1. ABSTRACT

Stem cell therapies, such as bone marrow transplantation, are a promising strategy for the treatment of stroke. Bone marrow-derived stem cells (BMSCs) including both hematopoietic and mesenchymal stem cells (HSCs and MSCs) can exhibit tremendous cellular differentiation in numerous organs. BMSCs may also promote structural and functional repair in several organs such as the heart, liver, brain, and skeletal muscle via stem cell plasticity. Interestingly, ischemia is known to induce mobilization of BMSCs in both animal models and humans. The tissue injury is “sensed” by the stem cells and they migrate to the site of damage and undergo differentiation. The plasticity, differentiation, and migratory functions of BMSCs in a given tissue are dependent on the specific signals present in the local micro-environment of the damaged tissue. Therefore, the ischemic micro-environment has critical patho-biological functions that are essential for the seeding, expansion, survival, renewal, growth and differentiation of BMSCs in damaged brain remodeling. Recent studies have identified the specific molecular signals, such as SDF-1/CXCR4, required for the interaction of BMSCs and damaged host tissues. Understanding the exact molecular basis of stem cell plasticity in relation to local ischemic signals could offer new insights to permit better management of stroke and other ischemic disorders. The aim of this review is to summarize recent studies into how BMSCs reach, recognize, and function in cerebral ischemic tissues, with particular regard to phenotypical reprogramming of stem cells, or “stem cell plasticity”.

2. INTRODUCTION

Despite advances in medical and surgical treatment, stroke is still a leading cause of death and disability worldwide (1). Sudden interruption of blood flow to the brain can be caused by the occlusion of a cerebral artery. Atherosclerosis of cerebral vessels leads to focal ischemia and subsequent degeneration in the restricted central nervous system region with acute loss of neurons, astrocytes and oligodendrocytes (2). This process finally results in tissue necrosis and possibly irreversible impairment of brain functions. It has been known for many years that neuronal regeneration can be induced in response to brain damage, but overall neuronal proliferation usually fails to repair the damaged brain completely.

In neurological disorders the aim of cell therapy is to replace, repair or enhance the biological function of damaged cells in order to restore brain function. Recent studies showing that new neurons can be generated from stem cell transplantation and that the adult brain produces new neurons in response to injury has raised hopes for the development of a stem cell based therapy for patients suffering from brain damage (3, 4).

Stem cells possess differentiation potential, a capacity for self-renewal and, depending on their origin, the ability to repopulate into multiple cell types. Several different types of stem cells, such as those from peripheral blood, bone marrow, and embryonic stem cells, have been successfully used to induce neurogenesis and functional recovery in various experimental models of ischemia (3, 4).
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Fetal-derived neural stem cells have been successfully delivered to the damaged brain, either by direct intracerebral injection or via intra-arterial routes (5). Unfortunately, the transplanted allogenic cells survived for only a short time due to immunorejection (5).

An attractive alternative to improve functional recovery after ischemic brain lesion is the use of autologous BMSCs, either after stimulation of endogenous stem cell pools or after exogenous stem cell transplantation, to generate new neurons (6, 7). Bone marrow contains precursors for hematopoietic stem cells and mesenchymal stem cells. BMSCs are multipotent, and some transplanted BMSCs can differentiate into neuronal cells and endothelial cells in the damaged brain (3). Special conditions including tissue injury may trigger those responses. BMSCs appear to have the ability to pass through the blood brain barrier and selectively migrate to the ischemic hemisphere of the damaged brain to improve neurological functions in experimental stroke (3, 8). This functional recovery of the damaged brain is dependent on the response to specific signals present in the local ischemic tissue microenvironment. The beneficial effects are considered to be mediated by two factors: [1] increasing endogenous angiogenic, neurogenic and anti-apoptotic factors due to interactions between BMSCs and host brain parenchymal cells, and/or the same angiogenic, neurogenic and anti-apoptotic factors secreted by BMSCs (9-13); [2] differentiation of BMSCs themselves directly into both neuronal and endothelial cells that restore brain function (3). Hanabusa et al. (14) have recently demonstrated in vivo that BMSCs induced angiogenesis, neurogenesis and inhibited cell apoptosis in the ischemic penumbra. Thus, BMSCs have neuroprotective effects not only directly, through their differentiation, but also through their ability to induce angiogenic, neurogenic and anti-apoptotic factors either by themselves or by interaction with host cells.

