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CELL-BASED THERAPIES FOR STROKE

Intravascular Stem Cell Transplantation for Stroke

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Abstract Stroke is the third leading cause of death and the leading cause of adult disability in North America. Emphasis has been placed on developing treatments that reduce the devastating long-term impacts of this disease, and preclinical research on stem cell therapy has demonstrated promising results. However, questions about the optimal cell delivery method and timing of cell transplantation are not fully answered. Recent findings suggest that intravascular stem cell delivery is a safe and efficacious alternative to stereotactic cell injections. It also offers advantages should repeat treatments prove beneficial. Recent reports further suggest that intra-arterial injection results in a wider distribution of cells throughout the stroked hemisphere with a significantly greater cell engraftment compared to intravenous injection. In this review, we describe the benefits and potential risks associated with intravascular stem cell delivery and compare intra-arterial to intravenous cell transplantation methods. We discuss the importance of cell biodistribution and timing of transplantation in driving cell survival. We examine current proposed mechanisms involved in cell migration and functional recovery and discuss future directions for intravascular stem cell therapy research.

Keywords Stroke · Stem cells · Neural stem cells · Intravascular · Intra-arterial · Cell therapy

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Introduction

Stroke is the third leading cause of mortality and the leading cause of disability in North America. The significant cost to the health care system will continue to increase as the population ages and the prevalence of risk factors grow (obesity, hypertension) [1]. Despite great efforts aimed at developing treatments for stroke, only one intervention has been approved for clinical use [2]. While intravenous tissue plasminogen activator (tPA) may provide some benefits for stroke, only about 2% of ischemic stroke patients are eligible to receive tPA given the limited time window available for effective thrombolysis [3]. Interventions focused on improving recovery in the post-acute period are needed, and stem cell therapies are a promising solution.

Cell-based therapies have shown a considerable ability to improve functional outcome when administered after experimental stroke [4, 5]. Many types of stem cells, including mesenchymal stem cells (MSCs)/bone marrow stromal cells (BMSCs), umbilical cord blood cells (UCBCs), embryonic stem cells (ESCs), fetal neural stem cells (FNSCs), and neural progenitor cells (NPCs) have been tested in animal models of stroke [6–9]. Although several clinical trials have indicated that stem cell treatment is safe and well tolerated by stroke patients [10–13], the ideal method of cell delivery remains an important unanswered question for future cell transplantation paradigms. While stereotactic transplantations seem to yield the best cell survival [14], intravascular injections are less invasive and result in a wider distribution of cells into the injured territory [6, 14–16]. Currently, several intravascular cell transplantation approaches are being investigated including intravenous, intra-arterial, and intracardiac injections. Although studies have suggested that the release of

diffusible trophic factors by cells transplanted intravenously provide sufficient benefit to the host [17, 18], intra-arterial transplantation results in over 100 times greater physical engraftment of cells into the brain [12], which potentially allows more targeted release of trophic factors directly into the site of injury.

While many important questions remain about stem cell therapy for stroke, we review the current preclinical evidence supporting intravascular stem cell transplantation as a potentially effective treatment for stroke. We focus on intra-arterial and intravenous cell transplantation approaches and consider how delivery method affects the biodistribution of transplanted cells. Mechanisms guiding stem cell migration and distribution in the brain are discussed and related to how cell engraftment characteristics impact the ideal timing of treatment. Benefits and potential risks associated with intravascular therapy are also addressed, followed by a description of mechanisms potentially contributing to treatment efficacy. Finally, we consider important future directions for stroke research on intravascular stem cell therapy.

Intravascular Cell Transplantation

Two main strategies currently under investigation for stem cell delivery are direct stereotactic transplantation into the brain and extracranial intravascular transplantation. Stereotactic transplantation places the cells either directly within the brain parenchyma or the ventricular system, often in close proximity to or within the site of injury. Vascular transplantation can be achieved through either intravenous or intra-arterial injection. In addition to the less invasive nature of intravascular injection, intra-arterial and intravenous transplants result in diffuse distribution of cells throughout the ischemic hemisphere [14, 15]. While stereotactic transplantation results in local migration in the rodent brain [19, 20], migration in the human brain appears to be quite limited [21]. Despite lower engraftment rates seen with the intravenous approach, functional recovery has been frequently reported. This indicates that benefits can occur with few or no cells migrating to the site of injury and that recovery is possibly driven by the release of soluble factors [18]. In contrast, the intra-arterial approach has been shown to result in higher engraftment of cells throughout the injured hemisphere compared to intravenous administration [15, 16]. The potential advantage of intra-arterial transplantation over intravenous or stereotactic transplantation is greater interaction in a wider area between transplanted and endogenous cells.

Few studies have directly compared alternative stem cell transplantation techniques in stroke (Table 1). Jin et al. [14] evaluated migration and engraftment of NPCs injected 24 h after middle cerebral artery occlusion (MCAO) and found that ischemia greatly increased migration of transplanted cells into and within the brain for multiple methods of cell delivery. They also found that intracerebral transplantation resulted in the largest number of cells at the lesion site, followed by intraventricular and intravenous delivery. Li et al. [15] used SPIO-labeled NPCs and magnetic resonance imaging (MRI) to compare intra-arterial, intravenous, and intracisternal cell delivery 24 h after MCAO. Repeated imaging of SPIO-labeled cells demonstrated greater infiltration and distribution after intra-arterial delivery than with the other methods. Recently, we used bioluminescence imaging (BLI) and histology to quantitatively determine the distribution of mouse NPCs transplanted intra-arterially or intravenously 24 h after hypoxia–ischemia [16]. Intra-arterial injection resulted in both superior initial engraftment and survival of NPCs in the ischemic brain (Fig. 1). We found that 69% of the BLI signal was detected in the brain early after intra-arterial injection, whereas 94% of the BLI signal was detected in the lungs immediately after intravenous injection. At 1 week, there was a 32% signal loss in the intra-arterial group versus a 91% signal loss in the intravenous group. These studies demonstrate that intra-arterially delivered stem cells enter the brain and migrate to sites of injury much more efficiently than cells injected intravenously.

Mechanisms of Cell Engraftment

Although cells transplanted intravascularly clearly localize to the ischemic hemisphere, the mechanisms involved in cell migration from the vasculature into the brain are not fully understood. Many endogenous chemokines and adhesion molecules upregulated early after stroke are highly expressed in the penumbral region [22–27] and could potentially contribute to migration of transplanted cells. Evidence supports the idea that intravascularly delivered stem cells are initially recruited by chemotactic signals and then undergo transendothelial movement and intraparenchymal migration to reach the ischemic lesion [6, 9, 28]. In order to exit the vasculature, transplanted cells likely utilize a process similar to that used by inflammatory cells involving rolling, tight binding, and diapedesis.

Initial chemoattraction of stem cells to an area of injury in the central nervous system (CNS) may involve stromal derived factor (SDF-1) [24, 29], monocyte chemotactic protein (MCP-1) [23, 30, 31], and other chemokines (Fig. 2). We have recently investigated the role of the MCP-1/CCR2 interaction in transendothelial migration of neural stem cells to areas of stroke. We demonstrated that neural stem cells lacking the CCR2 receptor (stem cells harvested from $CCR2^{-/-}$ knockout animals) had a 50% reduction in migration to the ischemic brain compared to

Table 1 Summary of studies comparing different stem cell administration methods

Study	Model	Cell type	Cell delivery	Treatment delay	Outcome measures	Final time point	Cell distribution	Additional findings
Chua et al. 2010	tMCAO (2 h) and SHAM rat	NPCs (immortalized mouse Cl7.2)	IA, 1×10^6; catheter or microneedle	24 h	BLI, LDF	24 h	Similar with the two methods; BLI signal in brain, no signal from peripheral organs	Microneedle method did not alter blood flow; catheter method significantly and persistently lowered blood flow; catheter method had indications of microstrokes
Jin et al. 2005	tMCAO (1 h) and SHAM rat	NPCs (embryonic mouse)	LV, 6.0×10^5; IP, 3.6×10^6; IV, 3.0×10^6	24 h	IHC	6 days	Cells migrated into the ischemic striatum and cerebral cortex with all methods, cell #. : IP> LV>IV	Most cells expressed GFAP or DCX; cells did not migrate in the SHAM animals
Lappalainen et al. 2008	tMCAO (2 h) and SHAM rat	ESCs (human HS181 or rat hippocampal)	IV, 1.0×10^6; IA, 1.0×10^6	30 mm	SPEC/CT, ^{111}In-oxide	24 h	IV: liver>spleen>kidneys, no signal in brain; IA: majority of signal in peripheral organs weak signal in brain; no difference between cell types	
Li et al. 2010	tMCAO (2 h) rat	NPCs (adult rat)	IC, 1.0×10^5; IV, 1.0×10^5; IA, 1.0×10^6	24 h	MRI	4 days	IA: significantly more cells and migration, and a more diffuse distribution	Mortality: IA, 41%; IC, 17%; IV, 8%; smaller lesions at the time of infusion had fewer cells in the parenchyma
Pendharkar et al. 2010	Hypoxia/ischemia (30 min) mouse	NPCs (immortalized mouse C17.2)	IP, 5.0×10^5; IV, 5.0×10^5; IA, 5.0×10	24 h	BLI	2 weeks	IV: lungs 94% of BLI signal at 1 day, IA: brain 69% of BLI signal at 1 day	IA: Persistently higher levels of cells in the ischemic brain
Walczak et al. 2008	tMCAO (2 h) rat	MSCs (adult rat)	IV, 1.0×10^6; IA, 1.0×10^6	30 min	MRI, LDF	10 days	Variable number of cells in brain after IA; few cells in brain after IV	IA had a risk of microvascular occlusion and an increased risk of mortality

tMCAO temporary middle cerebral artery occlusion, *NPCs* neuronal progenitor cells, *ESCs* embryonic stem cells, *LV* lateral ventricle, *IA* intra-arterial, *IP* intraparenchymal, *IV* intravenous, *IC* intracistern, *BLI* bioluminescence imaging, *LDF* laser Doppler flow, *IHC* immunohistochemistry, *MRI* magnetic resonance imaging, *GFAP* astrocytes, *DCX* immature neuronal marker

