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PERSPECTIVES

Cell-Based and Exosome Therapy in Diabetic Stroke

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SUMMARY

Stroke is a global health concern and it is imperative that therapeutic strategies with wide treatment time frames be developed to improve neurological outcome in patients. Patients with diabetes mellitus who suffer a stroke have worse neurological outcomes and long-term functional recovery than nondiabetic stroke patients. Diabetes induced vascular damage and enhanced inflammatory milieu likely contributes to worse post stroke outcomes. Diabetic stroke patients have an aggravated pathological cascade, and treatments that benefit nondiabetic stroke patients do not necessarily translate to diabetic stroke patients. Therefore, there is a critical need to develop therapeutics for stroke specifically in the diabetic population. Stem cell based therapy for stroke is an emerging treatment option with wide therapeutic time window. Cell-based therapies for stroke promote endogenous central nervous system repair and neurorestorative mechanisms such as angiogenesis, neurogenesis, vascular remodeling, white matter remodeling, and also modulate inflammatory and immune responses at the local and systemic level. Emerging evidence suggests that exosomes and their cargo microRNA mediate cell therapy derived neurorestorative effects. Exosomes are small vesicles containing protein and RNA characteristic of its parent cell. Exosomes are transported by biological fluids and facilitate communication between neighboring and remote cells. MicroRNAs, a class of naturally occurring, small noncoding RNA sequences, contained within exosomes can regulate recipient cell's signaling pathways and alter protein expression either acting alone or in concert with other microRNAs. In this perspective article, we summarize current knowledge and highlight the promising future of cell based and exosome therapy for stroke and specifically for diabetic stroke. STEM CELLS TRANSLATIONAL MEDICINE *2018;7:451–455*

SIGNIFICANCE STATEMENT

Patients with diabetes mellitus who suffer a stroke have worse neurological outcomes and long-term functional recovery than nondiabetic stroke patients. Given the differential challenges of treating strokes in diabetics compared to nondiabetics, this perspective summarizes current knowledge and highlights promising cell based and exosome therapy for diabetic stroke.

INTRODUCTION

Ischemic stroke and diabetes mellitus (DM) are both major and global health issues. DM is a strong predictor of ischemic stroke incidence, particularly in DM patients younger than 65 years of age [1]. Approximately 30% of stroke patients have DM [2]. Stroke is a known leading cause of death and disability, and DM stroke patients battle higher mortality rates, worse neurological functional deficits, and higher risk of recurrent strokes which hinders their long-term recovery and return to independent living compared to non-DM stroke patients [3–5]. Comorbidity of stroke with DM induces exacerbated microvascular and macrovascular damage, which can have a profound impact on multiple organs, as well as aggravate the pathological cascade after stroke [6–10]. While both diabetic men and women face high risk of stroke, higher Hba1c levels in men, and microvascular complications in women, were found to particularly increase stroke incidence [11]. Pre-clinical studies demonstrate that rodents with DM subjected to stroke exhibit worse neurological outcome, increased blood brain barrier (BBB) dysfunction, white matter damage, and inflammatory responses in the ischemic brain compared to stroke in non-DM animals, as summarized in Figure 1 [6–10]. Therefore, developing treatments specifically for DM stroke is necessary and challenging.

Tissue plasminogen activator (tPA) remains the only pharmacological agent approved by the FDA to treat ischemic stroke. Unfortunately, a significant majority of stroke patients do not receive tPA within the therapeutic time window of 3–4.5 hours from stroke onset [6, 9]. Diabetic stroke rats exhibit resistance to thrombolytic reperfusion, and are susceptible to developing intracerebral hemorrhage [12, 13]. Several novel therapeutics that improve functional outcome and promote neuroprotection in non-DM stroke have failed to yield similar outcomes when tested

