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FROM BONE MARROW TO BRAIN: STEM CELLS IN NEUROPROTECTION, PLASTICITY, AND NEUROREGENERATION

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At present, curative therapies for neurological diseases are limited, even though they are prevalent worldwide. So far, molecular strategies developed for brain disorders act through one single molecular mechanism, yet, these diseases are multifactorial and highly complex, as to why a successful therapy likely calls for a more multifaceted and cell-based approach. The bone marrow contains a mixed stem and progenitor cell population including hematopoietic stem cells (HSC) and mesenchymal stromal stem cells (MSC), which are potential endogenous candidates for cell-based therapy in various brain disorders like stroke, trauma, and neurodegeneration. Unlike the neural stem cells (NSC), bone marrow HSC are readily isolated, mobilized and expanded by means of treatment with granulocyte-colony stimulating factor (G-CSF) and CXCR4-antagonist plerixafor. Once in the blood circulation, the cells preferentially home to injured tissues including the brain. Bone marrow cells may convey neuroprotection, plasticity, and neuroregeneration by different mechanisms of action, which include either transdifferentiation or cell-cell fusion with resident brain cells. Bone marrow cells also benefit the injured brain by secreting bioactive factors, which in a paracrine manner convey intrinsic repair and enhance neurogenesis. Furthermore, transplanted MSC may activate the astrocytes leading to increased glial secretion of neurotrophic growth factors and enhanced proliferation and migration of the resident NSC. These neuroregenerative mechanisms of action are not mutually exclusive, in fact they may provide a multifaceted therapeutic approach, which is requested in order to move neurorestorative and protective strategies into the clinic. ***Biomed Rev*** **2011; 22: 1-6.**

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INTRODUCTION: STEM CELLS IN BRAIN

In animal and human central nervous system (CNS), neurons are generated in intracerebral germinal zones such as the subventricular zone (SVZ) and the dentate gyrus (DG) of hippocampus (1-3). Inside the germinal zones, neural stem cells (NSC) proliferate and migrate in response to brain injury and various pathological conditions including: inflammation, degeneration and ischemia (2,4-6). However, in the adult human brain, significant neurogenic activity and NSC recruitment from the germinal zones to an injured area remain controversial (1,3). While some groups report NSC and their proliferative capacity

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intact in the human parkinsonian brain as compared to those of human adult controls (3), others suggest that proliferating and migrating NSC are abundant in the human brain only before 18 months of age, after which the neurogenic capacity subsides and by adulthood, it is almost extinct (1). Other limitations in neurogenesis include that the differentiation of endogenous NSC might be restricted to glial cell types (2,7).

To address some of these hurdles, NSC activation in SVZ and DG as well as their regulation and recruitment have been extensively studied in experimental studies (2,5-7). Even if adult neurogenesis can provide neuroregeneration after some types of acquired brain injuries (5,6), this approach remains controversial, since the NSC found in brains from e.g. patients suffering Alzheimer's Disease (AD) were demonstrated to be implicated in AD pathogenesis (8). Particularly, NSC in the adult human brain were reported to generate aneuploid cells, characteristic for histopathology of neurodegenerative disorders (8,9). Accordingly, the therapeutic use of human endogenous NSC for neurodegenerative diseases, in particular AD remains contentious and not yet realistic. It is needed to reconsider the current stem cell-based concepts and their therapeutic application in neuroregenerative medicine as well as to investigate non-NSC-derived stem cell sources. For this purpose, stem and/or progenitor cells derived from other tissues than the brain, such as the bone marrow, could prove suitable. Bone marrow-derived stem and progenitor cells offer significant advantages over resident NSC in the diseased brain, since they are: 1. devoid of intrinsic cellular dysfunctions relating to neurodegenerative disorders; 2. easily obtained, expanded, and mobilized from host bone marrow into blood; 3. show preferential homing to the inflamed brain; 4. able to cross the blood-brain barrier due to chemotactic migration; and 5. interact with resident neuroglia and the neural micromilieu leading to improved neuroplasticity, repair and functional recovery (8-13). Significant crosstalks between the nervous and hematopoietic systems might involve the CNS' neuroendocrine regulation of host immune responses (13), although the possible neurotherapeutic use of bone marrow in CNS pathologies is still not completely elucidated.