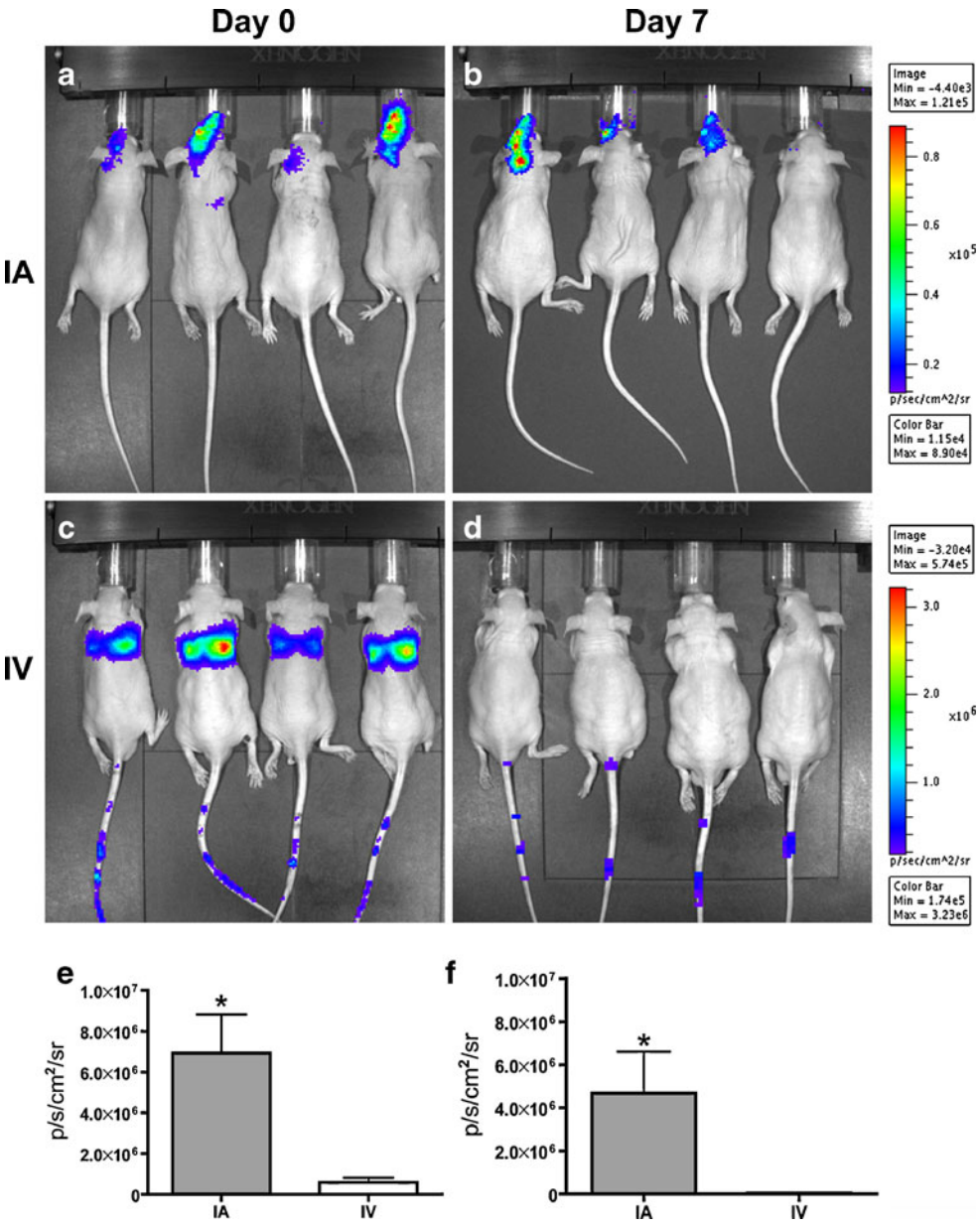

Fig. 1 Luciferase activity in transplanted NPCs detected using BLI. Photon flux indicated that, immediately after transplant, a high concentration of cells localized to the head in the intra-arterial group (**a**), while most cells localized to the torso in the intravenous group (**c**). Signal decreased in both groups 1 week after transplant; however, significant signal was still seen in the head of the intra-arterial group (**b**). Almost no signal was detected in the intravenous group 1 week after transplant (**d**). Quantification of photon flux found a significant increase in BLI signal from the head in the intra-arterial group compared to the intravenous group at both day 0 (**e**) and day 7 (**f**). Adapted from [16] with permission from *Stroke* (2010)

$CCR2^{+/+}$ cells [30]. In addition, lacking the CCR2 receptor significantly decreased the targeted intraparenchymal migration following transendothelial migration [30]. Therefore, it appears that chemoattraction plays an essential role for the targeted homing of stem cells to the injured brain and that this process is an active mechanism rather than solely a breakdown of the blood–brain barrier. Adhesion molecules including cell surface integrin CD49d (VLA-4), vascular cellular adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) facilitate migration of many cell types. Upregulation of VCAM-1 seen in patients [22, 32] and animal models [22] after stroke promotes binding and adhesion of cells expressing the VCAM-1 ligand CD49d. Pluchino et al. [33] demonstrated that blocking CD49d reduced the number of NPCs homing to the brain by 60% in a model of neuroinflammation. Our group has demonstrated that intra-arterial injection of 3×10^6 FACS-sorted $CD49d^+$ NPCs 48 h after hypoxia–ischemia produces significantly greater cell engraftment than similar injection of $CD49d^-$ cells [9]. Total cell engraftment was 46% higher in the cortex, 52% higher in the hippocampus, and 68% higher in the subventricular zone when $CD49^+$ cells were injected. Distribution of the two different phenotypes in the brain was similar and corresponded to stroked areas with elevated VCAM-1. Several other studies have demonstrated that NPCs express surface integrins and that blocking these adhesion molecules reduces stem cell migration into the brain [9, 20, 29, 33, 34].

All of these studies support the idea that stem cells respond to endogenous chemoattractant signals and utilize