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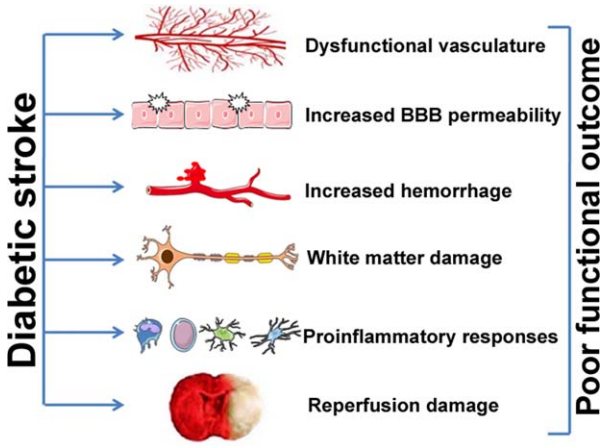

Figure 1. Diabetes exacerbates stroke pathology and results in poor neurological functional outcome. Abbreviation: BBB, blood brain barrier.

in DM-stroke animals as well as in human clinical trials [14, 15]. Given the differential challenges of treating strokes in diabetics compared to nondiabetics, this review summarizes current knowledge and highlights of promising cell based and exosome therapy for diabetic stroke.

Cell-Based Therapies and Exosome Therapy for Diabetic Stroke

Cell-Based Therapy

Neurorestorative therapies using stem cell based and stem cell derived exosomes hold promise as either stand-alone or as combination treatments with pharmacological agents to improve stroke outcome in non-DM and DM patients. Cell based and stem cell derived exosome therapy also has therapeutic benefits in other neurological diseases such as traumatic brain injury, which has been reviewed previously [16]. Since DM stroke induces extensive neural and vascular damage, it is critical for therapeutic interventions to promote remodeling of the neurovascular unit, which fundamentally describes the structural and functional interactions between neurons, capillaries and glia in the brain. Pre-clinical studies in animal models of stroke and DM stroke have shown that cell therapies have long treatment time windows ranging from several hours to days after stroke onset and improve neurological functional outcome by amplifying endogenous brain repair mechanisms such as neurovascular remodeling, white matter remodeling, and attenuating local and systemic inflammatory and immune responses [17–23].

Several types of stem/progenitor cells from different sources have been investigated in preclinical studies to test feasibility, efficacy, and mechanisms of therapeutic effects in stroke. There are a number of sources for stem cells such as mesenchymal stromal cells (MSCs), human umbilical cord blood cells (HUCBCs), induced pluripotent stem cells, neural stem cells and embryonic stem cells, with the advantages and disadvantages of each discussed elsewhere [24, 25]. In this brief review, we will discuss the therapeutic effects of bone marrow derived MSCs (BMSCs), HUCBCs and exosomes derived from BMSCs and HUCBCs in diabetic stroke due to the ease of harvesting exosomes from these cells, lack of ethical barriers and clinical trials indicating safety, therapeutic efficacy and feasibility of employing BMSCs and HUCBCs in patients with other diseases.

Exosome Therapy for Diabetic Stroke

Exosomes are nanosized vesicles (~30–100 nm in diameter) which facilitate intercellular communication and are capable of regulating cell function by delivering proteins, lipids, and nucleic acids. Over the last few years, exosomes have emerged as a major mediator of cell therapy derived therapeutic benefits, personalized targeted drug delivery vehicles, as well as a biomarker and promising treatment option for several neurological diseases [26–29]. Transplanted stem cells and exosomes stimulate host brain parenchymal cells to generate a plethora of cytokines, growth factors and trophic factors which promote endogenous brain repair mechanisms while suppressing apoptotic signaling and inflammatory responses [27], as summarized in Figure 2. As a result, functional recovery following cell based and exosome therapy is often observed as early as several days after treatment. Employing exosomes as therapeutic agents has several advantages over cell therapy. Exosomes have no vascular obstructive effect, low risk of secondary microvascular thrombosis and have a low risk of tumor formation. Favoring clinical translation, a large quantity of exosomes can be derived from a small quantity of cells; exosomes are stable and can be stored; exosomes can pass the BBB; and exosomes do not elicit immune rejection. Exosomes mediate benefit by transferring genetic instructions, often via microRNA to concurrently stimulate and activate multiple restorative pathways [28, 29]. Therefore, by modifying microRNA content, exosomes can be programmed to target specific restorative and protective pathways within recipient and target cells and systemic administration of exosomes may be a means to deliver designer genetic instructions as well as the active components of cell-based therapy to the central nervous system [27].