Our research team focuses on understanding how stem cells home to the injured brain (15). We believe that molecular pathways exist that are up-regulated immediately following ischemic brain damage causing stem cells to home into the damaged brain. A detailed analysis of the biological responses to brain injury would not only give us insight into how damage occurs, but would also yield insight into how the body attempts to repair itself. This review will focus on our recent studies on stem cell-based therapies for application in stroke patients, the possible mechanisms that may be involved in stem cell-based therapies, and how stem cells reach the site of cerebral ischemia and function there in the context of stem cell plasticity.

3. RECENT STUDIES ABOUT STEM-CELL BASED THERAPIES

Animal studies conducted by us and other groups have demonstrated that either direct injection of BMSCs in the infarct border zone at the time of cerebral infarction (6, 7), or mobilization of these stem cells after cerebral infarction results in regeneration of neurons with improved survival rate and brain function after cerebral ischemia (15).

Granulocyte colony-stimulating factor (G-CSF), a 20-kDa glycoprotein, is the major growth factor responsible for regulating granulopoiesis and promoting the survival, proliferation, functional activation, and maturation of cells of the neutrophil lineage (16, 17). G-CSF is able to mobilize stem cells from bone marrow into the peripheral blood (PB) (16). Recombinant G-CSF is also a common treatment after hematologic disease, or chemotherapy, when white blood cell counts tend to be dangerously low and there is a risk of infection (18). G-CSF is also widely used to induce mobilization of blood precursors in different clinical settings such as chemotherapy-induced myelosuppression and peripheral blood stem cell recollection for autologous and allogeneic bone marrow transplantation (19). In addition, it has been reported that G-CSF in combination with stem cell factors (SCF) improves ventricular remodeling after myocardial infarction (MI) through myocyte regeneration by way of transdifferentiation of BMSCs (20). This treatment significantly improved cardiac function and reduced mortality.

We have recently demonstrated that G-CSF can enhance tissue regeneration and improve the survival rate after stroke by mobilizing BMSCs from bone marrow into peripheral blood (15). Our earlier study showed that subcutaneous injections of G-CSF, starting one day after cerebral ischemia and continuing for up to 5 days, promote BMSC migration to the injured brain and enhance neural repair in rats suffering from cerebral ischemia (15). Infarction volume was markedly reduced, and there was also significant recovery of neurological dysfunction. Our animal data also suggested that mobilized BMSCs may contribute to tissue regeneration after focal cerebral ischemia (15). Neurogenesis occurs in discrete regions of the central nervous system (CNS) continuously during adulthood (21), and is increased after cerebral ischemia (22, 23). G-CSF may enhance this process by increasing the number of circulating BMSCs, and their infiltration into the CNS. In addition, a sufficient number of BMSCs, mobilized by G-CSF, could home in on cerebral ischemic injuries to promote neuronal repair and recovery of brain function; this would provide a basis for the development of a non-invasive autologous therapy for cerebral ischemia. Inflammation, a major contributor to cell death after cerebral ischemia, has been shown to be detrimental to neurogenesis (24). The anti-inflammatory properties of G-CSF may suppress inflammation and, in turn, create a more supportive environment for circulating BMSCs to migrate into, repopulate, survive and differentiate. Our findings (15) also suggest that the injured brain may be capable of self-renewal or that a chemokine may be able to draw progenitor cells into the infarcted brain. If G-CSF treatment can mobilize autologous BMSCs into circulation, enhance their translocation into the ischemic brain and thus significantly improve lesion repair, it represents an attractive strategy for the development of a clinically significant non-invasive stroke therapy. Our recent pilot clinical trial demonstrated that G-CSF could mobilize
BMSCs in patients after acute stroke in a safe, feasible manner and provide a neurological outcome superior to conventional treatment (Shyu et al. manuscript submitted).