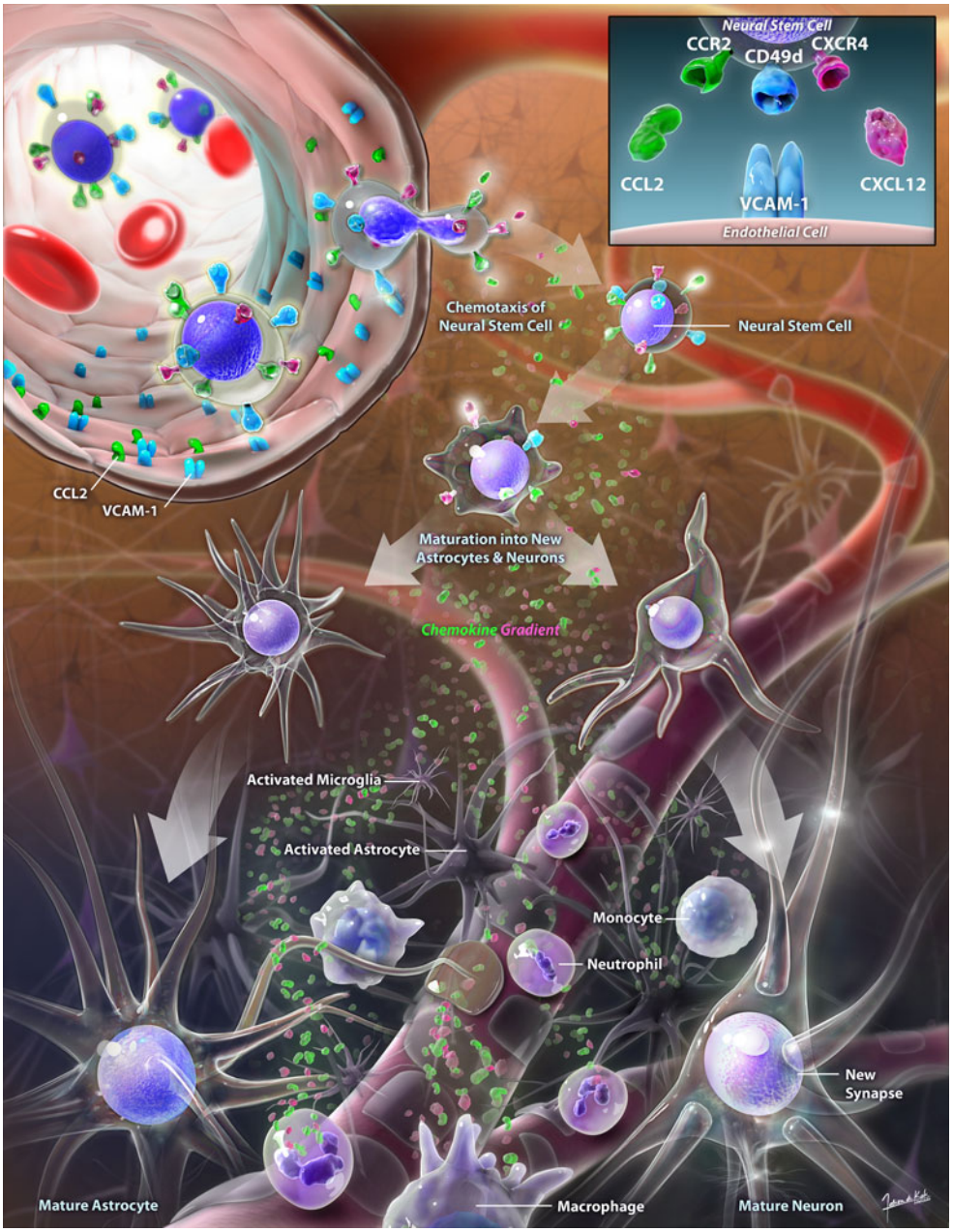

Fig. 2 Neural stem cell (*NSC*) migration into the brain is facilitated by adhesion molecules and chemokine gradients. Expression of chemokine receptors CCR2 and CXCR4 allow NSCs to respond to MCP-1 (*CCL2*) and SDF-1 (*CXCL12*) signals from the ischemic brain. Upregulation of VCAM-1 on endothelial cells after stroke promotes binding and transendothelial migration of $CD49d^{+}$ NSCs. Continued migration within the brain parenchyma and NSC maturation occurs in response to endogenous signaling

cell adhesion mechanisms to migrate into the injured brain. As other chemokines and adhesion molecules important for cell migration are identified, understanding the spatial and temporal changes in the expression of these molecules after stroke will become an important step in maximizing cell engraftment.

Timing of Cell Transplantation

Cell transplantation should be timed to take advantage of the rapid expression of adhesion molecules and chemoattractants that occurs after the onset of a stroke. In addition to maximizing cell migration and engraftment, timing of cell delivery should be coordinated with the development of pathological changes in the brain.

Given the rapid progression of CNS injury following stroke, treatments designed to be neuroprotective are most effective when administered soon after onset of injury. Neuroprotection occurs when stem cell treatment is administered 3 days after injury in experimental stroke models [35]; however, reductions in atrophy are seen even when stem cell treatments are delayed until 3 weeks after stroke [36]. Most transplantation studies demonstrating neuroprotection or reduced lesion volume deliver stem cells within the first 48 h of injury [7, 9, 37, 38]. Treatments designed to enhance the ischemic brain's endogenous repair

mechanisms remain effective even when delivered at later times [39].

Since cell adhesion and chemoattraction appears to be relevant for transendothelial migration and subsequent engraftment, timing of cell injection becomes a crucial variable. Acute upregulation of cytokines, chemokines, and adhesion molecules within the penumbra early after stroke creates a rich environment poised to direct transplanted cells to the region of injury. Elevated VCAM-1 levels have been detected in acute stroke patients [22, 32], and VCAM-1 has been shown to peak around 24 h after experimental stroke [22]. ICAM-1 is elevated as early as 4 h and is sustained at high levels for up to 1 week after stroke in animal models [40]. SDF-1 is upregulated soon after CNS injury [24, 38, 41], while MCP-1 expression increases 3 days after stroke and returns to baseline after 1 week [40]. With strong chemotactic signaling starting a few days after stroke and increased adhesion molecule expression during the first post-stroke week, transplanting stem cells within the first 7 days following stroke may yield the best cell chemoattraction and engraftment. The majority of intravascular studies in animal models delivered stem cells during this critical period, most completing the transplant within the first 3 days after stroke [7, 9, 14, 15, 28, 38, 42–45].

However, maximizing cell migration and engraftment may not be necessary to achieve functional improvement. Intravenous studies have found functional and histological improvements with minimal migration of cells into the brain, indicating that peripheral mechanisms may have an important role in stem cell-mediated recovery [18]. If cell engraftment in the brain is not required for treatment efficacy, intravascular stem cell delivery could improve clinical outcomes even when treatment is delayed until well after the acute chemotactic signals have subsided. Combining early and late interventions could facilitate acute neuroprotective processes as well as enhance the delayed plasticity changes. Both methods of intravascular delivery are particularly attractive for this multiple treatment approach as they can be performed repeatedly over time without the need for extensive surgery.

Benefits and Risks of Intra-Arterial Delivery

Transplanted cells can home to sites of CNS injury after stereotactic intraparenchymal, intracerebroventricular, intravenous, and intra-arterial transplantation [14, 15, 46]. Among these treatment methods, intra-arterial delivery is particularly promising as it eliminates the risks of intracranial surgery associated with stereotactic transplantation, leads to a more widespread cell distribution, and results in more efficient cell engraftment than intravenous transplantation [16]. In addition to the risks inherent to stereotactic transplantation [10, 11], the ideal injection location is not known and the possibility of repeated treatments is limited. Both clinical and experimental stroke studies avoid injecting cells directly into the core of the infarct because this area can be particularly hostile to cells [20]. Most cells are transplanted around the core of the infarct; however, bolus injection into this region can disrupt the extensive natural compensatory changes known to occur after stroke [47]. In addition to being less invasive, intravascular delivery allows cells to engraft according to endogenous signals thereby avoiding these issues.

Cell Engraftment and Distribution

Comparison of intraparenchymal, intraventricular, and intravenous transplantation methods indicates that more cells are found in the brain following stereotactic intraparenchymal cell delivery than after intraventricular and intravenous transplantations [14]. When comparing intravascular methods alone (Table 2), a number of studies have found that intra-arterial injection yields a higher total engraftment than intravenous injection [6, 15, 16]. However, a full appreciation of the benefits of each treatment cannot be gained from total cell engraftment numbers alone as distribution of cells within the tissue is also important. Intravascular delivery allows cells to access the brain from the extensive intracranial vasculature, facilitating a more advantageous distribution of cells than intraparenchymal or intraventricular injections [16, 19].

We recently demonstrated that intra-arterial delivery results in higher initial engraftment and sustained presence of NPCs in the ischemic brain compared to intravenous injection [16]. Walczak and colleagues also reported that intra-arterial, but not intravenous, treatment resulted in successful cerebral engraftment of SPIO-labeled MSCs [6]. Recently, Li and colleagues compared delivering NPCs intra-arterially, intracisternally, and intravenously and reported that intra-arterial delivery resulted in the highest brain engraftment [15]. They also noted that intracisternal injection produced greater engraftment than intravenous injection and that cells reached the brain at different times depending on the method of transplant. Cells were detected in the brain 4 h after intra-arterial delivery, but were not detected in the brain until 1–2 days after intracisternal injection and 2–3 days after intravenous injection. Although the cells preferentially migrated throughout the ischemic hemisphere for all administration routes, intra-arterially delivered cells spread to more of the injured hemisphere than cells transplanted into the venous system or cisterns.

Table 2 Summary of studies intravascular administration of stem cells for the experimental treatment of stroke

Author	Model	Cell type	Cell delivery	Treatment delay	Final time point	Functional outcome	Cell survival and migration	Effect on lesion size	Differentiation	Other
Brenneman et al. 2010	tMCAO (180 min) rat, 3 or 12 months old	Autologous bone marrow mononuclear cells	IA (carotid), 1.0×10^7	24 h	7 days	Cylinder and corner turn improved at 7 days in both young and old group	Peri-infarct area at 1 h and exponentially decreased over the week after injection; no cells in contralateral hemisphere or cerebellum; no differences between ages	Reduced	Not assessed	Decreased negative cytokines (IL-1α, IL-1β, IL-2, TNF-α, and IL-6); increased IL-10
Chung et al. 2009	Thromboembolic (permanent) canine	Human umbilical cord blood-derived MSCs	IA (basilar), 1.0×10^6	24 h	4 weeks	Rating scale (motor function, head turning, circling, hemianopsia) faster recovery 7–10 days	At 4 weeks, cells were in penumbra area	Reduced at 1 week (MRI) no difference at latter times	NeuN and GFAP	Cells released BDNF and VEGF at 4 weeks
Guzman et al. 2008	Hypoxia/ischemia (20 min) mouse	NPCs (immortalized mouse C17.2; $CD49d^+$ vs CD49d−)	IA (carotid), 3.0×10^5	48 h	2 weeks	Rotarod improved in $CD49^+$ group at day 10 vs $CD49^-$ and control	Cells found in cortex and striatum adjacent to the Stroke in SVZ and hippocampus of ipsilateral hemisphere; More $CD49^+$ cells in cortex, hippocampus and SVZ of ischemic hemisphere	No difference	Iba-1; nestin; GFAP; DCX; β-tubulin III	More lectin positive cells and Ibal activation in $CD49^+$ group compared to $CD49^-$ and control
Keimpema et. al. 2009	tMCAO (1 h) rat	BMSCs (adult rat)	IA (carotid), 1.0×10^6	48 h	2 weeks	None	Most cells (95%) in spleen shortly after injection, at 6 h some cells in lesioned hemisphere; number increases during first 12 h but starts decreasing at 24 h; at 2 weeks, only a few cells in and around lesion	Reduced	No neurons	Cells were surrounded by activated and phagocytotic microglia
Li et al. 2001	tMCAo (2 h) rat	BMSCs (adult rat)	IA (carotid), 2.0×10^6	24 h	2 weeks	mNSS and sticky tape improved at 14 days	21% of injected cells were found in multiple areas of ischemic hemisphere: cortex, rostral–caudal axis of the striatum, most (90%) were in ischemic core and boundary zone	No difference	GFAP (10%); MAP-2 (1%)	
Shen et al. 2006	tMCAO (2 h) rat	BMSCs (adult rat)	IA (carotid), 2.0×10^6	24 h	28 days	mNSS, sticky tape and corner turn persistently improved starting at 14 days	Not assessed	No difference, but corpus callosum area in both hemispheres was increased	Not assessed	Increased vessel sprouting, synaptophysin expression and NG2 positive cell numbers and density in cortical peri-infarct area; Ki-67 and oligo precursors in corpus callosum increased
Shen et al. 2007	tMCAO (2 h) rat	BMSCs (adult rat)	IA (carotid), 2.0×10^6	24 h	Up to 1 year	mNSS and sticky tape persistently improved starting at 14 days	Cells distributed throughout ipsilateral hemisphere, majority close to injured tissue; few cells in heart, lung, liver, spleen, or kidney	No difference, but reduced axonal loss and thickness of lesion scar wall	GFAP (22%), MAP2 (17%), 1B4 (5%), and VWF (<1%)	Increased synaptophysin and reduced number of Nogo-A positive cells