MSCs and MSC-Exosome Therapeutic Effects

MSCs are constituted by a heterogeneous collection of mesenchymal stem and progenitor cells that can differentiate into osteoblasts, adipocytes, chondroblasts, myocytes, and neurons [30]. In vitro, exposure of human BMSCs to ischemic rat brain tissue increases their secretion of growth factors, and in vivo intravenous administration of BMSCs in rodents, induces a time dependent release of neurotrophins and angiogenic growth factors like brain-derived neurotrophic factor, vascular endothelial growth factor (VEGF), nerve growth factor, hepatocyte growth factor, and glial cell derived neurotrophic factor [31–33]. In non-DM rodents, intravenous, intra-arterial, intra-carotid or intra-striatal administration of BMSCs at 1 or 7 days after stroke improves functional outcome, enhances synaptogenesis, stimulates nerve regeneration, mediates immunomodulatory effects, and reduces inflammation [34–37]. Human BMSCs administered intravenously at 3 days after stroke in type 2 DM rats, significantly improves neurological function, increases neurovascular remodeling and decreases inflammatory responses without increasing BBB leakage or cerebral hemorrhage [23]. However, intravenous administration of BMSCs at 1 day after stroke in type 1 DM rats does not improve neurological function, and instead increases brain hemorrhage and BBB leakage [15, 21]. These adverse effects have since been attributed to an early and acute role of VEGF signaling and treatment initiation time point. Exogenously administered MSCs can secrete VEGF [32], as well as stimulate brain parenchymal cells such as astrocytes to secrete VEGF [31], and while VEGF plays an

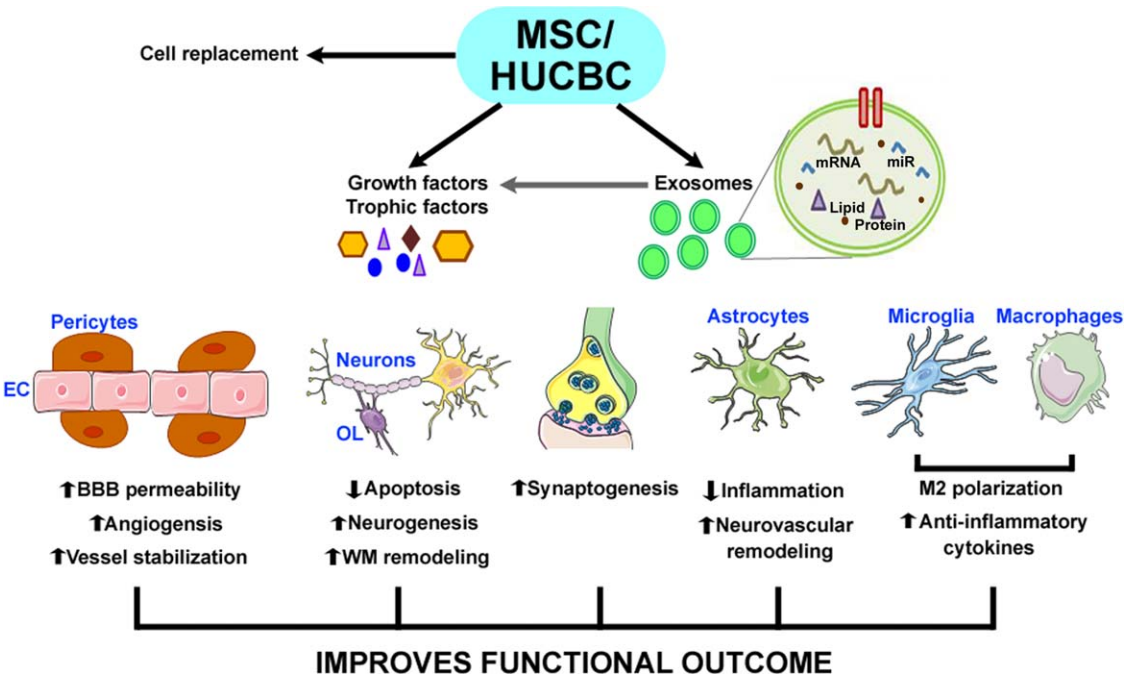

Figure 2. Mechanisms of cell-based and exosome therapy induced neurorestorative effects after stroke in diabetic rodents. Abbreviations: BBB, blood brain barrier; EC, endothelial cell; HUCBC, human umbilical cord blood cells; MSC, mesenchymal stromal cell; OL, oligodendrocyte.

