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# REGENERATION OF NEURONAL CELLS FOLLOWING CEREBRAL INJURY

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## Abstract

Stem cells possess a definitive role in neuronal rejuvenation following a cerebral injury. Whether endogenous, from the neurogenic niches of subventricular zone and subgranular zone, or recruited from the bone marrow through peripheral circulation, accumulating evidence demonstrates that stem cells ameliorate the consequences of cerebrovascular events, particularly cerebral ischemia. In this chapter, we review milestone studies implicating the role of stem cells in response to disease. Furthermore, we outline specific mechanisms of action along with their clinical potential as therapeutic treatments for ischemic stroke.

### Keywords

hematopoietic stem cells; mesenchymal stem cells; endothelial progenitor cells; very small embryonic-like stem cells; stroke; cell therapy; migration; homing; neurogenesis; angiogenesis

## Introduction

The idea that stem cells may reconstitute regions of neuronal damage has prompted much research interest in using bone marrow (BM) as a donor source for transplantation therapy in neurological disorders, notably stroke. The heterogeneous mixture of cells populating the BM includes: hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and very small embryonic-like stem cells (VSELs). A collection of in vivo and in vitro research suggests these cells may mobilize into the peripheral blood upon cerebrovascular injury and respond by secreting essential growth factors for survival [1], along with possibly undergoing neuronal differentiation on exposure to inducing regimens [2].

As stroke remains a primary cause of death worldwide, this chapter pursues the possibility of the aforementioned cell lines to afford brain plasticity and remodeling [3]. In the clinic, minimally invasive intravascular transplantation is an appealing approach for stem cell therapeutic measures. However, this scheme requires concerted mechanisms to ensure that cells, or their secreted therapeutic molecules, reach the site of injury. The mechanisms involved in migration, homing, isolation, and the potential therapeutic effects of these cells will be discussed within this theme.

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## Hematopoietic stem cells (HSCs)

In addition to the defining feature of repopulating ablated BM [4], HSCs can also migrate to peripheral blood (PB) in response to injury. During homeostasis, hematopoietic stem cells are quiescent and low in number, a characteristic attributed to chemokine regulation. Yet, in response to injury, these cells can become motile, with increased migration into blood circulation [5]. Stromal derived factor-1 (SDF-1, also termed CXCL12) contributes to an essential chemoattractant pathway via the receptor CXCR-4 [5]. When SDF-1 is active, HSCs cross the endothelial blood-BM barrier and populate the peripheral blood [6]. The SDF-1/CXCR-4 interface is highly expressed in several stem cell niches, notably the brain endothelium [7]. With the central nervous system (CNS) contributing to HSCs motility, conditions of stress (i.e. stroke) can amplify recruitment of HSCs into the brain [4, 5].

One such mechanism of CNS control in the migration of BM-derived HSCs is the induction of cytokines. Recently proposed, the neurotransmitter catecholaminergic signaling pathway may promote HSC mobilization through sympathetic secretion into the blood or via a more paracrine fashion from the bone marrow nerve endings [4, 8]. This neurotransmitter mediated interaction is bidirectional. Accumulating evidence suggests human HSCs can affect the nervous system and modulate its action. The homing of BM-derived stem cells through the catecholaminergic system involves multiple signaling pathways, including Wnt and beta-catenin, as well as specific migratory molecules, such as membrane-bound enzyme MT1-MMP and SDF-1, which all contribute to proliferation, increased motility, and engraftment capability of CD34 HSCs [8]. In terms of clinical stroke data, it is noted that following human acute stroke, the extent of PB immature hematopoietic CD34+ (HSCs) mobilization directly correlates with the recovery of function [9]. Following neurorestorative events such as neoangiogenesis, the up-regulation of SDF-1 within ischemic tissue will recruit CXCR4+ hematopoietic stem cells from peripheral blood.

