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High-Mobility Group Box 1: An Amplifier of Stem and Progenitor Cell Activity After Stroke

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Abstract

Stroke induces a highly complex web of pathophysiology that usually leads to serious long-term disability. Molecules from the damage-associated molecular pattern (DAMP) family immediately increase after stroke. DAMPs are known to cause massive inflammation and brain damage. Thus, they may be targets for neuroprotection. However, emerging data now suggest that DAMPs may not always be detrimental. The high-mobility group box1 (HMGB1) protein is discussed as an example of this idea. During the acute phase after stroke, HMGB1 amplifies neuroinflammation. But during the brain remodeling phase of stroke recovery, HMGB1 can mediate beneficial plasticity and enhance stem and progenitor cell recruitment, proliferation, and differentiation within damaged brain. These emerging findings support the hypothesis that HMGB1 might be an important molecule for regulating stem and progenitor cell therapies in stroke patients.

Keywords

Stroke; HMGB1; Inflammation; Stem cells; Brain remodeling

Introduction

Stroke is the second leading cause of death worldwide and is a very challenging clinical and scientific problem. Therapeutic options for stroke patients are still limited. For example, in ischemic stroke, acute treatments are limited to reperfusion with tissue plasminogen activator or mechanical catheter devices [56]. Many targets and drugs seem exciting at the level of molecular, cellular and animal models. But it has been exceedingly difficult to translate these pre-clinical ideas into clinical efficacy [47, 57].

The biological processes after cerebral ischemia are complicated. Energy deprivation and excitotoxicity are the main causes of the initial tissue damage. In response to initial injury, damage-associated molecular patterns (DAMPs) released into the extracellular micro-environment amplify the secondary processes of blood–brain barrier (BBB) disruption and

post-ischemic inflammation [50]. Nevertheless, damaged brain can be surprisingly plastic, and neurogenesis, angiogenesis, and dendritic and axonal remodeling may all provide substrates for compensation and repair. A paradigm shift from symptomatic control for secondary prevention against stroke to amplifying brain regenerative mechanisms may provide a therapeutic opportunity to improve long-term disability after stroke [57].

As a potential concept in brain regenerative medicine, cell-based therapies may become an exciting new frontier for promoting endogenous brain plasticity after stroke [10, 44]. Accumulating evidence suggests that the recruitment of endogenous stem and progenitor cells or exogenously transplanted cells may support brain remodeling following stroke. Intriguingly, high-mobility group box 1 (HMGB1, a member of the DAMP family) can act as a key regulator of stem cell migration, proliferation and differentiation. In this mini-review, we discuss multi-potential actions of HMGB1 (also known as amphoterin or HMG1) as a “case study” for how endogenous mediators might regulate and influence cell-based stroke therapies.

HMGB1 in the Acute Phase After Stroke

HMGB1, a highly conserved non-histone nuclear DNA-binding protein, is widely expressed in most eukaryotic cells, including neural cells in several animal species including humans [87]. Nuclear HMGB1 is able to bind to DNA to stabilize nucleosome formation and maintains nuclear homeostasis. HMGB1 enables bending of DNA and facilitates transcription to regulate gene expression [66, 69, 89]. Genetic knockout of HMGB1 causes severe energy deficits, and mice die shortly after birth owing to hypoglycemia caused by impaired glucocorticoid receptor [87]. Overall, intracellular HMGB1 plays an essential role in regulating activation of basal transcriptional machinery [20, 83] and energy homeostasis. Although the function of HMGB1 in the nucleus is not quite understood, the role of extracellular HMGB1 has been extensively studied.

Traditionally, HMGB1 has acted as a nuclear and cellular danger signal [50]. HMGB1 exerts different functions depending on its cellular localization. It can be passively released from damaged cells or actively secreted from stimulated cells. Release of HMGB1 is observed after traumatic brain injury and ischemic stroke. In rodent middle cerebral artery occlusion models, levels of HMGB1 in the ischemic core are immediately decreased, and in turn, serum HMGB1 is rapidly increased [24, 36, 70]. In clinical stroke patients, HMGB1 is upregulated in serum up to day 7 after stroke onset [59]. HMGB1 is also increased in cerebrospinal fluid of subarachnoid hemorrhage patients on days 3, 7, and 14 after onset [59]. In addition, plasma HMGB1 in patients is acutely elevated 30 min after severe trauma in comparison to healthy subjects [17]. These findings suggest that HMGB1 might be highly relevant to human stroke.

