Chapter 8
Stem Cell-Paved Biobridge: A Merger of Exogenous and Endogenous Stem Cells Toward Regenerative Medicine in Stroke

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Abstract Stroke is a significant unmet clinical need with therapeutic options limited to tissue-type plasminogen activator (tPA), which has a small therapeutic window and risk for hemorrhagic transformation. Stroke is a multiphasic disease with a complex pathology. After the initial insult, a cascade of events occur causing secondary cell death and the expansion of the penumbra. The major contributing factors to this secondary cell death are depletion of growth factors, neuroinflammation, and disruption of the neurovascular unit. There is a need for more innovative and effective therapies that can target the diverse pathological consequences of stroke. To this end, stem cell therapy is a promising approach for stroke. Pre-clinical studies have demonstrated the potential of stem cells for treating neurological disorders, including stroke. Here, we discuss diverse stem cell types which have generated encouraging results for advancing to the clinic. Then, we examine the mechanisms of action of stem cells—cell replacement, by stander effect, and a novel biobridge concept advanced by our laboratory. These mechanisms work in concert to afford the neuroprotection and neuroregeneration after stroke. We envision that an in-depth understanding of the benefits and drawbacks of various stem cells and their mechanisms of action will guide the translational entry of stem cell therapy from the laboratory into the clinical setting.

Keywords Adult-derived stem cells • Ischemia pathology • Stem cell mechanisms • Stem cell migration • Neuroregeneration • Neuroprotection • Extracellular matrix remodeling • Stem cell therapies • Translational research

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Abbreviations

BDNF	Brain-derived neurotrophic factor
BM-MSC	Bone marrow-derived mesenchymal stem cells
CCI	Controlled cortical impact
ECM	Extracellular matrix
EGF	Epithelial growth factor
FGF	Fibroblast growth factor
GDNF	Glial cell line-derived neurotrophic factor
hBMSCs	Human bone marrow stromal cells
IA	Intra-arterial
IC	Intracranial
IL-1β	Interleukin-1-beta
iPSCs	Induced pluripotent stem cells
IV	Intravenous
MCAO	Middle cerebral artery occlusion
MMP	Metallomatrix protein
NGF	Nerve growth factor
NSCs	Neural stem cells
SCF	Stem cell factor
SDF-1	Stromal-derived factor 1
SGZ	Subgranular zone
SVZ	Subventricular zone
TBI	Traumatic brain injury
TNF-α	Tumor necrosis-alpha
tPA	Tissue-type plasminogen activator
VEGF	Vascular endothelial growth factor

1 Introduction

Stroke continues to be a leading cause of death and disability in America, with approximately 800,000 people being affected annually [1]. Responsible for roughly 5% of American deaths—the fifth leading cause [2]—long term consequences for stroke survivors can range from mild functional impairments to severe disability [1]. Accounting for healthcare costs and loss of productivity, an economic burden of $33.6 billion is attributed to stroke, with this figure projected to increase in the future [1]. In fact, the economic burden of stroke has increased notably in recent years, largely due to improved treatment protocols and a resulting decreased mortality rate [1]. Despite posing such a prevalent medical and economic burden, therapeutic options for stroke have been limited to tissue-type plasminogen activator (tPA) and physical therapies to alleviate symptoms. Unfortunately, the clinical benefits of tPA are minimized by its narrow therapeutic window, with the risk of

hemorrhagic transformation rising sharply and its efficacy decreasing significantly over the initial 1–6 h timeframe [3–5]. As a result, the search for innovative and effective therapies which maintain their therapeutic value over the acute, sub-acute, and chronic pathological stages of stroke continues.