important role in mediating angiogenesis, in the acute phase of stroke in non-DM rats, administration of VEGF increases cerebral microvascular perfusion, BBB permeability, hemorrhage, and infarction volume [38]. Astrocyte derived VEGF-A has been implicated in promoting BBB disruption in the acute inflammatory lesions of multiple sclerosis in mice [39]. The adverse effects of VEGF are likely to be aggravated in DM stroke due to the extensive vascular damage induced by DM and stroke [40]. However, at delayed time points such as 48 hours after stroke, VEGF administration was found to enhance angiogenesis in the ischemic penumbra and contribute to neurological recovery in non-DM rats [38]. Compared to non-DM rats, in the ischemic brain of type 2 DM rats VEGF follows a trend of increase by day 1 and decrease by day 3 after stroke [23]. In concert, these studies indicate that treatment initiation time point is critical and requires optimization when treating DM stroke. MSC treatment in DM stroke rats was found to increase cerebral artery wall thickness while decreasing artery internal diameter indicating that MSC treatment may increase atherosclerosis-like vascular changes [15, 23].

Treatment with MSC derived exosomes at 24 hours after stroke in non-DM rats, was at least equivalent to the therapeutic effects of MSCs, and MSC-exosome treatment improves functional recovery, enhances neurite remodeling, neurogenesis, and angiogenesis in the ischemic brain [27]. In type 2 DM rats subjected to stroke, treatment with exosomes derived from bone marrow of type 2 DM rats initiated 3 days after stroke, was found to significantly improve long-term functional recovery, decrease BBB leakage and hemorrhage and increase white matter remodeling [41].

MicroRNAs play vital roles in stem cell function and therapeutics. MicroRNAs are a class of naturally occurring, small noncoding RNA sequences. MicroRNAs contained within exosomes are transported to both neighboring and remote sites via body fluids, and exosomal microRNA can alter multiple genes and signaling pathways in target cells by acting either alone or in concert with other microRNAs [42]. Therefore, tailored exosomes with enriched beneficial microRNA can potentially enhance the therapeutic effects of stem cell derived exosomes for treatment of stroke and other diseases. In vitro studies show that microRNA-17–92 cluster promotes oligodendrogenesis, neurogenesis, and axonal outgrowth [28]; and miR-133b transferred via exosomes to astrocytes and neurons increases neurite outgrowth [43]. In addition, treatment of stroke with exosomes derived from miR-133b-overexpressed MSCs as well as miR-17–92 cluster enriched exosomes were found to significantly improve brain plasticity and post stroke functional outcome compared to treatment with MSC derived exosomes [28, 29]. Optimizing the microRNA content of MSC derived exosomes to maximize therapeutic potential is of prime interest.

HUCBCs and HUCBC Derived Exosome Therapy of Diabetic Stroke

HUCBCs are a rich source of hematopoietic, mesenchymal and neural stem/progenitor cells, are easily obtainable without any ethical concerns; and have low risk of graft-versus-host disease. Non-DM rodents subjected to stroke and treated with intravenous HUCBC therapy 1, 7 or even 30 days after stroke exhibit significant functional recovery [44, 45]. HUCBCs administered intravenously to type 1 and type 2 DM rats and mice at 1 or 3 days after stroke significantly promotes functional recovery [17–19]. HUCBC treatment after DM stroke also significantly increases the expression of Angiopoietin-1, an important protein in the regulation of angiogenesis, vascular maturity and stabilization [19]. HUCBC derived therapeutic effects are mainly derived from stimulation of endogenous brain repair mechanisms via parenchymal cell stimulation and release of trophic factors and modulation of inflammatory responses such as attenuating pro-inflammatory T helper cell type 1 (Th1) response, amplifying anti-inflammatory T-helper 2 (Th2)