Extracellular HMGB1 binds to its receptors and activates a downstream pathway that leads to upregulation of cytokines and other pro-inflammatory molecules. HMGB1 acts as and acts in an autocrine/paracrine fashion. It is secreted by activated macrophage [11], natural killer cells [73], and myeloid dendritic cells [19] in response to endotoxin and other inflammatory stimuli. Besides active secretion, HMGB1 can be released into the

extracellular space from damaged cells or necrotic cells [36, 70]. In this case, the membranes of necrotic or damaged cells become “leaky” and HMGB1, which is normally bound loosely to chromatin, diffuses from nucleus to cytoplasm, then into the extracellular matrix. Interestingly, most cytokines start to increase a few hours after stroke [51], while HMGB1 release occurs in the ischemic core of striatum as early as 30 min after artery occlusion [36]. Furthermore, blockade of HMGB1 reduces inflammation and BBB disruption [36, 45, 90]. These results suggest that HMGB1 might be an early mediator that triggers inflammatory cascades.

HMGB1 may signal via its putative receptors, such as receptor for advanced glycation end products (RAGE), toll-like receptor-2 (TLR2), and TLR4. These receptors are expressed in brain cells [13, 27, 79]. RAGE expression is usually low under normal conditions, but enhanced expression of RAGE was observed in diabetic vasculature and other inflammatory diseases [8]. TLR2 and TLR4 have been shown to play a critical role in infectious diseases [79]. Higher constitutive expression levels of TLR4 have been found in brain regions that lack a tight BBB, such as the circumventricular organs and choroid plexus [40, 54]. Activation of HMGB1 receptors leads to activation of NF- κ B and MAP kinase [29]. Blockade of either RAGE or TLR4 results in a reduction of cytokine and nitric oxide production and a decrease in inflammation [36, 58, 79], suggesting that HMGB1 might potentially contribute to the induction of inflammation.

HMGB1 in Recovery and Remodeling

Most stroke patients show some degree of recovery over time. Damaged brain can be surprisingly plastic, and cross-talk between various types of remodeling brain cells takes place after brain injury [15, 16]. In the late phase after cerebral ischemia, the generation of new blood vessels underlies a highly coupled neurorestorative process that links neurogenesis, synaptogenesis, and angiogenesis [77, 92]. These phenomena are clinically relevant – markers of neurogenesis and angiogenesis have been observed in the adult human brain [39, 53].

In recent years, emerging data suggest that neurovascular repair might be induced by dynamic interactions among cerebral endothelial cells, glia, neurons, and extracellular matrix from days to weeks after stroke [3]. Of these neuro-vascular cells, astrocytes are the most numerous nonneuronal cell type in the mammalian brain [81]. Traditionally, it was assumed that reactive astrocytes after stroke or brain injury contribute to glial scarring, which impedes neuronal remodeling and recovery [75]. However, it is now recognized that reactive astrocytes may also release many trophic factors, such as nerve growth factor, basic fibroblast growth factor, platelet-derived growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, neuropilin-1, vascular endothelial growth factor, and others [55, 76, 80]. Many of these trophic factors may in fact be beneficial in that they promote neuronal survival and synaptogenesis, neurogenesis, and angiogenesis after stroke or brain injury [26, 65].

In contrast to its negative effects, as described in the earlier sections, HMGB1 may also possess beneficial actions. HMGB1 signaling can promote endothelial activation [82] and

sprouting [72], and it has also been reported that HMGB1 may increase neurite outgrowth and cell survival in neurons [30, 31, 67]. Intriguingly, stimulated astrocytes have been shown to induce and release HMGB1 protein into the extracellular medium [23, 67]. In a recent study, we showed that low levels of IL-1 β potentially upregulate HMGB1 in astrocytes. The prototypical MAP kinase ERK provided the upstream signal. The nuclear export protein chromosome region maintenance 1 (CRM1) enabled nuclear HMGB1 to translocate from nucleus to cytoplasm before release [23]. In vivo study, HMGB1 immunoreactivity was also observed in reactive astrocytes, which are concentrated in the ischemic penumbra in an in vivo ischemic model [35]. Importantly, metabolic inhibition of reactive astrocytes suppressed HMGB1-positive astrocytes in peri-infarct cortex, and that disrupted various neurovascular markers, such as CD31, synaptophysin, and PSD95, which correlated with functional recovery after cerebral ischemia in a mouse focal ischemia model [25]. These findings suggest that reactive astrocytes produce HMGB1, which may promote neurovascular repair in brain after stroke.

Stem and Progenitor Cells Research in Stroke

Over the past two decades, cell-based therapy has been widely proposed as a way of repairing damaged brain after stroke. A variety of cell types, such as hematopoietic stem cells (HSCs) [12, 78], mesenchymal stem cells (MSCs) [6, 85, 86], endothelial progenitor cells (EPCs) [21, 88], very small embryonic-like cells (VSELs) [71], and neuronal stem cells (NSCs) [2, 7, 74] have all been demonstrated to show therapeutic potential in stroke. More recently, human umbilical tissue-derived stem cells (hUTCs) [43, 91] and induced pluripotent stem cells (iPSCs) [33, 34] have been considered to be a candidate source of cells for restorative therapy in stroke. The strategies for stem cell therapy show that different stem cells and their derivatives of rodent and human origin can survive, differentiate into neurons, astrocytes and oligodendrocytes, and induce brain remodeling after stroke (Fig. 1) [44].