Stem cell therapies have been explored as a possible treatment to this unmet clinical need, having demonstrated both neuroprotective effects in the acute stage, as well as regenerative capacity in later stages of stroke [6–10]. Furthermore, stem cell therapies offer unique advantages over traditional pharmaceuticals by providing a dynamic and adaptive therapeutic profile—a likely requisite for any intervention capable of providing substantial functional recovery from the complex neurodegenerative pathology of stroke [11–15]. Apparent from the completed clinical trials of stem cell transplantation is their relative safety via both intracerebral and intravenous administration [16] (NCT01501773, NCT00535197, NCT00859014, NCT01716481). Unfortunately, clearly demonstrating their efficacy has proven more difficult due to a number of practical difficulties in outcome measurements, patient enrollment numbers, and trial design [17, 18]. As a result, basic and translational laboratories have engaged in a concerted effort to better understand the mechanisms by which stem cells offer their therapeutic effects in the hopes of inspiring more successful clinical trials. Following the recent *in vitro* and *in vivo* studies of our laboratory, we have proposed a third mechanism by which stem cells convey therapeutic effects, the *biobridge*, which works cooperatively with the two well-established mechanisms of cell replacement and bystander effects (secretion of trophic factors, cytokines, and anti-inflammatory molecules, among others) [19–21]. This novel mechanism, whereby transplanted stem cells assist the migration of endogenous stem cells from neurogenic niches in the subventricular zone (SVZ) and the subgranular zone (SGZ) to the region of damaged tissue via extracellular matrix remodeling, was demonstrated in a controlled cortical impact (CCI) model of traumatic brain injury (TBI) [21]. Here, we expand this concept by revealing preliminary data which indicate the formation of a similar structure in the middle cerebral artery occlusion (MCAO) model of ischemic stroke. When contemplating the clinical feasibility of cell-based therapies for the treatment of stroke, the biobridge concept advances the notion that transplanted stem cells can work in synchrony with endogenous stem cell repair mechanisms. This provides a clearer understanding of the mechanisms by which stem cells confer their therapeutic benefits, and also supports their safety by demonstrating that long-term effects generated by cell therapy may not require transplanted stem cell survival per se, but rather endogenous stem cells can subsequently continue the regenerative process despite non-survival fates of the grafted cells.

2 The Many Facets of Stroke Pathology

Stroke is defined as a pathological state whereby a reduction in blood flow effects one or more regions of the brain, which may be caused by an obstructed vessel resulting in ischemic stroke or a ruptured blood vessel, leading to hemorrhagic

stroke [1]. Ischemic stroke is more common and has a lower mortality rate [1]. The cells that directly lose their supply of glucose and oxygen die quickly, as neurons are exceedingly sensitive to metabolic stress. This ischemic tissue region comprises the infarct core; these cells are vulnerable to primary cell death processes and are less amenable to therapeutic intervention [22, 23]. Oxygen and nutrient deprivation causes mitochondrial damage and an increase in reactive oxygen species, both of which contribute to cell death cascades [22]. Additionally, without proper energy supply, the cell membrane is no longer able to uphold ionic homeostasis, which drives improper calcium ion concentrations within the cell, further contributing to cell death pathways [24]. The acute damage to these cells ultimately leads to cell death, with little opportunity for intervention.

Despite stroke being an acute event, the resulting pathophysiology of this event persists chronically, a product of a phenomenon known as secondary cell death [23]. The necrotic cells within the infarct core leave in their wake a toxic microenvironment. Leaked substances from these cells have the capacity to reach adjacent healthy cells and cause harm [23]. For example, following stroke, high levels of glutamate are released into the microenvironment and reach concentrations that lead to excitotoxicity in neighboring cells [23]. This region of cells susceptible to secondary cell death is referred to as the penumbra. Researchers often focus on this region of cells due to a higher likelihood of restoration and a wider therapeutic window. Unlike the infarct core, the penumbra is not fixed—this region of secondary cell death may continue to expand over weeks, months and even years [25, 26].