4. MECHANISM THAT ALLEVIATE STROKE SYMPTOMS IN G-CSF THERAPY

It is likely that the mechanisms providing therapeutic benefit for stroke rats using G-CSF treatment in our recent study are multi-dimensional (15). First, we found that administration of G-CSF increased the mobilization of circulating BMSCs to damaged areas of the brain; this increase may in turn stimulate cell division in the penumbra of the ischemic brain. Second, it is possible that interaction of BMSCs with ischemic tissue may lead BMSCs and/or parenchymal cells to produce trophic factors (25) that may contribute to the recovery of neural functions lost as a result of tissue injury (13). BMSCs have been shown to constitutively express interleukins, such as IL-1beta, IL-8, IL-16, FGF-2, VEGF, IGF-1, GM-CSF and TNF-alpha (26). In addition, it has been demonstrated that several factors, such as FGF-2, EGF, VEGF, SCF, erythropoietin, BDNF, caspase inhibitors, and anti-inflammatory drugs, can increase adult neurogenesis by stimulating formation or improving survival of new neurons (23, 27-32). These cytokines may act as survival, growth, and/or differentiation factors for neuronal and vascular progenitor cells, which may in turn proliferate, migrate and differentiate following brain injury, thus contributing to recovery processes. Furthermore, neurotrophic factors have been shown to enhance neuronal sprouting (33), synaptogenesis (34) and neurotransmission (35), as well as increase neurotransmitter release (36). The injection of GDNF into the brain was found to greatly diminish infarction volume and improve neurological functions in animals suffering cerebral ischemia (37). Thus, it is plausible that BMSCs themselves may be directly involved in promoting the plasticity of ischemically damaged neurons and endothelial cells. Some G-CSF-mobilized BMSCs may enter the cerebral ischemic region and interact with penumbra cells; and this interaction may enhance the production of trophic factors such as GDNF and BDNF, which may in turn promote tissue repair (4) of damaged parenchymal cells after stroke.

5. HOMING FACTORS

In models of traumatic or ischemic brain injuries, BMSCs administered intravenously, intra-arterially, or intracerebrally could preferentially migrate into the injured region of the brain (3, 10, 11, 38, 39). Therefore the ischemic microenvironment may express signals that promote the incorporation of circulating BMSCs to the ischemic area where they further differentiate into mature brain tissue. In addition, it is noteworthy that in ischemic rat brain, a number of neurotrophic factors are released that have been shown to result in human BMSC growth factor production (4, 40). We speculate that these trophic factors may be released as a result of ischemic damage to the brain tissue, which may, in turn, target BMSCs to damaged tissue (15). Recent studies (3, 41) have also demonstrated that intravenous administration of BMSCs in rats results in their accumulation in the injured region. These findings suggest that the injured brain might specifically attract BMSCs. It is important to identify which signaling molecules attract BMSCs and direct their migration to damaged areas in order to understand the BMSC-mediated cell therapy for cerebral ischemia and possibly other ischemic related diseases. While stem cell homing to bone marrow has been well studied, and is known to be critical for normal fetal development (42), until recently no ischemic-stem cell homing factor has been yet identified.

6. STROMAL CELL-DERIVED FACTOR 1 AND CXC CHEMOKINE RECEPTOR 4

Stromal cell-derived factor 1 (SDF-1 or CXCL12) and its receptor CXC chemokine receptor 4 (CXCR4) have been demonstrated to play an important role in the mobilization and homing of stem cells to bone marrow (43-45), endothelial cell migration, as well as adult vasculogenesis (46-49), neurogenesis and neuronal migration (50-52). The up-regulation of SDF-1 after early focal cerebral ischemia (53) and myocardial infarction (54, 55) indicate that this chemokine may have a similar signaling pathway for the adhesion and migration of BMSCs both to bone marrow and to ischemic tissue. Regenerating tissues may exert regulatory functions on circulating stem cells via locally induced SDF-1. Since local SDF-1 expression is up-regulated in a variety of damaged tissues, the interaction between damaged tissues induced SDF-1/CXCR4 on circulating BMSCs may recover tissue function.