Emerging data suggest that stem cells might repair the damaged brain by increasing blood supply by the creation of new vessels, reducing inflammation and ongoing brain cell death, enhancing endogenous repair mechanisms. For example, intravenous injection of human NSCs has been shown to induce improvements after hemorrhagic stroke in rats through anti-inflammatory actions [41]. Intravenously administered human MSCs have been found to reduce stroke-induced deficits in rats by inducing angiogenesis and improving cerebral blood flow [61]. Human ES cell-derived MSCs injected intravenously in rats have been shown to migrate to the infarct region, express neuronal and endothelial cell markers, provide neuroprotection, and improve recovery [46]. Importantly, mouse NSCs delivered intravenously 3 days after stroke in mice have been shown to suppress inflammation and glial scar formation and give rise to delayed neuroprotection and improved functional recovery, starting 18 days after the insult [4]. Clinical reports of MSC transplantation in stroke patients reveal that MSCs may improve the functional recovery of patients without adverse side effects [5]. Accumulating evidence now suggests that stem and progenitor cell-based therapy might hold promise for regenerative medicine, even beyond stroke per se.

A few initial clinical trials delivering stem cells in stroke have been completed [48]. For example, an immortalized human teratocarcinoma cell line implanted into ischemic/hemorrhagic infarcts affecting the basal ganglia and in some cases also the cerebral cortex [37, 38, 60] – these effects may be related to slight improvements in some patients. No substantial clinical improvements were detected after intravenous injection of autologous MSCs in patients with an ischemic lesion in the territory supplied by the middle cerebral artery [5]. Several clinical studies using intravenous or intra-arterial (into damaged territory) infusion of autologous bone marrow-derived stem cells in stroke patients are ongoing or planned (www.clinicaltrials.gov). The company ReNeuron, based in the United Kingdom, is planning a clinical trial in stroke patients involving transplantation of clonal, conditionally immortalized NSCs isolated from human fetal cortex.

Role of HMGB1 on Various Stem and Progenitor Cells Activation

HMGB1 has also had a beneficial effect under a number of pathological conditions in terms of its ability to recruit stem cells and promote their proliferation [62]. HMGB1 was found to enable endothelial progenitor cells to home to ischemic muscle in animal models of hind limb ischemia [14]. If HMGB1 was injected into the infarct area of the heart, it promoted tissue regeneration, and a significant recovery of cardiac performance was indeed mediated by RAGE signaling [22, 42]. More recently, it was reported that endogenous HMGB1 was crucial for ischemia-induced angiogenesis and that HMGB1 protein administration enhanced collateral blood flow in the ischemic hind limbs of diabetic mice through a VEGF-dependent mechanism [9]. It is now recognized that HMGB1 may have beneficial effects on tissue regeneration and remodeling through the recruitment of stem and progenitor cells.

Recent findings suggest that HMGB1 might be a key regulator for MSC activity [68]. It has been demonstrated that HMGB1 not only attract MSCs, but also enhances the proliferation of MSCs and inhibits the ability of MSCs to produce the IFN- γ -induced immunosuppressive molecule indoleamine-2,3-dioxygenase (IDO). These phenomena mediated by HMGB1 were neutralized by specific polyclonal antibodies to HMGB1 or by the soluble receptor for advanced glycation end products (sRAGE) [49]. On the other hand, others reported that HMGB1 inhibited the proliferation of MSCs, but promoted migration and differentiation into osteoblasts. Moreover, HMGB1-treated MSCs displayed unchanged suppressive activity on in vitro lymphocyte cell proliferation elicited by ConA [52]. Hence, there may also be involvement of TLR2 and/or TLR4 receptors in the stimulation of MSC migration mediated by HMGB1 [52]. Indeed, MSCs may be a target of HMGB1 signaling, but many more studies are needed to properly define the mechanisms involved.

In terms of connections between brain and blood, HMGB1 is also an essential factor of non-cell-autonomous enhancement in hematopoietic stem cell (HSC) activity [18]. HMGB1 released by MSCs after treatment with 12 Gy gamma-ray irradiation induced the proliferation and differentiation capacities of cord blood hematopoietic stem cells. This effect was partially inhibited by TLR2 and TLR4 antibodies [84]. In addition, HMGB1 promoted endothelial progenitor cells (EPCs) to home to ischemic muscle in animal models of hind limb ischemia [14]. HMGB1 increased EPC adhesion to the immobilized integrin ligands intercellular adhesion molecule-1 and fibronectin in a RAGE-dependent manner,

suggesting that HMGB1-RAGE signaling in EPCs might be a novel target for improving vascular remodeling after stroke.