There are many components contributing to secondary cells death after stroke including depleted growth factors, neuroinflammation, and blood-brain barrier (BBB) breakdown [27–30]. Appropriate growth factor levels within the microenvironment must be sustained for cell survival, with loss of these factors resulting in apoptosis. Several types of growth factors contribute to neuron homeostasis including, but not limited to, glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), stromal-derived factor 1 (SDF-1), epithelial growth factor (EGF), and stem cell factor (SCF). Highlighting their importance, preclinical studies have displayed neuroprotective benefits using GDNF, BDNF VEGF, SDF-1, and SCF treatments following cerebrovascular injury [30].

The neuroinflammatory response after stroke is a double-edged sword. While inflammation plays an important neuroprotective role in the acute phase, chronic inflammation perpetuates secondary cell death [22]. The neuroinflammatory process is triggered by damage-associated molecular patterns (DAMPs) propagated by dying and dead cells. Some of the DAMPs are high mobility group box-1 (HMGB1), heat shock proteins, and hyaluronan [31]. Once the inflammation process is initiated, the vulnerable cells within the penumbra are activated and secrete pro-inflammatory cytokines including tumor necrosis-alpha (TNF-α), interleukin-1-beta (IL-1β), and interleukin-6 (IL-6), among others [30]. This stroke-induced inflammatory response further exacerbates cell death and BBB breakdown. The BBB is a part of the dynamic neurovascular unit which is composed of vascular cells (endothelial cells, pericytes, and smooth muscle cells), supporting glial cells (astrocytes,

microglia, and oligodendrocytes), neurons, and extracellular matrix [32]. Aberrant neuroinflammation dramatically disrupts the interactions between components of the neurovascular unit. Pro-inflammatory cytokines interfere with the tight connections between the astrocytic end-feet, pericytes, and endothelial cells, causing a leaky BBB [33]. The damaged BBB permits the entry of circulating cells and substances which are typically excluded or tightly-regulated from the brain, inducing further inflammation and upsetting the homeostatic solute balance, which results in intracranial edema [33]. Also, a number of molecular factors which are upregulated following injury—such as Notch, HMBG1, and SPARC—prompt microglia toward an M1-like phenotype, favoring mobility, phagocytosis, and the section of additional pro-inflammatory cytokines [34–36]. Finally, the inflammatory cytokines promote the upregulation of adhesion molecules (i.e. ICAM1, E-selectin, P-selection) on the endothelial cells and attract peripheral immune cells to adhere and enter the brain [32, 35–38]. Altogether, this inflammation contributes to a hostile environment which, if prolonged, can cause further damage to neural cells.

The pathology of cerebrovascular diseases is not isolated within the central nervous system (Fig. 8.1). Peripheral body systems have received increasing recognition for their role in cerebrovascular disease progression. Inflammatory signals that result post-injury travel through the circulatory system and impact systemic inflammation which may propagate cerebral inflammation. This brain-to-periphery interplay is both permitted and heightened by BBB breakdown, as peripheral lymphocytes and monocytes easily pass through compromised vessels, migrating toward the inflammatory signals originating from the site of injury. Treatment options will be