BMSCs bone marrow stromal cells, *MSCs* mesenchymal stem cells, *NPCs* neural progenitor cells, *IA* intra-arterial, *mNSS* modified neurologic severity score, *SVZ* subventricular zone, *MAP2* neuronal marker, *GFAP* astrocytes, *vWF* endothelial cell marker, *IB4* microglia, *DCX* immature neuronal marker, *NeuN* neuronal specific nuclear protein, *tMCAO* transient middle cerebral artery occlusion, *Iba-1* microglia, *Ki-67* marker of proliferation

Peripheral Organ Cell Entrapment

Although both intravascular transplantation methods result in a broad distribution of cells throughout the brain, peripheral organ cell entrapment and cell redistribution remain an issue for intravenous delivery techniques. The lower brain engraftment rates seen following intravenous injection corresponds with a high peripheral distribution of cells. Studies using intravenous delivery techniques consistently report high levels of cell entrapment in peripheral organs with only a very small portion of cells entering the brain [16, 48].

Borlongan and colleagues demonstrated that injection of 2×10^5 human UCBCs during MCAO results in significant cell localization to the kidneys, lungs, and spleen, while no cells were found in the brain [18]. Given that cells are thought to utilize chemokines and adhesion molecules to migrate into the brain, infusion during or too soon after ischemia may contribute to the small number of cells homing to the brain. Even with a 24-h delay, few cells migrated into the brain after intravenous transplantation and most ended up in peripheral organs [15, 16].

Immediately after intravenous injection, cells are concentrated primarily in the lungs; however, later, they begin diffusing to other peripheral organs including the brain [35, 48, 49]. Clinical evidence is in agreement with this change in distribution, and patients show a similar high uptake in the lungs 30 min after intravenous infusion of peripheral blood stem cells. However, cells localized to the spleen (42.12%), liver (21.3%), and lungs (5.8%) of these patients 4 h later [50]. These findings suggest that the distribution of stem cells following intravenous injection is dynamic, with redistribution commonly occurring after the initial infusion. Despite this ability to redistribute, intravenously delivered stem cells still fail to consistently or substantially enter the ischemic brain [15, 16].

Alterations in Blood Flow and Microemboli

Most concerns about the safety of intra-arterial delivery centers around potential changes in cerebral blood flow and the development of microstrokes after the injection. Preliminary studies in animal models indicate that the impact on cerebral circulation and development of microemboli depend on the specific injection technique [46]. Two previous studies using intra-arterial stem cell injection reported higher mortality rates during and immediately after stem cell transplantation [6, 15]. It was suggested that the injected stem cells lead to microembolic occlusion of capillaries contributing to worsening stroke and mortality [6]; however, complete occlusion of the internal carotid artery during injection and downstream disruption of laminar blood flow could also cause deleterious effects. We conducted a study to compare intra-arterial injections using two different injection techniques for intra-arterial stem cell delivery: a microneedle- and a catheter-based approach (Fig. 3) [46]. Both methods resulted in NPC brain engraftment; however, only the catheter method produced a persistent decrease in cerebral blood flow (Fig. 4). This change in blood flow ultimately resulted in microstrokes and increased mortality. In contrast, the microneedle method did not alter blood flow (Fig. 4), produced no microembolic strokes, and was not associated with premature mortality [46]. We hypothesized that the key point for intra-arterial injection included meticulous single cell dissociation protocols, appropriate cell dilution and speed of injection, and preserved flow in the parent artery during cell administration. Although these findings indicate that stem cells can be delivered intra-arterially with careful attention to injection technique, further studies will be warranted to prove its safety.

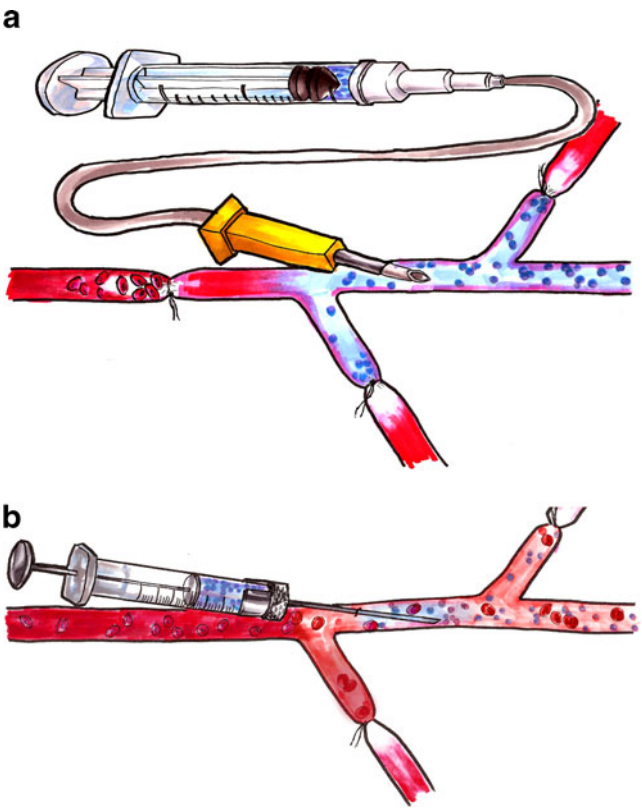

Fig. 3 Schematic of catheter cell injection method (**a**). After preparation of in the injection site by occlusion of the common carotid artery (*CCA*) and ligation of the pterygopalatine artery (*PPA*) and external carotid artery (*ECA*), the catheter is inserted into the CCA. A low concentration of cells is infused slowly. Schematic of microneedle cell injection method (**b**). The CCA remains patent while the PPA and ECA are ligated. A high concentration of cells can be infused due to the substantial blood flow in the CCA. Adapted from [46] with permission from *JCBFM* (2011)

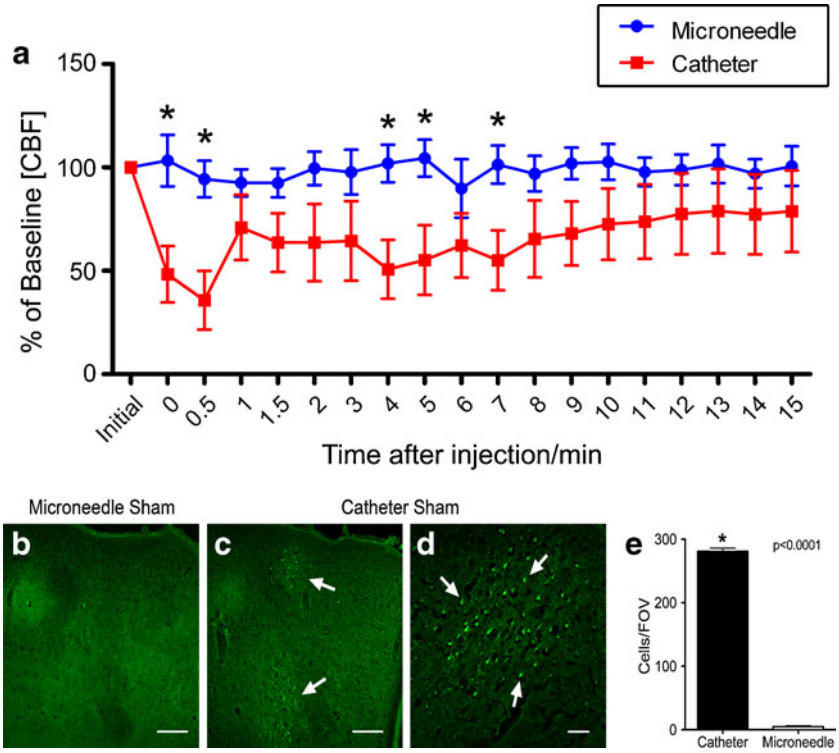

Fig. 4 Changes in cerebral blood flow (*CBF*) following intra-arterial transplantation (**a**) with a catheter or microneedle. A 65% decrease in CBF was noted in the first minute following catheter injection with recovery to about 60% of baseline 15 min later. CBF remained close to 100% of baseline during the microneedle injection and did not change after injection. Fluoro-Jade C staining found minimal neural degeneration in animals receiving microneedle injections (**b**), but did reveal substantial neural degeneration in the catheter group at low (**c**) and high (**d**) magnifications. Quantification of Fluoro-Jade C staining revealed significantly more cells staining positive in the catheter group (**e**). Adapted from [46] with permission from *JCBFM* (2011)

Clinical Trials Utilizing Intravascular Techniques

Several clinical studies using a variety of cell types to treat stroke patients have been started using intravenous, intra-arterial, or stereotactic stem cell delivery (Table 3). Results of these studies will provide further insight into which stem cell transplantation strategies are most promising. Most clinical studies to date employ stereotactic transplantation even though intravascular delivery yields a broader cell distribution within the brain. Evaluation of intra-arterial and intravenous techniques is becoming more important as minimal migration is seen away from the injection site in patients receiving stereotactic stem cell transplants [51].