Migration of mesoangioblast stem cells may also involve HMGB1. Full-length HMGB1 and a fragment that lacks only the acidic tail chemoattracts mesoangioblast stem cells in a concentration-dependent manner [64], whereas the individual boxes (box A or box B), or both boxes (box A + B) had no significant chemotactic effect, suggesting that the segment from the end of box B to the beginning of the acidic tail (amino acids 165–185) might be necessary for the chemotactic effect in mesoangioblast stem cells [64]. Interestingly, this segment significantly corresponded to the surface recognized on HMGB1 by the RAGE [28, 32]. In transmigration assay of mesoangioblast stem cells, HMGB1 in the lower compartment induced a higher number of mesoangioblasts to migrate across the endothelial monolayer and filter than in the presence of VEGF [62]. In vivo migration assay also showed that HMGB1 could chemoattract both mesoangioblast stem cells and DiI-labeled lin^- hematopoietic stem cells [62]. The cell signaling of HMGB1-induced mesoangioblast migration may be involved in the nuclear factor kappaB pathway via extracellular signal-regulated kinase phosphorylation [63].

What makes HMGB1 particularly important is its pleiotropic actions in harnessing the power of stem and progenitor cells. Multiple cell sources may be affected. In zebrafish brain development, HMGB1 may be a critical factor enabling survival and proliferation of neural progenitors in amphotericin-induced gene and orf (AMIGO) dependent manner [93]. Neuronal progenitors have the capacity to replace lost neurons and enhance neurogenesis, angiogenesis, axonal sprouting, and synaptogenesis. HMGB1 multipotency may contribute to neuronal regeneration by enhancing the potential of neuronal progenitor cells after stroke.

Perspective for Stem and Progenitor Cells Therapy in Stroke

To date, cell-based approaches have been extensively investigated as potential treatments for neurodegenerative disorders. Indeed, transplantation of stem cells or their derivatives in animal models of neurodegenerative diseases can improve function by replacing the lost neurons and glial cells and by mediating remyelination, trophic actions, and modulation of inflammation. Endogenous stem and progenitor cells are also potential therapeutic targets for producing neurons, glial cells, and endothelial cells in response to injury. However, in order to translate stem cell therapy into clinical reality, many hurdles still remain. For example, it is necessary to determine the optimal cell types to use. Perhaps even more importantly, we need to learn how to control/regulate the proliferation, survival, migration, differentiation, and functional integration of transplanted and/or endogenous cells or factors (i.e., HMGB1) in the damaged brain after stroke. Ultimately, however strong the biology, we may also need to develop procedures and criteria for cell delivery, scaling up, appropriate manufacturing and quality control of target cells, optimal functional recovery, and patient selection and assessment [1]. Clearly, longer-term studies are needed to translate cell transplantation therapy to the clinic in a timely but safe and effective manner so that the remarkable potential already shown for cell transplantation to aid recovery from experimental stroke can become a reality for the patients [10].

Conclusion

Extensive data from molecular, cellular, and animal models support the existence of a potent pro-inflammatory effect of HMGB1 in acute ischemic stroke. In fact, therapeutically beneficial effects of monoclonal HMGB1 antibody have been described in several animal models of cerebral ischemia. However, it is now important to realize that HMGB1 might possess biphasic actions during stroke, brain injury, and neurodegeneration. During delayed phases of stroke recovery, extracellular HMGB1 may not only enhance the crosstalk among neurovascular cells such as neurons, astrocytes, and endothelial cells, but may also contribute to stem cell migration into the ischemic region and promote stroke recovery through neurogenesis, vasculogenesis, and angiogenesis. Ultimately, a more nuanced approach to modifying the HMGB1 response after stroke may be needed in order to optimize inhibition during acute stages of injury without interfering with the beneficial mechanisms of neurovascular remodeling driven by stem and progenitor cells.

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Stem cell therapy

- (a). Neuron precursors or neuroblasts generated from stem cells
- (b). Compounds to enhance endogenous neurogenesis and angiogenesis
- (c). Stem cells for neuroprotection and modulation of inflammation

Fig. 1.

Overall strategy of cell-based therapies for stroke. Stroke induces multiple cell death in neurons, astrocytes, oligodendrocytes, and endothelial cells in the cortex and subcortical regions. **(a)** Stem cell-based therapy could be used to repair damaged tissue by transplanting neuronal stem cells/multipotent stem cells. **(b)** Exogenous molecules could be infused to promote neurogenesis, axonal regeneration, and angiogenesis. **(c)** Stem and progenitor cells could be injected systemically for neuroprotection and modulation of inflammation. HMGB1 provides an example of how molecular regulation of these cell therapies may be critically important in optimizing these approaches for clinical utility