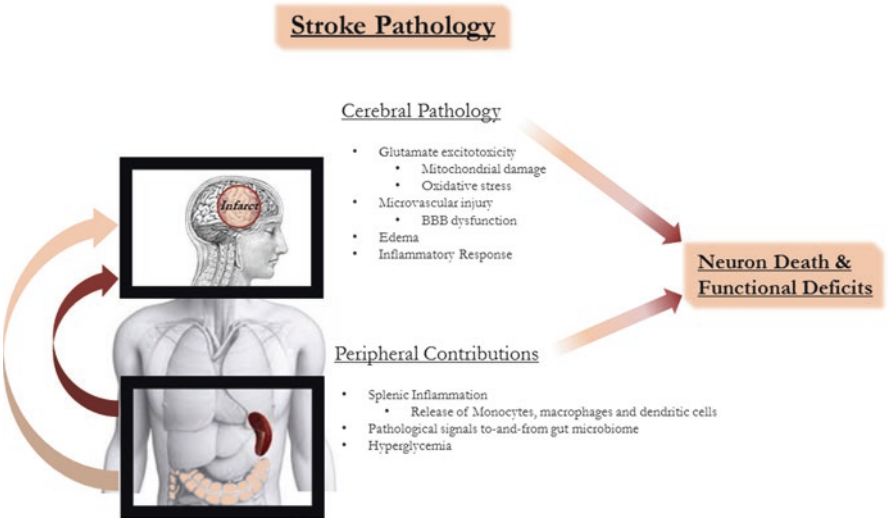

Fig. 8.1 A diagram of stroke pathology which includes both cerebral damage and peripheral contributions. Importantly, the loss of BBB fidelity permits the transfer of pathologically relevant molecules to and from the periphery. Changes in the peripheral organs—especially the spleen and gut—have been shown to accompany and contribute to worsening outcomes

rendered most effective if they consider peripheral body systems, due to their capacity to exacerbate brain injury. For example, preclinical studies suggest that mitigation of peripheral inflammation—particularly in the spleen—may be a primary mechanism of intravenous stem cell injection after stroke [9]. Indeed, the spleen is a significant contributor to systemic inflammation as a consequence of cerebral insult [9, 39, 40]. Following stroke, the physical size and function of the spleen alter, impacting brain health [41–43]. Under pathological conditions, the spleen will release splenocytes into circulation causing further neurodegeneration. Animals that receive splenectomies prior to cerebrovascular insults display improved cognitive function and decreased lesion volumes [44]. While this method is not practical for clinical use, this knowledge of the spleen-brain inflammatory axis highlights the critical role of the spleen in neuropathology. In addition to the spleen, research has also revealed that the gut microbiome plays a vital role in stroke pathology. Depletion of the proper intestinal flora leads to poorer outcomes in animal models of stroke, testifying to the significance of the microbiome to global health [45]. Our understanding of stroke as a global disease-state gives insight on how to properly assess and develop effective treatments [46, 47]. As we will discuss in great depth later, intravenous transplantation of stem cells has the unique ability to utilize trophic mechanisms to abrogate central and peripheral inflammation, in addition to forming the biobridge structure which helps facilitate repair by way of endogenous stem cell optimization, all working to reduce the pathological consequence of stroke.

Stroke pathology is complex and multiphasic. The initial metabolic restriction and glutamate toxicity are not the only factors that cause damage to the neurovascular unit. In fact, the subsequent secondary cell death in the form of growth factors deficiency, neuroinflammation, and BBB breakdown can further exacerbate the injury for an extensive period of time. Current stroke treatment is limited to restoration of the blood flow through tPA or mechanical means which is only effective when targeting the supracute stage of pathology [3]. While the body has a small capacity to repair and regenerate neural damage, these efforts are insufficient to overcome the overwhelming damage of secondary cell death. Therefore, there is a tremendous need for novel therapeutic strategies that can address this multifaceted pathology of stroke. The complexity of stroke pathology necessitates a therapy that has as an equally complex and diverse array of therapeutic mechanisms. To this end, we and others have proposed stem cell therapy as a promising therapeutic strategy. Briefly, stem cells exert their therapeutic benefits through replacing loss or damaged cells, providing trophic factors and anti-inflammatory cytokines, and via the novel concept of the stem cell-paved biobridge. These mechanisms will be expanded upon in later sections.

3 The Evolution of Stem Cell Research

Stem cells are a small population of cells which possess specific characteristics, including the ability to self-replicate, to differentiate into various cell lineages, and to express specific cell markers [48]. Self-replication gives stem cells the ability to

preserve their characteristics and maintain a reservoir population of stem cells within several niches of the body. The capability of stem cells to differentiate into different cell types is vital to their role in preserving homeostasis and to the maintenance of various body systems [49]. For example, the body continually regenerates red blood cells to replace the old by using stem cells within the bone marrow. Collectively, the capacity of stem cells to self-replicate and differentiate into various lineages is referred to as the property of *stemness* [50]. Each type of stem cell has a characteristic level of stemness which is an important factor to be considered when contemplating any potential therapeutic treatment.