Currently, only two clinical trials have been completed that utilize intravenous injection of autologous MSCs to treat stroke (Table 4). No adverse effects were reported in either study [12, 13, 52]. One study including 85 patients with middle cerebral artery strokes found that the treatment group remained free of adverse effects, demonstrated a significant reduction in the modified Rankin score, and had a lower mortality rate compared to the control group 5 years after treatment [12].

Mechanisms of Stem Cell Mediated Recovery

Initial studies into the therapeutic effect of stem cell transplantation focused on the potential to replace damaged circuitry. Several studies proved fruitful, including one in which transplanted NPCs were observed to differentiate

Table 3 Ongoing intravascular clinical stem cell trials for stroke

Clinical identifier	Phase	Cell type	Estimated enrollment	Stroke age	Delivery	Country
NCT00761982	I/II	Autologous $CD34^+$bone marrow stem cells	20	5–9 day	Intra-arterial	Spain
NCT00875654	II	Autologous mesenchymal stem cells	30	<14 days	Intravenous	France
NCT00535197	I/II	Autologous $CD34^+$bone marrow stem cells	10	<7 days	Intra-arterial	UK
NCT00859014	I	Autologous mononuclear bone marrow stem cells	30	24–72 h	Intravenous	USA
NCT01297413	I/II	Allogeneic adult mesenchymal bone marrow stem cells	35	>6 months	Intravenous	USA
NCT00473057	I	Autologous bone marrow cells	12	<90 days	Intra-arterial	Brazil
NCT01091701	I/II	Allogeneic mesenchymal stem cells	78	<10 days	Intravenous	Malaysia
NCT01310114	II	Human placenta-derived cells	44	Acute	Intravenous	USA

Table 4 Completed intravascular clinical stem cell trials for stroke

Study	Cell type	Group size	Stroke age	Delivery	Result
Bang et al. [52]; Lee et al. [12]	Autologous bone marrow-derived stem cells	16 cell and 36 control	4–5 weeks, 7–9 weeks	Intravenous ×2	No cell treatment-related or long-term (5 years) adverse events. mRS reduced, lower mortality
Honmou et al. [13]	Autologous mesenchymal stem cells	12	36–133 days	Intravenous	No adverse events; lesion volume reduced at 1 week post-transplant

mRS modified Rankin Scale

into mature $NeuN^+$ neurons that express the synaptic transport protein synaptobrevin [53]. Additional studies reported presynaptic association of differentiated human NPCs with post-synaptic terminals of endogenous neurons in damaged tissues [54]. Many studies have shown differentiation of transplanted stem cells into neurons, astrocytes, and to a lesser extent, oligodendrocytes. However, there is only limited data on neuronal communication and oligodendrocyte remyelination of endogenous neurons that shows transplanted NPCs can be functionally integrated and replace lost circuitry. In fact, with the diverse array of cell types being used in preclinical transplantation studies (MSCs/BMSCs, ESCs, and NPCs) [55] and the mounting evidence of acute behavioral recovery [56], it seems unlikely that cell transplantation offers therapeutic benefits through functional integration and replacement of lost circuitry. Support for this idea includes the finding that functional recovery is possible with limited or no cell infiltration into the brain parenchyma [18], suggesting that functional and structural improvement occurs through a variety of mechanisms including trophic support [57].

Neuroprotection

Several studies on CNS injury have reported neuroprotection and reduced cell apoptosis after stem cell transplantation. Reduction of lesion size with intravascular stem cell therapy is most commonly reported when cells are administered within 2 days of stroke onset [8, 37, 54] and may result from the combined neuroprotective and anti-apoptotic effects.

The neuroprotective role of stem cells is not surprising given their ability to neutralize free radicals (H_2O_2) following CNS injury [58] and to enhance neurotrophin expression in the ischemic brain [56]. Either through stimulation of endogenous cell secretion or direct secretion as micropumps, transplantation of stem cells is often marked by the increase in bioavailable neurotrophins such as interleukin-10 (IL-10), glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), and brain-derived neurotrophic factor (BDNF) [55, 59–61]. These increases are seen following transplantation of many cell types, including MSCs/BMSCs, NPCs, and UCBCs [56], and likely contributes to the observed neuroprotection. One study evaluating intra-arterial transplantation of human UCBC-derived MSCs in a canine model of cerebral ischemia found evidence of neuroprotective factor secretion in addition to a reduction in lesion size on MRI [8]. This group found that transplanted stem cells expressed BDNF and VEGF in vivo, supporting the idea that transplanted cells themselves may secret neuroprotective factors. Recently, Daadi and colleagues reported in vivo increases in neurotrophins insulin growth factor 1 (IGF-1), neurturin, GDNF, and fibroblast growth factor 2 (FGF-2) in neonatal rats transplanted with human neural stem cells [62]. Perhaps most intriguingly, they reported an increase in chemokine receptor CXCR4, oligodendrocyte markers Olig2, and myelin marker MBP endogenously. CXCR4 and its cognate ligand SDF-1 (CXCL12) play a vital role in oligodendrocyte regeneration and remyelination [63], suggesting that stem cells may mitigate loss of myelin or facilitate remyelination after stroke. SDF-1 is also known to play a direct neuroprotective role by neutralizing peroxide free radicals and may contribute to decreased infarct volumes through upregulation of anti-apoptotic factors Bcl-2 and Bcl-xL and inhibition of active caspase-3 [64].

There is some evidence that stems cells may exert their neuroprotective effects in a secondary fashion by influencing endogenous neurogenesis or by increasing the recruitment of endogenous progenitors. Endogenous neurogenesis has been reported after transplant of BMSCs, UCBCs, MSCs, and recently in human embryonic-derived NPCs [28, 37, 65, 66]. Additionally, a variety of stem cells have been shown to directly secrete or mediate the secretion of chemokines (SDF-1) known to stimulate endogenous neural and endothelial progenitor migration to damaged tissue [64].

Angiogenesis

Following CNS injury, transplanted stem cells are often implicated in the improvement of endogenous angiogenesis during the recovery process, and post-stroke angiogenesis is observed primarily in the penumbra [67]. The penumbra remains in a highly sensitive state for several days after

stroke, and recovery by stem cell-induced neovascularization is believed to be a key component in observed functional gains [55, 57, 68]. Cell-induced blood vessel formation has been reported following transplantation of BMSCs, NSCs, UCBCs, and peripheral blood cells [65, 68–71]. Increased secretion of angiogenic factors (VEGF, BDNF, and FGF) and chemoattractant factors (SDF-1) may contribute to this neovascularization. In fact, direct injection of SDF-1 into the stroke-affected rat brain resulted in increased recruitment of BMSCs and increased vascular density, and fluorescein isothiocyanate dextran perfusion studies revealed enhanced cerebral microvasculature perfusion [64]. Recently, Horie and colleagues transplanted NPCs that secrete VEGF and found neovascularization in the stroked hemisphere [68]. In addition to seeing elevated VEGF expression in the penumbra, they also observed that VEGF increased blood–brain barrier integrity 1 week after transplantation.

Immunomodulation

Transplanted stem cells can improve functional outcome after CNS injury by decreasing inflammatory damage and mediating inflammatory effector cells. Pluchino and colleagues highlighted this immunomodulatory strategy by demonstrating that NPCs can promote neuroprotection through release of anti-inflammatory chemokines and by expressing immunomodulatory molecules (FasL and TRAIL) [33, 72]. Inflammatory cells (macrophages, neutrophils, and T cells) have been shown to accumulate in the peri-infarct zone after stroke, and NPCs accumulate in the same area [73]. In fact, intravascular administration of human NPCs decreases inflammatory infiltration and attenuates proinflammatory tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and nuclear factor-kappa B [74]. Similar downregulation of infiltrating immune cells is seen following intravenous administration of human umbilical cord blood cells (hUCBCs) [75]. While less well characterized, a similar trend has been demonstrated in bone marrow mononuclear cells administered via the common carotid artery [59]. This study demonstrated a similar decrease in proinflammatory cytokines IL-1α, IL-1β, IL-2, TNF-α, and IL-6 and an increase in neurotrophin/anti-inflammatory cytokine IL-10 [59]. Stem cells can also modulate T cell proliferation [55, 76, 77], possibly by the direct secretion or stimulation of endogenous anti-inflammatory cytokines and chemokines such as those mentioned above. Many studies have focused on the positive outcomes of decreasing inflammation. However, Lo et al. [55, 78] demonstrated that inflammation has a dual role. Unlike the acute inflammatory response, inflammation during the post-acute phases of recovery can be beneficial in sustaining metabolic balance and remodeling.