HSC mobilization may also serve an integral role in early host repair mechanisms for many other neurological disorders. Endogenous reparative responses have been seen in pathological conditions such as: elevated BM CD34+ HSCs accompanying chronic spinal cord injury, cord blood (CB) CD34+ cells reducing heat stress symptoms upon injection, delays in disease progression of amyotrophic lateral sclerosis upon injection of human CB mononuclear cells into mice, and CB mononuclear injection decreasing beta-amyloid deposits in animal Alzheimer models. With the experimental evidence surmounting, influence of the central nervous system in the mobilization of HSCs suggest potential for the maintenance and repair of the nervous system upon insult. Furthermore, HSCs have been proposed as an ideal donor graft source because of their safety and efficacy profile in the clinical treatment of other diseases [10].

## Mesenchymal stem cells (MSCs)

MSC transplantation has been utilized in experimental stroke models and demonstrates improvement in functional recovery of neurological deficits induced by cerebral ischemia. The following sections within this topic will expand upon potential mechanisms that may mediate the therapeutic effect of MSCs in cerebral vascular incidents.

The proposition of stem cell differentiation into neuronal cells remains controversial. Upon transplantation, via intravenous, intracarotid, or intracerebral delivery, the graft survival is modest at best, therefore adequate levels for differentiation seems unlikely [11]. A more plausible mechanism involves the production of trophic factors such as: hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF, FGF-2), and insulin growth factor-1 (IGF-1), which may each activate ischemic brain endogenous repair

through particular mechanisms [12]. For example, early increases (1 hour after stroke) could increase BBB leakage, exacerbating ischemic cell damage, but when administered 48 hours post-stroke, VEGF could enhance angiogenesis in the ischemic border zone (IBZ) to improve recovery [13].

HGF has demonstrated an influential role in vascularization. Upon treatment with HGF, the amelioration of BBB destruction without exacerbating cerebral edema, decreased intracranial pressure, and induction of angiogenesis have all been reported. Although it seems unlikely that MSCs differentiate into neurons themselves, research indicates that transplantation with MSCs may promote migration and induction from subventricular zone (SVZ) and subgranular zone (SGZ) neurogenic sites within the brain to regions of ischemia [12]. This process of neurogenesis appears to be regulated by the neurotrophic factors being secreted by the transplanted MSCs.

A limitation of human mesenchymal stem cells (hMSCs) is their lack of telomerase activity, leading to a population doubling of approximately 18, with decreased expectations upon passaging of the stem cells [14]. A mechanism to circumvent this issue is use of retroviral transfection of hHSCs with the human telomerase gene, termed hTERT-MSCs [15]. Expanding gene manipulation of hMSCs, transfection of genes such as: FGF-2, HGF, and BDNF have also been incorporated into hTERT-MSCs before transplantation to extend and increase neurotrophic efficacy [15].

As an alternative to genetic manipulation, studies have also utilized the use of trophic factors as adjuvants with MSC delivery. Studies show that transplantation with BDNF markedly improved stroke recovery in the animal models [16]. The use of other adjuvants, such as cell permeable inhibitor of caspases (Z-VAD), enhances graft survival and behavioral recovery when intracerebrally infused with MSCs into the region of ischemia [17]. Additionally, intravenous infusion of MSCs with a nitric oxide donor (DETA/NONOate) demonstrates enhancement of vessel perimeter and endothelial cell proliferation, leading to improved functional recovery in stroke animals [18]. Nitric oxide donor adjuvants have also contributed to increased SVZ neurogenesis alongside VEGF and bFGF expression within ischemic regions [18]. The use of grafted MSCs may also impart benefits by way of glial cell proliferation including neuron remyelination as well as synaptogenesis and a reduction in apoptosis.

As previously mentioned, the SDF-1/CXCR-4 chemoattractant pathway serves as a homing signal for stem cell populations. In the nonhematopoietic system, SDF-1 similarly serves as a signal from injured organs to influence migration of CXCR-4 cells. SDF-1 expression is regulated by the hypoxia-responsive transcription factor HIF-1 (hypoxia-inducible factor 1). With transplanted MSCs expressing CXCR-4, the SDF-1 gradient pattern associated with the hypoxia gradient provides a signal for attracting both hematopoietic and nonhematopoietic stem cells [19] to migrate from the periphery to the site of ischemic injury.

