Opin Neurobiol* 2003;13:127–132.
- 62 Macas J, Nern C, Plate KH, Momma S: Increased generation of neuronal progenitors after ischemic injury on the aged adult human forebrain. *J Neurosci* 2006;26:13114–13119.
- 63 Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, Tamura A, Kirino T, Nakafuku M: Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* 2002;110:429–441.
- 64 Bliss TM, Andres R, Steinberg GK: Optimizing the success of cell transplantation therapy for stroke. *Neurobiol Dis* 2010;37:275–283.
- 65 Nakano-Doi A, Nakagomi T, Fujikawa M, Nakagomi N, Kubo S, Lu S, Yoshikawa H, Soma T, Taguchi A, Matsuyama T: Bone marrow mononuclear cells promote proliferation of endogenous neural stem cells through vascular niches after cerebral infarction. *Stem Cells* 2010;28:1292–1302.
- 66 Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T: Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest* 2004;114:330–338.
- 67 Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M: Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. *J Neurosci Res* 2003;73:778–786.
- 68 Mathieu P, Battista D, Depino A, Roca V, Graciarena M, Pitossi F: The more you have, the less you get: the functional role of inflammation on neuronal differentiation of endogenous and transplanted neural stem cells in the adult brain. *J Neurochem* 2010; 112:1368–1385.
- 69 Ekdahl CT, Kokaia Z, Lindvall O: Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience* 2009;158: 1021–1029.
- 70 Saino O, Taguchi A, Nakagomi T, Nakano-Doi A, Kashiwamura S, Doe N, Nakagomi N, Soma T, Yoshikawa H, Stern DM, Okamura H, Matsuyama T: Immunodeficiency reduces neural stem/progenitor cell apoptosis and enhances neurogenesis in the cerebral cortex after stroke. *J Neurosci Res* 2010;88:2385–2397.
- 71 Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR: Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. *Cerebrovasc Dis* 2005;20:101–107.
- 72 Rabinovich SS, Seledtsov VI, Banul NV, Poveschenko OV, Senyukov VV, Astrakov SV, Seledtsova GV, Samarin DM, Poveschenko OV: Cell therapy of brain stroke. *Bull Exp Biol Med* 2005;39:126–128.
- 73 Yang QC, Zhang XD, Liang CC, Du Y, Li HW: Functional evaluation of stroke patients 6 months after intrathecal injection of neural stem cells. *Chinese J Clin Rehab* 2005; 9:208–210.
- 74 Man Y, Li J, Yang B, Ma J: Vein transplantation using human umbilical cord blood stem cells in the treatment of stroke sequela. *Neural Regener Res* 2006;1:618–621.