This review highlights the recent and major findings in the field of neuroregeneration by means of recruitment of non-neural cells from the bone marrow. It summarizes the putative mechanisms of actions comprising 1. cellular replacement by putative transdifferentiation, 2. cell-cell fusion leading to hybrid brain cells, 3. indirect signalling through activated astroglia, and 4. paracrine secretion of neurotrophic growth factors supporting neuronal survival and brain repair.

STEM CELLS FROM BONE MARROW TO BRAIN

In the adult bone marrow reside multipotent and easily mobilized stem cells. Accumulating data support the observation that bone marrow-derived hematopoietic and non-hematopoietic stem cells transdifferentiate into cell types of different tissues including glia and neurons (2,10-15). This degree of plasticity, self-renewal, and pluripotency with the ability to undergo neurodifferentiation is a hallmark of bone marrow-derived stem cells, although the same wide-ranging neuroplasticity can be found in a range of other stem cell lineages such as adipose-derived stem cells (16). The 'cell replacement' concept is likely not the only therapeutic mechanism provided by stem cells. Accumulating data have shown that expression and paracrine secretion of neurotrophic factors from bone marrow-derived stem cells also provide support for neuronal survival, intrinsic brain repair, and neuroregenerative processes (10,17,18). These mechanisms open up new possibilities regarding the neurotherapeutic use of mobilized stem cells from the bone marrow.

Advantage of the bone marrow stem cells

As compared to other stem cell lineages, the bone marrow-derived CD34+ hematopoietic stem cells (HSC) possess a major advantage as compared to other stem cell types, as the HSC are easily expanded and mobilized endogenously leading to their blood-borne circulation, from which these cells are particularly able to home to injured or inflamed tissues in any compartment of the human body including the CNS (17-22). The damaged brain displays high levels of chemokines like stromal cell-derived factor-1 (SDF-1), which attract circulating HSC, due to their surface expression of CXC-chemokine receptor-4 (CXCR4; also known as fusin or CD184) (10,14,15,17-21). In the bone marrow, stromal expression of SDF-1 that binds CXCR4 on HSC is a key factor in HSC residency (2,14,15,18-20). However, by means of granulocyte colony-stimulating factor (G-CSF) combined with synergistic agent plerixafor (a rapidly degraded, reversible CXCR4 antagonist), hypermobilization of endogenous CD34+ HSC into the circulation is obtained (13-15,17-20,23). After HSC egress to the circulation, the HSC home to the injured brain by means of increased neuroglial expression of SDF-1 (14,15,18-23). Accordingly, CD34+ cells are recruited in significant numbers to the injured brain (17,22). Both recruitment to injured or inflamed brain as well as their homing to a depleted bone marrow are characteristic for HSC and combined with their feasible G-CSF/plerixafor-mobilization, these cells are highly