SDF-1 is a member of the alpha (CXC) chemokine family, which are small chemo-attractant molecules involved in inflammatory responses (56). SDF-1 is a strong chemo-attractant for CD34+ cells that express CXCR4, the receptor for SDF-1, and it plays an important role in BMSC trafficking between peripheral circulation and bone marrow (57). This factor is, therefore, suggested to play a major role in successful BMSC engraftment in the bone marrow (58). SDF-1 regulates adhesion/chemotaxis of bone marrow hematopoietic progenitor cells through activation/regulation of specific integrin molecules (59-61). In vivo gene inactivation of SDF-1 and its receptor CXCR4 in mice leads to early embryonic lethality due to abnormal cerebellar, gastrointestinal vasculature, and hematopoietic development (42, 62, 63). A role for SDF-1 in BMSC recruitment from bone marrow (BM) to peripheral blood (PB) has been proposed, based on the administration of G-CSF activated neutrophil elastases, which then cleave the membrane-bound SDF-1 of stromal cells in the bone marrow and provoke an efflux of stem cells that express CXCR4 (57). Furthermore, plasma elevation of SDF-1 is involved in BMSC mobilization through matrix metalloproteinase-9 (MMP-9)–mediated conversion of kit ligand factor (SCF) from membrane-bound to soluble form (64). Over-expression of SDF-1 in ischemic tissues has recently been found to enhance BMSC recruitment from PB and to induce neo-angiogenesis (46, 65). A possible role for SDF-1 in the homing of stem cells to damaged sites has also been unraveled by studies in animal models of liver, limb, and heart damage (46, 54, 66, 67). SDF-1 is secreted
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primarily by bone marrow fibroblasts and is required for BMSC homing/retention in the bone marrow microenvironment. A concentration gradient of SDF-1 across the endothelium in the bone marrow is the major mechanism for homing of BMSCs to bone marrow (58). Physiologically, bone marrow has a higher SDF-1 concentration than any other tissue, and circulating BMSCs home to bone marrow proficiently. Since mobilized peripheral blood stem cells are increasingly used for clinical cell transplantation, it is becoming clear that proteolytic degradation of SDF-1 and CXCR4 on stem cells is an important step in stem cell release and homing (68).

Since SDF-1 receptors are present on bone marrow stem cells (69), up-regulation of SDF-1 in the local ischemic damage after injury (70-72) may be related to homing and engraftment of stem cell to the injured tissue. SDF-1 peptides produced in ischemic tissue could represent the link between BMSCs and the ischemic tissue microenvironment. Therefore, cross-talk between ischemically produced SDF-1 and CXCR4 receptors on BMSCs in circulation could contribute to tissue regeneration in many organs. We hypothesize that SDF-1 is up-regulated in ischemic tissues and that ischemia-associated hypoxia causes an imbalance between plasma and bone marrow SDF-1 concentration, resulting in the transient establishment of an SDF-1 gradient that favors stem cell translocation into ischemic tissue, thereby enhancing angiogenesis and functional recovery.

SDF-1 is also secreted in several other organs and the secretion of SDF-1 increases during tissue damage (46, 73). De Falco and colleagues (74) have reported that SDF-1 expression following hind-limb ischemia in mice was up-regulated in plasma and down-regulated in bone marrow, thus providing a novel potential mechanism for mobilization of BMSCs in peripheral blood. Therefore, SDF-1 secreted during tissue damage may play an important role in directing transplanted or mobilized BMSCs to damaged tissues. The fact that circulating stem cells home to loci of high SDF-1 concentration, is supported by in vitro data showing transmigration of BMSCs towards regions of high SDF-1 concentration (75).