## Endothelial progenitor cells (EPCs)

Although hematopoietic in origin, EPCs can be found in both the peripheral blood of adults and derived from umbilical cord blood (UCB). In pioneering studies, transplanted EPCs isolated from human UBC, populated endothelial neovascularization in regions of ischemia. The ability for endothelial progenitor cells to participate in re-endothelialization during neovascularization makes EPCs an exceptional candidate for management of cerebrovascular disease.

Currently, endothelial progenitor cells display a variety of markers for isolation such as: CD31, VE-cadherine, E-selectine, eNOS, and von Willebrand factor [20], however, substantial evidence suggests that only the CD34+ EPCs from BM or UCB are capable of differentiating into mature endothelium [21]. A contributing factor for the lack of clearly defined methods for cell isolation may be due to the rare prevalence of EPCs in adult peripheral blood (0.01%). Until recently, neovascularization, the formation of new blood vessels, was though to occur exclusively from proliferation and migration of pre-existing endothelial cells; this process is known as angiogenesis. Juxtaposing neovascularization, vasculogensesis (also known as vascularization) is the differentiation of endothelial cells from precursor cells and was thought to only occur in the embryo during development. Yet, current evidence suggests that BM-derived endothelial progenitor cells in circulation are capable of homing to neovascularization sites for proliferation and differentiation of subsequent endothelial cells [22].

Over the last few years, clinical research suggests that circulating EPCs as a biomarker may predict clinical outcome of cardiovascular disease, with low EPC counts correlating to more severe functional impairments. Expanding upon this observation, clinical studies have also been initiated to assess a higher risk for atherosclerotic events in populations with lower EPC numbers. In terms of clinical applications for neurovascular disease, the observational studies are limited and with notable discrepancies.

The primary mechanisms of stroke pathogenesis remain unclear, however, there is mounting evidence that implicates immunological attack upon the brain and/or its vasculature, which provides a novel therapeutic stroke target involving EPCs. This immunological attack could result in altered inter-endothelial junction integrity, leading to vascular endothelial damage and breakdown of the blood brain barrier (BBB). Therefore, restoration of this barrier through EPC therapy may serve to abrogate the consequences of stroke pathogenesis.

## Very small embryonic-like stem cells (VSELs)

Present in a variety of adult organs, specifically the brain, VSELs express several progenitor stem cell (PSC) markers. These very tiny stem cells can be mobilized into the peripheral blood following tissue and organ injuries. Human VSELs, smaller than an erythrocyte, belong to the non-hematopoietic fraction of leukocytes (Lin−/CD45− cells) expressing CD34, CD133, and CXCR-4 antigens [23]. Due to their low constitution of peripheral blood, special flow cytometric protocols have been established for identification. VSEL phenotypic markers include: CD45 (mouse and human), positive expression of Sca-1 (mouse), CXCR-4, CD133, and CD34+ (mouse and human), positive PSC markers (i.e. Oct-4, Nanog, and SSEA), and express markers of epiblast/germ line stem cells [23]. In addition to peripheral blood, purified VSELs can be isolated from bone marrow.

With the notion being that VSELs are epiblast-derived stem cells deposited early in embryonic development, these stem cells may present as a good candidate for tissue rejuvenation and regeneration. The ease of harvesting should also be considered for therapeutic potential with VSELs. The patients own BM, stored UCB, and mobilized PB are sources readily accessible in harvesting VSELs for autologous transplantation. With respect to allotransplantation, histocompatible-related or unrelated donors could serve as another source. Yet, despite the ease of harvesting these cells, expansion strategies must be employed due to the relatively low number of cells yielded.

Treatment strategies for the acute and subacute stage (time 0 to one week post injury) appear to provide the best opportunity to initiate therapeutic intervention. Due to this immediate need for intervention, purifying these cells from BM aspirates, UCB, or mobilized PB through multi-parameter staining and regular high speed sorting may not be feasible [24]. To

counter this challenge, the Ratajczak group proposed a relatively short and economical three-step method for isolation that allowed approximately 60% recovery of the initial number of Lin−/CD45−/CD133+ UCB-VSELs. This novel procedure takes 2--3 hours per UCB unit (ideally applicable using BM aspirates and mobilized PB as well) and should produce VSELs freshly isolated from BM, PB, or UCB that are pre-committed to neurological lineage in ex vivo cultures [25].