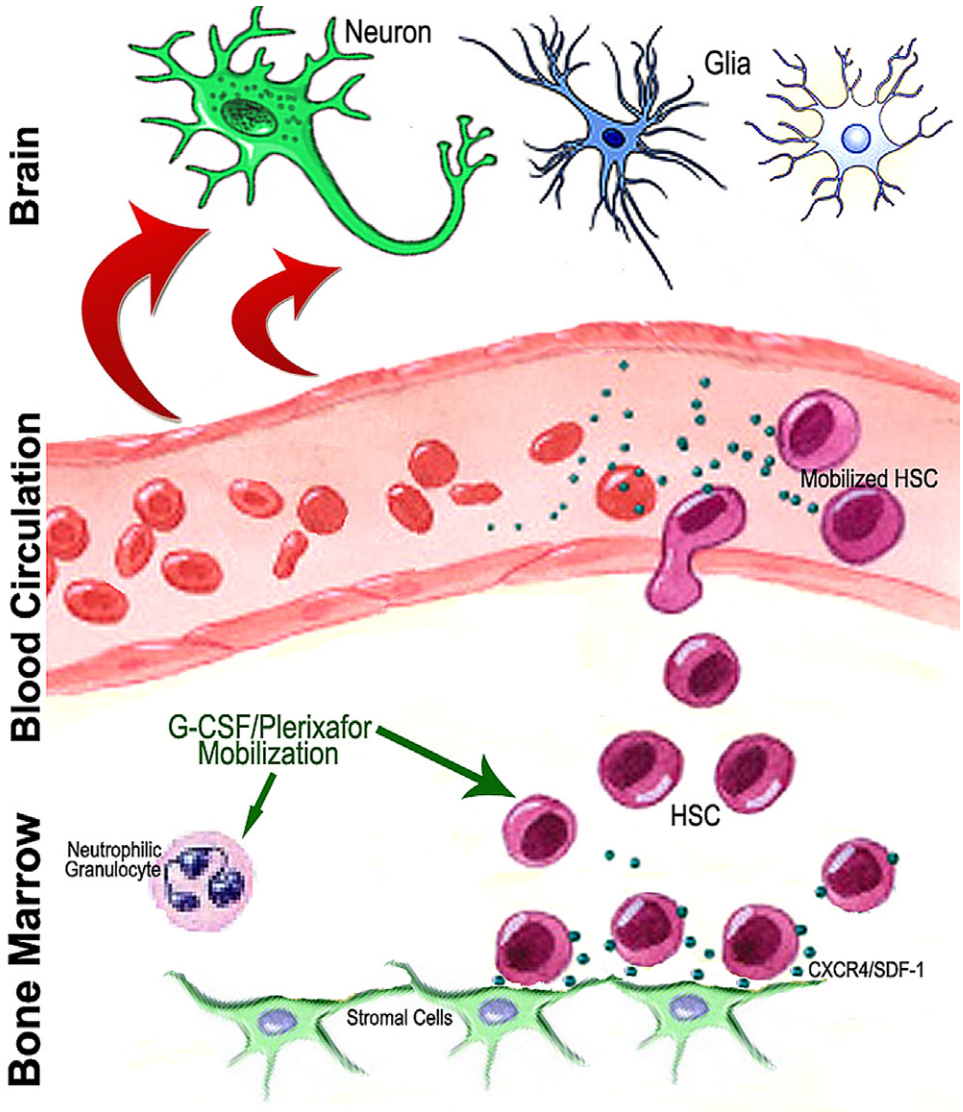

Figure 1. *Illustration of the bone marrow, blood, and brain tissues. CXCR4+ HSC are anchored to SDF-1+ stromal cells of the bone marrow. The administration of G-CSF combined with CXCR4-antagonist plerixafor leads to hypermobilization of endogenous HSC into the blood circulation along with stimulation of neutrophilic granulocytes. The blood-borne HSC are recruited to the injured brain by chemotaxis. Inside CNS, bone marrow cells improve neuroprotection, plasticity, and brain repair.*

suitable for regenerative medicine. Moreover, since this approach allows a strictly endogenous strategy, the well-known ethical, technical and biological problems relating to allogenic and donor-based approaches are bypassed.

Neuroregenerative mechanisms of action

As reviewed here, various independent researchers have reported that bone marrow-derived subpopulations of stem cells can generate neural cell types both *in vitro* and *in vivo* (2,25-29).

Hence, in mice without the capacity to generate myeloid and lymphoid cell lineages, administration of adult bone marrow resulted in transformation of the transplanted cells into neuronal phenotypes expressing neuron-specific antigens (25,27). Thus, by receiving male bone marrow-derived cells, female recipient mice displayed Y chromosome in neurons dispersed throughout their female brain (27). Likewise, olfactory bulb neurons contained bone marrow-derived graft cells as dem-

onstrated due to the genetic alteration of the transplants (25).

Moreover, bone marrow-derived MSC may *in vivo* adopt both neuronal phenotype and functions as shown in a study of transplanted bone marrow cells including their migration, phenotypic expression, and long-term survival in rats (2,28). Consequently, bone marrow-derived cells contain the capability to transform into CNS cell types that are viable within the microenvironment. Also, the cell grafts are likely differentiated into neural phenotypes as they express both markers of neural progenitors as well as mature neuronal markers such as e.g. neuronal-specific nuclear (NeuN) protein (2,28). In an interesting study, adult human bone marrow cells were demonstrated to give rise to adult human brain cells, as shown in cerebellum from patients, who suffered from malignant hematologic neoplasms for which they received radiation, chemotherapy, and bone marrow transplantation (29). In the Purkinje neurons of female patients receiving male bone marrow transplants, both an X and a Y chromosome were detected in the nuclei, which indicates that male donor cells had infiltrated the CNS, in which they either transdifferentiated into Purkinje neurons or fused with the host cerebellar cells (29).