Hill et al. (76) recently demonstrated that upregulation of SDF-1 was associated with endothelial cells when GFP-bone marrow transplanted mice underwent temporary middle cerebral artery occlusion (MCAO). SDF-1 expression was primarily localized to the ischemic penumbra and GFP cells were associated with SDF-1-positive endothelial cells. These results suggest that the interaction of SDF-1-CXCR4 may mediate trafficking of transplanted BMSCs to ischemically damaged tissue. Lesion-induced up-regulation of endothelial SDF-1 (53) and the appearance of increased CXCR4 expression in the ischemic hemisphere in our previous study (15), indicate that cerebro-endothelial SDF-1 could be a chemo-attractant for peripheral blood cells. Lataillade and colleagues (77) have also reported that a significant proportion of BMSCs, mobilized by G-CSF, express CXCR4 receptors on their cell surface and that SDF-1 induces directional migration of BMSCs. By attracting BMSCs to the ischemic region, a SDF-1/CXCR4 interaction may be directly involved in vascular remodeling, angiogenesis and neurogenesis, thereby alleviating stroke symptoms.

To further investigate this “homing phenomenon”, we used a neutralizing CXCR4 antibody to block SDF-1 binding to CXCR4 after induction of cerebral ischemia in a rat model. The administration of CXCR4 antibody compromised BMSCs homing in the penumbra area of cerebral ischemic rats, resulting in poor neurological recovery after stroke (15). The CXCR4 antibody inhibited homing and engraftment of bone marrow cells to a subsequent number of bone marrow-derived neuron-like cells in the damaged brain (15). This chemotaxis of SDF-1/CXCR4 may take place in a manner similar to the migration of leukocytes into damaged or inflamed tissues (78). Previous work suggesting SDF-1 plays a role in CXCR4 BMSC recruitment to injured tissues (46, 53, 66), together with our own observations, implies SDF-1 mediates BMSC homing and integration into regions of ischemic brain injury. Furthermore, SDF-1-CXCR4 interactions play an important role, not only in the homing of BMSCs into bone marrow, but also into ischemic limb, heart and neural tissues. It has recently been reported that several CXCR4-positive tumor cell types metastasize to bone tissue. Therefore, SDF-1 that is secreted by bone marrow stroma cells may play a key role in metastasis of CXCR4-positive tumor cells to bone (79, 80).

7. WHEN IS HOMING POSSIBLE?

In order to determine if a temporal window for brain regeneration and stem cell homing exists following focal cerebral ischemia, we have studied the effects of stem cell mobilization with G-CSF in rats with chronic cerebral ischemia, one week after cerebral infarction (Shyu et al., manuscript submitted). We found administering G-CSF this late after focal cerebral ischemia did not result in stem cell engraftment into the infarct zone or improvement in brain function in test rats despite a 25 fold increase in the number of circulating CD34+ cells. Similar results were obtained when BMSCs were infused at later time points other than the time of acute myocardial infarction (81). Thus despite clear evidence from animal models that BMSCs home to the infarct zone within days of a brain infarction, stem cell mobilization and/or infusion at times other than the time of infarct are not efficacious unless the expression of signaling molecules required for stem cell homing is re-established. These findings suggest that the time of stem cell transplantation subsequent to cerebral ischemia is critical for stem cell homing. Intravenous BMSCs increased stem cell homing, angiogenesis and improved cardiac function within 1 day post cerebral-ischemia, whereas no effects were found when infusion was initiated at 7 and 14 days post cerebral-ischemia. The same study also showed that SDF-1 expression in cerebral ischemic rats peaked the first few days post-ischemia and decreased thereafter (53). Interestingly, cerebral ischemia SDF-1 expression also decreased progressively 7 to 14 days post ischemia. The identification of BMSC homing factor(s) would allow for further optimization of the delivery of homing factors and
possibly increase our ability to help prevent ischemic stroke. This could also lead to the application of SDF-1 as a gene or protein therapy, for the mobilization of either exogenous or endogenous stem cells for the treatment of stroke and other ischemic diseases.