## Conclusion

The developmental biology research elucidating stem cell plasticity has served as the impetus for advancing regenerative medicine in many neurological disorders, including stroke. Some of the most commonly explored cell lines include: HSCs, MSCs, EPCs, and VSELs, all with specific therapeutic potential. Each of these cell lines does, however, impart their own individual challenges.

The ability for HSCs to develop into differentiated neurons has yet to be determined. Opposing this notion, transdifferentiation may be explained as a transient change in phenotypic expression induced by neural tissue-derived spherical membrane fragments called microvesicles. These fragments, also termed exosomes, may transfer neural cell surface receptors, mRNA, and miRNA to the HSCs employed for regeneration [26].

An emerging concern in the use of MSCs involves the potential to cause neoplastic tumor formation upon deposition into the brain. Similar to the initial impression of HSC differentiation, it was challenged whether MSCs are able to develop into neuronal cells. One possible explanation for this finding was in vitro contamination in the cell culture media that may alter the morphology of MSCs [27]. Therefore, the working postulate is, upon homing of the stem cell to the site of injury, the production of trophic factors influences the microenvironment. Evidence that grafted stem cells do not persist after delivery and are rapidly eliminated supports this proposal.

More recently, my research group has explored EPC transplantation for repair of the BBB after stroke [28, 29, 30]. The working hypothesis suggests that tissue-type plasminogen activator (t-PA) may exacerbate the breakdown of the all ready vulnerable BBB. Currently, much of the stroke therapy implemented does not consider the capacity of BBB damage after stroke. It is our contention that if EPC transplantation promotes restoration of the vascular endothelium, the clinical effects could be far reaching and substantially help a large population of patients that may be excluded from the current 3-hour guideline for tPA.

Lastly, another appealing therapy for stroke is the use of VSELs. A prominent restriction in cell therapy is their ability to cause embolism, especially accompanying the large quantity necessary for therapeutic effect. This caveat makes the use of very small stem cells appealing. Because the isolation and expansion of this cell line may be more tedious and longer, the use of allogenic transplants and faster expansion protocols are to be considered.

In summary, the plethora of accumulating stem cell research is quickly translating into clinical trials. The use of HSCs, MSCs, EPCs, and VSELs all appear to provide specific insight into treating neurological disease from many facets. However, it is important to acknowledge that these mechanisms are yet to be fully determined and there is still a gap in our translational laboratory-to-clinic understanding of stem cell therapy. Therefore, as the research transcends theory and progresses into treatment, we must ensure systematically designed preclinical studies precede initiation of clinical trials to allow rigorous investigations as to the safety and efficacy of these stem cells.

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## References

1. Hess DC, Borlongan CV. Stem cells and neurological diseases. Cell Prolif. 2008; 41(Suppl 1):94–114. [PubMed: 18181951]
2. Munoz-Elias G, Woodbury D, Black IB. Marrow stromal cells, mitosis, and neuronal differentiation: stem cell and precursor functions. Stem Cells. 2003; 21(4):437–448. [PubMed: 12832697]
3. Borlongan CV, et al. Potential of stem/progenitor cells in treating stroke: the missing steps in translating cell therapy from laboratory to clinic. Regen Med. 2008; 3(3):249–250. [PubMed: 18462048]
4. Lapidot T, Kollet O. The brain-bone-blood triad: traffic lights for stem-cell homing and mobilization. Hematology Am Soc Hematol Educ Program. 2010; 2010:1–6. [PubMed: 21239763]
5. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? Blood. 2005; 106(6):1901–1910. [PubMed: 15890683]
6. Dar A, Kollet O, Lapidot T. Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. Exp Hematol. 2006; 34(8):967–975. [PubMed: 16863903]
7. Stumm RK, et al. A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J Neurosci. 2002; 22(14):5865–5878. [PubMed: 12122049]
8. Kalinkovich A, et al. Blood-forming stem cells are nervous: direct and indirect regulation of immature human CD34+ cells by the nervous system. Brain Behav Immun. 2009; 23(8):1059–1065. [PubMed: 19341792]
9. Dunac A, et al. Neurological and functional recovery in human stroke are associated with peripheral blood CD34+ cell mobilization. J Neurol. 2007; 254(3):327–332. [PubMed: 17345048]
10. Bajada S, et al. Updates on stem cells and their applications in regenerative medicine. J Tissue Eng Regen Med. 2008; 2(4):169–183. [PubMed: 18493906]
11. Shen LH, et al. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab. 2007; 27(1):6–13. [PubMed: 16596121]
12. Chen J, et al. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res. 2003; 92(6):692–699. [PubMed: 12609969]
13. Zhang ZG, et al. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin Invest. 2000; 106(7):829–838. [PubMed: 11018070]
14. Zimmermann S, et al. Lack of telomerase activity in human mesenchymal stem cells. Leukemia. 2003; 17(6):1146–1149. [PubMed: 12764382]
15. Kurozumi K, 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. [PubMed: 15585410]
16. Chen J, Li Y, Chopp M. Intracerebral transplantation of bone marrow with BDNF after MCAo in rat. Neuropharmacology. 2000; 39(5):711–716. [PubMed: 10699437]
17. Chen J, et al. Caspase inhibition by Z-VAD increases the survival of grafted bone marrow cells and improves functional outcome after MCAo in rats. J Neurol Sci. 2002; 199(1--2):17–24. [PubMed: 12084437]