response, decreasing pro-inflammatory and increasing anti-inflammatory cytokines, and macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 phenotype [18, 19, 46]. In the ischemic brain parenchyma of rats subjected to stroke, HUCBC treatment significantly decreases infiltration of granulocytes and monocytes, and decreases astroglial and microglial activation [47]. HUCBC treatment after stroke can also modulate peripheral immune responses and rescue stroke induced gross spleen size and CD8+ T-cell number decrease which correlates with the extent of ischemic injury to the brain [48].

DM and stroke both alter microRNA-126 expression which regulates angiogenesis and endothelial cell function. Type 2 DM has been associated with decreased endothelial cell expression of microRNA-126 [49]. Stroke in type 2 DM mice significantly decreases microRNA-126 expression in blood serum and ischemic brain tissue compared to non-DM stroke mice [17]. In type 2 DM mice subjected to stroke, HUCBC treatment significantly increases microRNA-126 expression in serum as well as in ischemic brain tissue compared to non-DM stroke mice, while treatment with microRNA-126 knockdown HUCBCs drastically attenuates the therapeutic effects of HUCBCs; indicating that microRNA-126 substantially contributes to HUCBC derived therapeutic effects in DM stroke [17]. MicroRNA-126 is primarily expressed in endothelial cells. We found that exosomes derived from brain endothelial cells contain higher levels of miR-126 than exosomes derived from other types of cells such as smooth muscle cells, neurons, astrocytes and MSCs [50]. In type 2 DM stroke mice, brain endothelial cell derived exosome treatment increases brain and serum miR-126 expression, and significantly improves neurological outcome, cognitive function, and axon, myelin and vessel density [50]. Therefore, identifying key microRNA involved in meditating neurorestorative events after stroke and modulating microRNA content of exosomes by manipulation of parent stem cells, can potentially amplify the therapeutic effects of exosomes for the treatment of stroke.

Summary and Future Directions

Cells-based and exosome therapies are powerful tools to promote endogenous brain repair mechanisms after stroke. Cell and exosome therapy using BMSCs and HUCBCs are promising treatment options for DM stroke with low ethical barriers, wide treatment time frames, and high translational feasibility. Some of the challenges of cell therapy such as finding matching donor, low yield and adverse effects such as thrombosis, may be overcome by employing exosomes for stroke treatment. Largely, exosome therapy appears to be safe without adverse effects; however, exosomes may facilitate intercellular membrane exchange and the spread of infectious agents like prions [51].

The content of exosomes consisting primarily of proteins, non-coding RNAs, and lipids vary depending on the donor cells and cell culturing and exosome harvesting conditions. Since the therapeutic efficacy of exosomes depend on the intercellular transfer of their content, and microRNAs appear to play an important role in mediating exosome function, optimization of donor cell cultures and characterization of exosome content, particularly that of the key microRNAs mediating therapeutic benefits are warranted. The effects of exosome therapy on immune system need to be fully understood. To facilitate clinical translation, high purity, low cost, and large scale exosome isolation techniques need to be developed.

Two clinical trials have reported therapeutic benefits of administering culture-expanded autologous MSCs in patients with ischemic stroke compared to placebo control group [52, 53]. Another clinical trial reports that stereotactic placement of modified MSCs (SB623) at the margin of stroke in patients with chronic motor deficits at >6 months after their initial stroke was safe and improved clinical outcome at 12 months after stroke [54]. However, the study groups of these trials were small and large clinical trials to evaluate the safety and efficacy of MSCs (NCT01922908), adipose tissue derived MSCs (NCT01678534), HUCBCs (NCT02580019, NCT02397018, NCT03004976), MSC-exosomes enriched by miR-124 (NCT03384433) for treatment of stroke and intracerebral hemorrhage (NCT03371329) are still at a preliminary stage.

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Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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