Neuroplasticity

Neural plasticity and neural circuitry remapping is vital for recovery following CNS injury [79], and stem cells are thought to stimulate this rewiring. It was shown that intracarotid injection of BMSCs increases cortical sprouting, synaptophysin levels in the peri-infarct zone, and the number of NG2 positive cells, indicating endogenous remapping [28]. In another study, transplanted NPCs facilitated the removal of molecules that would otherwise inhibit remapping of injured tissue [80]. Andres et al. recently demonstrated that human fetal-derived NPCs promote axonal rewiring after stroke and that neutralization of thrombospondins 1 and 2 in noncontact cocultures of NPCs/neurons significantly decreased dendritic branching and axonal outgrowth [39]. Thrombospondins 1 and 2, secreted by immature astrocytes, are a necessary component of synapse development and are expressed by reactive astrocytes and activated microglia after ischemia [81, 82]. The secretion of thrombospondins by endogenous astrocytes after stroke is a possible mechanism of neurite remodeling that is enhanced by crosstalk between astrocytes, microglia, and stem cells. The importance of astrocyte–NPC crosstalk in CNS recovery is supported by the observation that transplanted BMSCs enhanced secretion of GDNF from astrocytes in the peri-infarct zone [71]. Additionally, transplanted MSCs and BMSCs can stimulate astrocytic release of tPA, resulting in tPA-induced neurite outgrowth [83, 84].

Intravascular Paradigms

Diverse intravascular stem cell transplant methods utilizing different injection techniques, cell types, cell quantities, and time of treatment are being evaluated. With benefits being observed with and without cell engraftment in the brain, two distinct paradigms have emerged to describe how transplanted stem cells facilitate functional recovery. The first paradigm asserts that intravenously administered NSCs [74], hUCBCs [18], and BMSCs [71, 83] exert their positive effects with little or no entry into the brain. One proposed mechanism of action suggests that stem cells secrete neuroprotective factors directly into the blood or stimulate trophic factor secretion after peripheral organ engraftment [18]. Lee and colleagues reported functional recovery driven by an immunomodulatory mechanism in which splenic engraftment of NPCs downregulated proinflammatory cytokines. Functional gains are lost if splenectomy is performed before transplantation [74], supporting the idea that trophic secretion in the periphery can impact the brain. The second paradigm asserts that cell recruitment into the ischemic brain following intra-arterial administration of NPCs maximizes functional recovery [9]. Recently,

intra-arterial injections of human UCBCs [8], bone marrow mononuclear cells [59], and NPCs [9, 30] were shown to engraft in the ischemic brain, leading to functional recovery. This supports the idea that cell engraftment is a key factor in the functional gains observed following intra-arterial stem cell therapy. Results are often difficult to compare given the variety of cell types, treatment time point, transplantation methods, and animal models used. More consistent and systematic evaluation of these two paradigms is needed to further clarify the importance of factor secretion and engraftment in functional recovery after stroke.

Future Directions for Intra-arterial Therapy

As we gain a better understanding of the mechanisms involved in cell engraftment and the crosstalk between transplanted and endogenous cells, bioengineering will become an important way to improve the efficacy of intravascular stem cell therapy. Modification of both surface molecules and secreted proteins can help maximize cell homing to the region of injury and stimulate functional recovery. Ensuring that cell survival, phenotype, and secretory profile are not negatively affected by genetic or epigenetic alterations will be a key step in optimizing stem cells for transplant. Accurately defining the host–graft interaction may identify specific trophic factors that may improve engraftment when cotransplanted with cells. Additionally, the ideal time of cell delivery after stroke and whether multiple serial transplants offer additional benefits over a single larger transplant must still be determined.

Improving Cell Engraftment

Increasing surface expression of adhesion molecules important for rolling, tight binding, and diapedesis of stem cells prior to intra-arterial injection may improve migration from the vasculature and increase overall engraftment to the ischemic region. While studies have found that multiple integrins are expressed on human MSCs, NSCs, and ESCs [85, 86], few studies have utilized cell engineering to improve adhesion molecule expression on transplanted cells. Recently, glycosyltransferase-programmed stereosubstitution of CD44 to an E-selectin ligand (HCELL) was used to enhance binding to E-selectin. This modification ultimately promoted CD49d binding to VCAM-1 and enhanced transendothelial migration of human MSCs into bone [87]. Our laboratory has previously shown that injection of $CD49d^{+}$ mouse NPCs selected through FACS results in higher cell engraftment in the cortex, hippocampus, and subventricular zone. Enrichment for cells expressing a particular adhesion molecule through FACS, overexpression of adhesion molecules through viral transfection, direct surface molecule modification, and epigenetic modification are all potential methods for improving the ability of transplanted cells to bind endothelial cells and successfully migrate from the vasculature into the brain.

Enhancing Cell Chemotaxis

In addition to stimulating adhesion molecule expression, cell homing may be further improved through increased presentation of receptors that mediate cell chemotaxis to inflammatory regions. Chemokines such as SDF-1 [24] and MCP-1 [30] among others have been shown to facilitate transplanted cell migration, suggesting that higher expression of CXCR4 and CCR2 may enhance the graft's responsiveness to endogenous signals. Understanding the temporal expression of chemokines following stroke will not only help identify additional receptors to target for overexpression, but will also provide guidance on the appropriate timing of cell injection following stroke.

Altering Cell Secretory Profile

Genetic modification of cells resulting in elevated secretion of factors known to be neuroprotective, angiogenic, immunomodulatory, or to facilitate communication with endogenous cells is a promising way to promote functional recovery. Understanding endogenous signals that dictate changes in trophic factor secretion from transplanted cells may provide a way to increase the sensitivity of cells to endogenous signals and remove the requirement of constitutive expression.

Two studies evaluating the effect human MSCs virally transfected to overexpress angiogenic factors angiopoietin-1 (Ang-1) [88, 89] and VEGF [89] found greater neovascularization and better functional recovery when Ang-1-modified or VEGF-modified cells were intravenously transplanted. They also reported that dual overexpression of Ang-1 and VEGF yielded the greatest structural improvements and the largest gains in a motor function test [89].

Increasing secretion of neurotrophins through viral transfection has been evaluated most extensively so far; however, further research is needed to clarify the impact of this process on cell engraftment and recovery. A few studies evaluating the effect of MSCs transfected to constitutively express BDNF found reduced lesion volumes and a reduction in motor deficits after intravenous transplant [17, 69]. Evaluation of GDNF overexpressing human MSCs and UCBCs delivered intravenously [17] found improvements on motor tests and modified neurological severity score. Similar results were seen following intrave-

nous injection of human MSCs overexpressing placental growth factor (PIGF) [69]. Studies involving stereotactic transplants of cells modified to overexpress BDNF [90], GDNF [91], HIF-1α [92], NT-3 [93], NGF [94], and Noggin [94] have also positively impacted lesion size, functional recovery, and angiogenesis.

Some studies have noted significantly higher levels of neurotrophic factors BDNF [95], GDNF [96], and PIGF [69] in the ischemic hemisphere following transplantation of the transfected cells, indicating that careful evaluation of post-transplant changes must be done to ensure that high levels of these factors do not result in undesirable or detrimental effects.

Pretreatment and Cotransplantation

In addition to direct cell modifications, pretreatment of cells prior to transplantation and cotransplantation with additional factors provide alternative methods for improving cell engraftment and behavioral recovery. A few studies have utilized hypoxic preconditioning of cells to enhance migration and improve survival [66], but long-term outcomes have not been evaluated. Injection of stem cells in a solution supplemented with specific factors may also improve cell engraftment and enhance functional recovery. One study evaluating the impact of transplanting mouse BMSCs simultaneously with a nitric oxide donor found not only significantly higher cell engraftment (attributed to increased cell expression of CXCR4 and endogenous expression of SDF-1), but also greater functional improvements [97].

One study evaluating mRNA expression of mouse BMSCs isolated from the ischemic hemisphere 14 days after intravenous transplant provided valuable information on key neurotrophic and angiogenic factors upregulated in cells after engraftment [98]. Additional studies evaluating changes in adhesion molecule, chemokine receptor, and chemokine expression in cells after transplant will help identify additional molecules and receptors that are potential targets for bioengineering.

Conclusion

Intravascular delivery of stem cells is a promising new method for the treatment of stroke. Preliminary data indicates that intra-arterial injection results in higher cell engraftment in the ischemic cortex; however, a number of important questions remain. Temporal changes in endogenous chemokine secretion and the mechanisms promoting migration of transplanted cells must be better understood in order to determine the ideal timing of transplantation and whether multiple transplants yield additional benefits. Given that trophic factors secreted by transplanted stem cells likely act over a very short distance, maximizing cell engraftment may be an important part of achieving the best functional outcomes after intravascular transplantation. Neuroprotection, neuroplasticity, immunomodulation, and angiogenesis remain key targets for stem cell therapy, and the ideal time for facilitating each of these mechanisms may vary. Most studies have utilized MSCs, ESCs, or NPCs; however, the ideal cell type for transplant may depend on the specific pathological condition as each cell type has unique advantages and disadvantages. Although initial clinical trials employing intravascular stem cell transplantation for stroke have reported no adverse outcomes, long-term studies following changes in functional recovery are needed. Some of the most exciting developments will likely come from understanding how the mechanisms through which exogenous stem cells interact with the endogenous post-stroke environment in order to elicit the structural and functional improvements seen following therapy. Results of the clinical trials currently under way and continued focus on understanding mechanisms through basic science will facilitate the successful transition of stem cell therapy from animal models to use in the clinic.