There are several ways to classify stem cells. The most common type of classification is based on the origin of the harvested stem cells. For example, umbilical cord stem cells and adipose stem cells are harvested from the umbilical cord and adipose tissue, respectively. Depending on a stem cell's potency, defined as the number of cell types a stem cell can differentiate into, a stem cell can be classified as totipotent, pluripotent, or multipotent [51]. Totipotent stem cells can become all cell types including extraembryonic cells, whereas, pluripotent stem cells can develop into all cell types except for extraembryonic and placental cells. Multipotent stem cells can give rise to various cell types, yet much more limited than totipotent and pluripotent stem cells [51]. In general, the earlier the cell is harvested within the developmental process, the higher the stem cells' potency (i.e. embryonic). Additionally, stem cells can be classified molecularly based on their profile of expressed cell markers. Bone marrow-derived mesenchymal stem cells, for example, are positive for CD29, CD44, CD105, CD73, CD90, CD106, and CD166 markers, while negative for CD14, CD34 and CD45 [52, 53]. In this section, we will discuss the unique properties and pros/cons of specific stem cell types which have shown promising preclinical results, with an emphasis on the relevance and feasibility for clinical translation.

3.1 The Early Era of Stem Cell Research and Initial Cell Sources

When the stem cell research field first developed, stem cells were primarily isolated from fetal tissues. Fetal stem cells have been shown to afford therapeutic benefits in preclinical models of many neurological disorders, including stroke, and were the cornerstone of early stem cell research in the 1970s and 1980s [54, 55]. These benefits include neuroprotective and neuroregenerative effects through secreting anti-inflammation molecules, releasing growth factors and differentiating into neuronal cells [56]. Furthermore, fetal stem cells demonstrate greater graft survival and ability to hone in on sites of injury when compared to adult stem cells [57, 58]. Unfortunately, fetal stem cells have been plagued by notions of immorality since their discovery, with opponents citing a lack of respect for human life and a possible justification for abortion as grounds for restricting research efforts [55]. From 1987 to 1992, these ethical concerns manifested as a moratorium—a legislative

suspension of all funds related to fetal stem cell research—which pushed scientists to search for non-fetal stem cell sources [55].

In an attempt to avoid these ethical concerns, varying methods have been used to develop and harvest alternative stem cell sources which produce potent therapies in lieu of fetal tissue. One such effort in the neurological field involved creating neuron-like hNT by exposing NT2-N embryonic carcinoma-derived stem cells to retinoic acid. These cells terminally differentiate into post-mitotic neurons, and were shown to survive and integrate into host neural networks [59]. Despite promising preclinical data [60], this line of cells was beset by concerns of tumorigenicity [61]. In a Phase I clinical trial, 12 patients—9 male and 3 female—with an age range from 44 through 74 years old, were transplanted with hNT cells developed by Layton Bioscience Inc. [62]. The study concluded that the transplantation of the hNT cells was safe and feasible, however consensus on the efficacy could not be reached due to small sample size [62]. The first postmortem analysis of a participant was reported 27 months after implantation [63]. The analysis showed that the hNT cells survived at 27 months after implantation with no evidence of tumor, additional infarcts, or neurodegenerative diseases [63]. However, this patient did not show motor recovery after transplantation [63]. While Phase I and Phase II clinical trials ultimately revealed the safety of these cells—with no adverse cell-related serological effects [60, 63], and moderate functional improvements—the inadequate patient sample size and ongoing concerns over their cancerous origin and high proliferative capacity would severely cripple investigations into this cell line. In light of cell lines such as hNT, the genetic modification of stem cells emerged as a potential solution to a number of issues which dampened progression into the clinic, such as artificially reducing proliferation/tumorigenicity, improving graft survival, and heightening anti-inflammatory effects [64].