8. HOW DO STEM CELLS FUNCTION AT THE SITE OF ISCHEMIA?

It is possible that BMSCs migrating to the ischemic hemisphere create local chemical gradients and/or localized chemokine accumulation, dictating a directional response in endothelial, neuronal and glial progenitor cells (46). As a consequence of this autocrine regulatory pathway, endothelial and neuronal progenitor cells may mobilize and connect with each other, a step required for subsequent formation of a structural network of branching vessels and neurons (82). For example, SDF-1 might also stimulate host endothelium progenitor cell (EPC) differentiation from pre-existing blood vessels and/or host EPCs derived from bone marrow (82). SDF-1 has also been shown to increase survival of cultured CD34+ cells (46) and to regulate endothelial cell branching morphogenesis (49). It is known that integrins mediate the homing of transplanted hematopoietic stem cells to bone marrow (83) as well as recruiting inflammatory cells to sites of inflammation. In addition, recent data has also demonstrated that β2-integrins play a role in the homing and neovascularization of BMSCs to areas of ischemia (84).

In our recent study of stroke rats treated with G-CSF (15), we found that mobilized stem cells or EPCs, labeled with BrdU contributed to “re-endothelialization and angiogenesis” in the perivascular area of the penumbra region of the brain. In order to further confirm this phenomenon, we conducted experiments to demonstrate that the proliferative marker ki67 (85) was present in FITC-dextran-perfused cerebral vessels of the penumbra region in the G-CSF treated rats (data not shown). The results of our study suggested that mobilized EPCs contributed to “collateral circulation”, and could even “line-up” and “build” new vessels in the ischemic brain (86). However, we also found that EPCs incorporate rate for the construction of new blood vessels in the absence of brain ischemia was relatively low, due to a lack of up-regulation of SDF-1/CXCR4. Furthermore, we concluded that the efficiency of neo-vascularization may not be solely attributable to EPC incorporation into new blood vessels, but may also be influenced by the release of angiogenic substances, including VEGF, in the surrounding microenvironment of the ischemic brain.

9. CONCLUSION

There is no doubt that cell therapy may serve as a future restorative therapy for stroke. Further studies are necessary to examine whether stem cells can have a therapeutic role, as supportive cells, a sole treatment and/or as vehicles of gene delivery, and to what extent these cells are capable of neuronal remodeling. In addition, it is still unknown what percentage of transplanted or stimulated progenitor cells differentiate into functional neurons rather than glial cells. Moreover, it remains to be shown how transplanted cells are integrated into neuronal circuits and promote functional recovery, and whether grafts survive for long periods of time.

Recent research has shown that specific types of neurons and glial cells suitable for transplantation can be generated from stem cells in culture. We have also seen that the adult brain produces new neurons from its own stem cells in response to stroke. Although these findings raise hope for the development of stem cell therapies for brain repair after stroke, many basic issues remain to be solved. Before clinical trials with stem cell-based approaches are initiated, we need to know much more about how to control stem cell proliferation and differentiation into specific phenotypes, induce their integration into existing neural and synaptic circuits, and optimize the functional recovery from stroke in animal models.

In summary, further investigation is necessary to understand the possible roles of endogenous BMSCs in organogenesis under normal conditions or in the repair processes of pathological conditions. It has been shown that BMSCs can differentiate into neurons and astrocytes in vivo, indicating that BMSCs are potentially useful as vectors for treating a variety of CNS disorders (87). Although some recent evidence has raised doubt about the issue of trans-differentiation (89), BMSCs have been shown to produce growth factors and neurotrophins that may improve the functions of the impaired brain (4, 40, 91). Therefore, the discovery of molecular signals that mediate BMSC trafficking could contribute to the development of BMSC-mediated cell therapy strategies in terms of facilitating site-specific migration of transplanted stem cells, thereby promoting functional improvement of the diseased or injured brain.

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