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References

1. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, et al. Heart disease and stroke statistics—2010 update: a report from the American Heart Association. Circulation. 2010;121(7):e46–215.
2. Sandercock PA, Counsell C, Gubitz GJ, Tseng MC. Antiplatelet therapy for acute ischaemic stroke. Cochrane Database Syst Rev. 2008;3:CD000029.
3. Otwell JL, Phillippe HM, Dixon KS. Efficacy and safety of i.v. alteplase therapy up to 4.5 h after acute ischemic stroke onset. Am J Health Syst Pharm. 2010;67(13):1070–4.
4. Chopp M, Li Y, Zhang J. Plasticity and remodeling of brain. J Neurol Sci. 2008;265(1–2):97–101.
5. Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. Stroke. 2007;38(2 Suppl):817–26.
6. Walczak P, Zhang J, Gilad AA, Kedziorek DA, Ruiz-Cabello J, Young RG, et al. Dual-modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. Stroke. 2008;39(5):1569–74.
7. Li Y, Chen J, Wang L, Lu M, Chopp M. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology. 2001;56(12):1666–72.

8. Chung DJ, Choi CB, Lee SH, Kang EH, Lee JH, Hwang SH, et al. Intraarterially delivered human umbilical cord blood-derived mesenchymal stem cells in canine cerebral ischemia. J Neurosci Res. 2009;87(16):3554–67.
9. Guzman R, De Los Angeles A, Cheshier S, Choi R, Hoang S, Liauw J, et al. Intracarotid injection of fluorescence activated cell-sorted CD49d-positive neural stem cells improves targeted cell delivery and behavior after stroke in a mouse stroke model. Stroke. 2008;39(4):1300–6.
10. Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, et al. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. J Neurosurg. 2005;103 (1):38–45.
11. Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, et al. Transplantation of cultured human neuronal cells for patients with stroke. Neurology. 2000;55(4):565–9.
12. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010;28(6):1099–106.
13. Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, et al. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain. 2011;134 (Pt 6):1790–807.
14. Jin K, Sun Y, Xie L, Mao XO, Childs J, Peel A, et al. Comparison of ischemia-directed migration of neural precursor cells after intrastriatal, intraventricular, or intravenous transplantation in the rat. Neurobiol Dis. 2005;18(2):366–74.
15. Li L, Jiang Q, Ding G, Zhang L, Zhang ZG, Li Q, et al. Effects of administration route on migration and distribution of neural progenitor cells transplanted into rats with focal cerebral ischemia, an MRI study. J Cereb Blood Flow Metab. 2010;30(3):653–62.
16. Pendharkar AV, Chua JY, Andres RH, Wang N, Gaeta X, Wang H, et al. Biodistribution of neural stem cells after intravascular therapy for hypoxic-ischemia. Stroke. 2010;41(9):2064–70.
17. Ou Y, Yu S, Kaneko Y, Tajiri N, Bae EC, Chheda SH, et al. Intravenous infusion of GDNF gene-modified human umbilical cord blood CD34+ cells protects against cerebral ischemic injury in spontaneously hypertensive rats. Brain Res. 2010;1366:217–25.
18. Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. Stroke. 2004;35 (10):2385–9.
19. Guzman R, Uchida N, Bliss TM, He D, Christopherson KK, Stellwagen D, et al. Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. Proc Natl Acad Sci U S A. 2007;104(24):10211–6.
20. Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, et al. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc Natl Acad Sci U S A. 2004;101(32):11839–44.
21. Kondziolka D, Steinberg GK, Cullen SB, McGrogan M. Evaluation of surgical techniques for neuronal cell transplantation used in patients with stroke. Cell Transplant. 2004;13(7–8):749–54.
22. Justicia C, Martin A, Rojas S, Gironella M, Cervera A, Panes J, et al. Anti-VCAM-1 antibodies did not protect against ischemic damage either in rats or in mice. J Cereb Blood Flow Metab. 2006;26(3):421–32.
23. Yan YP, Sailor KA, Lang BT, Park SW, Vemuganti R, Dempsey RJ. Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. J Cereb Blood Flow Metab. 2007;27(6):1213–24.
24. Beech JS, Reckless J, Mosedale DE, Grainger DJ, Williams SC, Menon DK. Neuroprotection in ischemia-reperfusion injury: an antiinflammatory approach using a novel broad-spectrum chemokine inhibitor. J Cereb Blood Flow Metab. 2001;21(6):683–9.
25. Hoehn BD, Palmer TD, Steinberg GK. Neurogenesis in rats after focal cerebral ischemia is enhanced by indomethacin. Stroke. 2005;36(12):2718–24.
26. Hill WD, Hess DC, Martin-Studdard A, Carothers JJ, Zheng J, Hale D, et al. SDF-1 (CXCL12) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury. J Neuropathol Exp Neurol. 2004;63(1):84–96.
27. Shen LH, Li Y, Chen J, Zacharek A, Gao Q, Kapke A, et al. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab. 2007;27(1):6–13.
28. Shen LH, Li Y, Chen J, Zhang J, Vanguri P, Borneman J, et al. Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. Neuroscience. 2006;137 (2):393–9.
29. Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A. 2004;101(52):18117–22.
30. Andres R, Choi R, Pendharkar A, Gaeta X, Wang N, Lee SE, Palmer TD, Steinberg GK, Guzman R. The CCR2/CCL2 interaction regulates therapeutic homing of neural stem cells after intraarterial delivery for stroke. Stroke. 2011;in press.
31. Wang L, Li Y, Chen J, Gautam SC, Zhang Z, Lu M, et al. Ischemic cerebral tissue and MCP-1 enhance rat bone marrow stromal cell migration in interface culture. Exp Hematol. 2002;30(7):831–6.
32. Blann A, Kumar P, Krupinski J, McCollum C, Beevers DG, Lip GY. Soluble intercellular adhesion molecule-1, E-selectin, vascular cell adhesion molecule-1 and von Willebrand factor in stroke. Blood Coagul Fibrinolysis. 1999;10(5):277–84.
33. Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, et al. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature. 2005;436(7048):266–71.
34. Belmadani A, Tran PB, Ren D, Miller RJ. Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. J Neurosci. 2006;26(12):3182–91.
35. Bacigaluppi M, Pluchino S, Peruzzotti-Jametti L, Kilic E, Kilic U, Salani G, et al. Delayed post-ischaemic neuroprotection following systemic neural stem cell transplantation involves multiple mechanisms. Brain. 2009;132(Pt 8):2239–51.
36. Jin K, Mao X, Xie L, Greenberg RB, Peng B, Moore A, et al. Delayed transplantation of human neural precursor cells improves outcome from focal cerebral ischemia in aged rats. Aging Cell. 2010;9(6):1076–83.
37. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, et al. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res. 2003;73(6):778–86.
38. Fan Y, Shen F, Frenzel T, Zhu W, Ye J, Liu J, et al. Endothelial progenitor cell transplantation improves long-term stroke outcome in mice. Ann Neurol. 2010;67(4):488–97.
39. Andres RH, Horie N, Slikker W, Keren-Gill H, Zhan K, Sun G, et al. Human neural stem cells enhance structural plasticity and axonal transport in the ischaemic brain. Brain. 2011;134(Pt 6):1777–89.
40. Zhang RL, Chopp M, Jiang N, Tang WX, Prostak J, Manning AM, et al. Anti-intercellular adhesion molecule-1 antibody reduces ischemic cell damage after transient but not permanent middle cerebral artery occlusion in the Wistar rat. Stroke. 1995;26 (8):1438–42. discussion 43.
41. Rubin JB, Kung AL, Klein RS, Chan JA, Sun Y, Schmidt K, et al. A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. Proc Natl Acad Sci U S A. 2003;100(23):13513–8.

42. Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A, et al. One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. Stroke. 2007;38(7):2150–6.
43. Minnerup J, Kim JB, Schmidt A, Diederich K, Bauer H, Schilling M, et al. Effects of neural progenitor cells on sensorimotor recovery and endogenous repair mechanisms after photothrombotic stroke. Stroke. 2011;42(6):1757–63.
44. Keimpema E, Fokkens MR, Nagy Z, Agoston V, Luiten PG, Nyakas C, et al. Early transient presence of implanted bone marrow stem cells reduces lesion size after cerebral ischaemia in adult rats. Neuropathol Appl Neurobiol. 2009;35(1):89–102.
45. Fujita Y, Ihara M, Ushiki T, Hirai H, Kizaka-Kondoh S, Hiraoka M, et al. Early protective effect of bone marrow mononuclear cells against ischemic white matter damage through augmentation of cerebral blood flow. Stroke. 2010;41(12):2938–43.
46. Chua JY, Pendharkar AV, Wang N, Choi R, Andres RH, Gaeta X, et al. Intra-arterial injection of neural stem cells using a microneedle technique does not cause microembolic strokes. J Cereb Blood Flow Metab. 2011;31(5):1263–71.
47. Ward NS. The neural substrates of motor recovery after focal damage to the central nervous system. Arch Phys Med Rehabil. 2006;87(12 Suppl 2):S30–5.
48. Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs. 2001;169 (1):12–20.
49. Daldrup-Link HE, Rudelius M, Metz S, Piontek G, Pichler B, Settles M, et al. Cell tracking with gadophrin-2: a bifunctional contrast agent for MR imaging, optical imaging, and fluorescence microscopy. Eur J Nucl Med Mol Imaging. 2004;31(9):1312–21.
50. Kang WJ, Kang HJ, Kim HS, Chung JK, Lee MC, Lee DS. Tissue distribution of 18F-FDG-labeled peripheral hematopoietic stem cells after intracoronary administration in patients with myocardial infarction. J Nucl Med. 2006;47(8):1295–301.
51. Nelson PT, Kondziolka D, Wechsler L, Goldstein S, Gebel J, DeCesare S, et al. Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation. Am J Pathol. 2002;160(4):1201–6.
52. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol. 2005;57 (6):874–82.
53. Toda H, Takahashi J, Iwakami N, Kimura T, Hoki S, Mozumi-Kitamura K, et al. Grafting neural stem cells improved the impaired spatial recognition in ischemic rats. Neurosci Lett. 2001;316(1):9–12.
54. Ishibashi S, Sakaguchi M, Kuroiwa T, Yamasaki M, Kanemura Y, Shizuko I, et al. 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 (2):215–23.
55. Bliss TM, Andres RH, Steinberg GK. Optimizing the success of cell transplantation therapy for stroke. Neurobiol Dis. 2010;37 (2):275–83.
56. Guzman R, Choi R, Gera A, De Los Angeles A, Andres RH, Steinberg GK. Intravascular cell replacement therapy for stroke. Neurosurg Focus. 2008;24(3–4):E15.
57. Guzman R. Cellular stroke therapy: from cell replacement to trophic support. Expert Rev Cardiovasc Ther. 2009;7(10):1187–90.
58. 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(11):1103–10.
59. Brenneman M, Sharma S, Harting M, Strong R, Cox Jr CS, Aronowski J, et al. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. J Cereb Blood Flow Metab. 2010;30(1):140–9.
60. Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci. 2006;7(5):395–406.
61. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, et al. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. Mol Ther. 2005;11(1):96–104.
62. Daadi MM, Davis AS, Arac A, Li Z, Maag AL, Bhatnagar R, et al. Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. Stroke. 2010;41(3):516–23.
63. Patel JR, McCandless EE, Dorsey D, Klein RS. CXCR4 promotes differentiation of oligodendrocyte progenitors and remyelination. Proc Natl Acad Sci U S A. 2010;107(24):11062–7.
64. Shyu WC, Lin SZ, Yen PS, Su CY, Chen DC, Wang HJ, et al. Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. J Pharmacol Exp Ther. 2008;324(2):834–49.
65. Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest. 2004;114(3):330–8.
66. Francis KR, Wei L. Human embryonic stem cell neural differentiation and enhanced cell survival promoted by hypoxic preconditioning. Cell Death Dis. 2010;1(2):e22.
67. Cai W, Guzman R, Hsu AR, Wang H, Chen K, Sun G, et al. Positron emission tomography imaging of poststroke angiogenesis. Stroke. 2009;40(1):270–7.
68. Horie N, Pereira MP, Niizuma K, Sun G, Keren-Gill H, Encarnacion A, et al. Transplanted stem cell-secreted VEGF effects post-stroke recovery, inflammation, and vascular repair. Stem Cells. 2011;in press.
69. Liu H, Honmou O, Harada K, Nakamura K, Houkin K, Hamada H, et al. Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. Brain. 2006;129(Pt 10):2734–45.
70. Zhang ZG, Zhang L, Jiang Q, Chopp M. Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. Circ Res. 2002;90(3):284–8.
71. Shen LH, Li Y, Chopp M. Astrocytic endogenous glial cell derived neurotrophic factor production is enhanced by bone marrow stromal cell transplantation in the ischemic boundary zone after stroke in adult rats. Glia. 2010;58(9):1074–81.
72. Pluchino S, Zanotti L, Brini E, Ferrari S, Martino G. Regeneration and repair in multiple sclerosis: the role of cell transplantation. Neurosci Lett. 2009;456(3):101–6.
73. Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat Biotechnol. 2002;20(11):1111–7.
74. Lee ST, Chu K, Jung KH, Kim SJ, Kim DH, Kang KM, et al. Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. Brain. 2008;131(Pt 3):616–29.
75. Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, et al. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. Stem Cells Dev. 2005;14(5):595–604.
76. Nasef A, Mathieu N, Chapel A, Frick J, Francois S, Mazurier C, et al. Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Transplantation. 2007;84 (2):231–7.
77. Einstein O, Fainstein N, Vaknin I, Mizrachi-Kol R, Reihartz E, Grigoriadis N, et al. Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. Ann Neurol. 2007;61(3):209–18.

78. Lo EH. A new penumbra: transitioning from injury into repair after stroke. Nat Med. 2008;14(5):497–500.
79. Carmichael ST. Translating the frontiers of brain repair to treatments: starting not to break the rules. Neurobiol Dis. 2010;37(2):237–42.
80. Emsley JG, Mitchell BD, Magavi SS, Arlotta P, Macklis JD. The repair of complex neuronal circuitry by transplanted and endogenous precursors. NeuroRx. 2004;1(4):452–71.
81. Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell. 2005;120 (3):421–33.
82. Lin TN, Kim GM, Chen JJ, Cheung WM, He YY, Hsu CY. Differential regulation of thrombospondin-1 and thrombospondin-2 after focal cerebral ischemia/reperfusion. Stroke. 2003;34(1):177–86.
83. Shen LH, Xin H, Li Y, Zhang RL, Cui Y, Zhang L, et al. Endogenous tissue plasminogen activator mediates bone marrow stromal cell-induced neurite remodeling after stroke in mice. Stroke. 2011;42(2):459–64.
84. Xin H, Li Y, Shen LH, Liu X, Wang X, Zhang J, et al. Increasing tPA activity in astrocytes induced by multipotent mesenchymal stromal cells facilitate neurite outgrowth after stroke in the mouse. PLoS One. 2010;5(2):e9027.
85. Krishna OD, Jha AK, Jia X, Kiick KL. Integrin-mediated adhesion and proliferation of human MSCs elicited by a hydroxyproline-lacking, collagen-like peptide. Biomaterials. 2011;32:6412–24.
86. Prowse AB, Chong F, Gray PP, Munro TP. Stem cell integrins: implications for ex-vivo culture and cellular therapies. Stem Cell Res. 2010;6(1):1–12.
87. Thankamony SP, Sackstein R. Enforced hematopoietic cell E- and L-selectin ligand (HCELL) expression primes transendothelial migration of human mesenchymal stem cells. Proc Natl Acad Sci U S A. 2011;108(6):2258–63.
88. Onda T, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. J Cereb Blood Flow Metab. 2008;28(2):329–40.
89. Toyama K, Honmou O, Harada K, Suzuki J, Houkin K, Hamada H, et al. Therapeutic benefits of angiogenetic gene-modified human mesenchymal stem cells after cerebral ischemia. Exp Neurol. 2009;216(1):47–55.
90. Lee HJ, Lim IJ, Lee MC, Kim SU. Human neural stem cells genetically modified to overexpress brain-derived neurotrophic factor promote functional recovery and neuroprotection in a mouse stroke model. J Neurosci Res. 2010;88(15):3282–94.
91. Chen B, Gao XQ, Yang CX, Tan SK, Sun ZL, Yan NH, et al. Neuroprotective effect of grafting GDNF gene-modified neural stem cells on cerebral ischemia in rats. Brain Res. 2009;1284:1–11.
92. Wu W, Chen X, Hu C, Li J, Yu Z, Cai W. Transplantation of neural stem cells expressing hypoxia-inducible factor-1alpha (HIF-1alpha) improves behavioral recovery in a rat stroke model. J Clin Neurosci. 2010;17(1):92–5.
93. Zhang ZH, Wang RZ, Li GL, Wei JJ, Li ZJ, Feng M, et al. Transplantation of neural stem cells modified by human neurotrophin-3 promotes functional recovery after transient focal cerebral ischemia in rats. Neurosci Lett. 2008;444(3):227–30.
94. Ding J, Cheng Y, Gao S, Chen J. Effects of nerve growth factor and Noggin-modified bone marrow stromal cells on stroke in rats. J Neurosci Res. 2011;89(2):222–30.
95. Nomura T, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. I.V. infusion of brain-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. Neuroscience. 2005;136(1):161–9.
96. Horita Y, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat. J Neurosci Res. 2006;84(7):1495–504.
97. Cui X, Chen J, Zacharek A, Li Y, Roberts C, Kapke A, et al. 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. 2007;25 (11):2777–85.
98. Yilmaz G, Alexander JS, Erkuran Yilmaz C, Granger DN. Induction of neuro-protective/regenerative genes in stem cells infiltrating post-ischemic brain tissue. Exp Transl Stroke Med. 2010;2(1):11.