Cell lines such as the conditionally immortalized human neural stem cell, CTX0E03 or CTX, developed by ReNeuron aimed to maintain all facets of stem cell therapeutic efficacy while eliminating tumorigenic risks [65]. ReNeuron utilized c-mycER(TAM) technology in human first trimester fetal cortical cells to develop conditional growth control with a fusion protein containing the growth promoting gene, c-myc, and a hormone receptor regulated by the synthetic drug, 4-hydroxytamoxifen (4-OHT) in producing the CTX-DP immortalized cell line [66]. This allowed the cells to be cultured to large quantities *in vitro* with 4-OHT-containing media, yet have their growth cycle arrested upon transplantation in the absence of 4-OHT [66]. With the support of promising preclinical data, CTX cells entered a phase 1 clinical trial named PISCES in 2010 (NCT01151124) and were shown to improve primary outcome measurements in male stroke patients [67]. A narrow patient pool of 11 males aged 60+, and the open-label, single-arm study design calls into question the extent to which reliable conclusions can be made regarding the efficacy of CTX cell implantation on functional recovery (NCT01151124). Arguably, the modified nature of these CTX cells may have negative effects on their stemness and therapeutic characteristics. In particular, with the lineage commitment

of the cells artificially restricted to neuronal phenotype, the ability of these neuronal-like cells to migrate is likely reduced, thus compromising their efficacy. This underscores the important balance which must be found when genetically modifying stem cells; being that stem cells are such complex biologics, scientists must be mindful not to unintentionally diminish major therapeutic mechanisms of stem cells by modifying dynamic and far-reaching pathways. When compared to unmanipulated or minimally-manipulated cell types, CTX cells (as well as SB623, which will be discussed shortly) took significantly longer to gain clinical approval, largely due to additional regulatory obstacles including long term *in vivo* preclinical studies and safety mechanism demonstrations which were required for all modified cell types [68]. The complications and dangers of genetic modification were first made evident in clinical trials of viral vector gene therapy which displayed the risk of fatal side-effects in some patients, producing an atmosphere of fear and apprehension surrounding all forms of genetically modified therapies [69]. This had the result of dampening and greatly delaying the clinical entry of genetically engineered stem cell types, such as CTX, which faced the skepticism of a wary Food and Drug Administration with the tragic loss of life fresh in their memory, and a negative public perception of all things genetically modified [70]. These unfavorable attitudes severely crippled the clinical progress of genetically modified stem cells—which objectively possess unique therapeutic potential.

Turning to embryonic stem cells, being from an early stage of development, these cells are considered the gold standard for stemness, with intrinsically high potency and high proliferative rates. In fact, only embryonic stem cells from the first few cell divisions after fertilization have true totipotent characteristics and are free from replicative senescence. These qualities make embryonic stem cells diverse in their applications. The use of embryonic stem cells arose from scientific efforts to steer clear of fetal-derived and cancer-derived cell lines, seeing as both were fraught with public image issues. Formed from *in vitro* blastocysts fertilization [71], embryonic stem cells evaded a portion of the moral issues surrounding fetal stem cells, yet fell short of acquiring complete public acceptance. While preclinical evidence has repeatedly demonstrated the efficacy of embryonic stem cells in neurological disorders [72], their wide-scale use has been similarly hindered by ethical, moral, and tumorigenic concerns.

Pressure from politicians and public opinion concerning embryonic and fetal stem cells, as well as the failed clinical trials of gene therapy which negatively affected the view of genetically engineered stem cells, have pushed scientists in the field of adult stem cells to look for alternative sources. For the past few decades, scientists have been able to identify and isolate adult-derived stem cells from various sources. These stem cells circumvent the ethical issues faced with embryonic stem cells, however, they pose challenges of their own. Some of the adult-derived stem cells which will be discussed are bone marrow-derived mesenchymal stem cells (BM-MSCs), extraembryonic stem cells, and induced pluripotent stem cells (iPSCs). While there are many other stem cells, these cell types currently hold the most potential to advance to the clinic.