In favor of the cell fusion concept are studies that have verified how co-culturing of embryonic stem cells with bone marrow-derived stem cells results in cellular fusion (30,31). Likewise, co-culturing of murine brain cells with embryonic pluripotent cells led to a spontaneous formation of hybrid cells due to cellular fusion (32). Accordingly, spontaneous fusion of cells may likely provide an explanation for the reported 'cell fate-switches' or the apparent "transdifferentiation" observations, which otherwise have been attributed to pluripotency and plasticity of stem cells (30,32). In line with this, it was shown that cell-cell fusion of donor and recipient cells is responsible for the regeneration of normal hepatic function and structure after bone marrow transplants in mice (33-35).

However, it is unlikely that this mechanism of cell-cell fusion is going to reduce the excitement relating to stem cell pluripotential properties and cell-based treatment strategies. Hence, formation of hybrid cells due to spontaneously fused bone marrow and resident cells is a process considered important within the field of stem cell therapy and regenerative medicine (33-35).The fusion of cells as a mechanism of cell replacement may however seem discouraging compared to the concept of transdifferentiation, where bone marrow stem cells are considered to undergo steps of de- and transdifferentiation into functionally complete neurons. However, bone marrow cell fusion in the CNS may in fact provide unpredicted advantages by endowing damaged or pre-apoptotic neurons with intact genes and gene regulatory machinery. Since it is not easy to reproduce *de novo* and appropriately replace the adult brain's highly specialized neurons and their neural networks, the fusion-mediated cell resuscitation could provide a quite compliant practice. Nevertheless, *in vitro* studies have demonstrated that bone marrow stem cells are capable of undergoing complete and functional transdifferentiation into neurons, and in these cell culture experiments, fusion mechanisms can be ruled out, since no co-culturing with other neuronal cell types was applied (2,10-15,25-29). In addition to the cell replacement mechanisms, the neurotrophic growth support mediated by bioactive factors secreted in a paracrine manner by stem cells may cause more significant and immediate treatment effects in the injured CNS (18). In fact, transplantation of bone marrow stem cells results in surprisingly low survival rates of the engrafted cells inside the brain tissue (10,17).

Among the bioactive factors secreted by bone marrow cells are brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF, FGF-2), nerve growth factor (NGF), and insulin-like growth factor-1 (IGF-1), which all are demonstrated to exert profound neuroprotective and neuroregenerative actions including the stimulation of both intrinsic neurogenesis and angiogenesis in the CNS (10,17,18,36,37). Another indirect molecular mechanism of action might convey the neuroprotective and -regenerative effects in the CNS of administered bone marrow. Hence, in a recent study it was shown that transplanted bone marrow MSC can activate resident astroglia in the ischemic brain leading to increased astrocytic secretion of glial cell derived neurotrophic factor (GDNF), which in the CNS promotes plasticity and recovery (36,37). Hence, the increased astroglial GDNF levels led to enhanced proliferation and migration of the resident NSC in the ischemic brain (37).

Reactive astroglia have several neuroprotective and regenerative actions in the brain and are the main source of neurotrophic growth factors, antioxidants, and neuronal survival signals as well as an astrocytic phenotype provides the endogenous source of NSC within the brain (36,38). Consequently, the study of transplanted MSC effects upon astroglia during brain ischemia (37) is fundamental, as it reveals how stem cell-based therapy may not only work through cellular replacement and/or cellular fusion. Hence, a range of molecular and/or paracrine mechanisms are likely contributing to the neurotherapeutic effects of bone marrow cells and

these involve secretion of bioactive factors, signal transduction mechanisms, extracellular cross-talking, and repair through activation of astroglia.

CONCLUSION

As reviewed here, the neurotherapeutic use of bone marrow-derived cells in brain injury and diseases has opened up new avenues of stem cell-based insight and treatment. Moreover, we will likely see in the near future that there are more options in terms of the different possibilities regarding how to apply which types of stem cells and/or their molecular repertoire in terms of secreted bioactive factors. Further elucidation of the diverse biological mechanisms by which various stem cells may exert neuroprotective, restorative and neuroregenerative functions will most likely result in the discovery of new and safer neurotherapies that can be applied in the clinic.

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