18. Chen J, et al. Combination therapy of stroke in rats with a nitric oxide donor and human bone marrow stromal cells enhances angiogenesis and neurogenesis. Brain Res. 2004; 1005(1--2):21–28. [PubMed: 15044060]
19. Ceradini DJ, Gurtner GC. Homing to hypoxia: HIF-1 as a mediator of progenitor cell recruitment to injured tissue. Trends Cardiovasc Med. 2005; 15(2):57–63. [PubMed: 15885571]
20. Lapergue B, Mohammad A, Shuaib A. Endothelial progenitor cells and cerebrovascular diseases. Prog Neurobiol. 2007; 83(6):349–362. [PubMed: 17884277]
21. Griese DP, et al. Isolation and transplantation of autologous circulating endothelial cells into denuded vessels and prosthetic grafts: implications for cell-based vascular therapy. Circulation. 2003; 108(21):2710–2715. [PubMed: 14597586]
22. Masuda H, Asahara T. Post-natal endothelial progenitor cells for neovascularization in tissue regeneration. Cardiovasc Res. 2003; 58(2):390–398. [PubMed: 12757873]
23. Kucia M, et al. Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood: preliminary report. Leukemia. 2007; 21(2):297–303. [PubMed: 17136117]
24. Zuba-Surma EK, et al. Optimization of isolation and further characterization of umbilical-cord-blood-derived very small embryonic/ epiblast-like stem cells (VSELs). Eur J Haematol. 2010; 84(1):34–46. [PubMed: 19758351]
25. Ratajczak J, et al. Stem cells for neural regeneration--a potential application of very small embryonic-like stem cells. J Physiol Pharmacol. 2011; 62(1):3–12. [PubMed: 21451204]
26. Ratajczak J, et al. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia. 2006; 20(9):1487–1495. [PubMed: 16791265]
27. Lu P, Blesch A, Tuszynski MH. Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? J Neurosci Res. 2004; 77(2):174–191. [PubMed: 15211585]
28. Kaneko Y, Tajiri N, Shinozuka K, Glover LE, Weinbren NL, Cortes L, Borlongan CV. Cell therapy for stroke: emphasis on optimizing safety and efficacy profile of endothelial progenitor cells. Curr Pharm Des. 2012; 18(25):3731–3734. [PubMed: 22574986]
29. Borlongan CV, Glover LE, Tajiri N, Kaneko Y, Freeman TB. The great migration of bone marrow-derived stem cells toward the ischemic brain: therapeutic implications for stroke and other neurological disorders. Prog Neurobiol. 2011; 95(2):213–228. [PubMed: 21903148]
30. Borlongan CV. Bone marrow stem cell mobilization in stroke: a ‘bonehead’ may be good after all! Leukemia. 2011; 25(11):1674–1686. [PubMed: 21727900]