3.2 Transitioning to Adult-Sourced Stem Cells

3.2.1 Bone-Marrow Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are a class of multipotent stem cells that can be harvested from many adult mesenchymal tissues such as bone marrow, adipose tissue, and placenta. Of these, BM-MSCs are the most common and also the most studied adult stem cells, with multiple pre-clinical studies showing their therapeutic benefits in various neurological disorders such as TBI, amyotrophic lateral sclerosis, and particularly in stroke [9, 73–75]. One of the major advantages of BM-MSCs is the ability for autologous transplantation. BM-MSCs can be harvested and amplified from a patient's own tissues, thereby eliminating the concern of post-transplant immunologic rejection. However, an argument can be made against using autologous stem cells because the patients' BM-MSCs might be less potent than healthy donors'. Recent studies have linked stroke neurological deficits with changes in the peripheral systems such as inflammation in the spleen or alterations in the gut microbiome [9, 76]. These peripheral alterations could negatively affect the health and therapeutic efficacy of the patient's BM-MSCs. In terms of tumorigenicity, some studies have reported that BM-MSCs may induce tumor formation [77–79], however, BM-MSCs have been deemed safe in both pre-clinical and clinical studies [70, 75, 79–82]. These advantages of BM-MSCs are particularly relevant in attempting to transition BM-MSCs into the clinic. Importantly, BM-MSCs can still exert their beneficial effects despite short survival time and lack of neuronal differentiation [9, 16, 81, 83]. While appearing paradoxical, BM-MSCs' mechanisms of action rely more heavily on immunomodulation, modifying the microenvironment, and secreting trophic factors rather than differentiating and integrating into neural networks [53]. This is a distinct advantage of these cells, as clinically it is more feasible to give a stem cell "booster shot" to compensate for the low survival rate rather than attempting to control the formation of tumors inherent in other cell types. However, BM-MSCs also pose challenges that must be considered. BM-MSCs may behave differently depending on their location, method of extraction, isolation, and culture [79]. Therefore, it could be difficult to have a consistent and homogenous pool of BM-MSCs in mass production. Another limitation of BM-MSCs is that it requires time to collect, isolate and amplify the autologous BM-MSCs before they can be transplanted back into the patient, limiting the accessibility of these stem cells and their availability to the population at large.

Homogenous subpopulations of BM-MSCs may offer distinctive benefits. One such cell type, multilineage-differentiating stress-enduring (Muse) stem cells can be found within bone marrow (in addition to all connective tissues), and have displayed characteristics which make them highly appealing for therapeutic exploration [84]. These pluripotent cells have shown a unique ability to remain viable within highly stressful microenvironments [84]. Furthermore, the asymmetrical divisions and low telomerase activity of Muse cells mean low tumorigenicity and minimal risk of teratoma formation. Another subpopulation of MSCs found within bone marrow is the

Very small embryonic-like (VSEL) stem cell [85]. These cells are roughly half the size of hematopoietic stem cells, and maintain the pluripotent ability to differentiate into cells from all three germ layers [86]. Additionally, these cells have been shown to form small clusters that resemble embryonic bodies *in vitro*, which could have implications in the efficiency these cells can be cultured, as well as preserving their highly potent characteristics [86].

BM-MSCs have experienced success within the clinic. A GDNF-releasing, Notch-induced human bone marrow-derived mesenchymal stem cell line—SB623—was employed in a Phase I clinical trial beginning in 2011 (NCT01287936), after displaying significant amelioration of stroke symptomology in preclinical animal models. GDNF—glial cell line-derived neurotrophic factor—confers potent pro-survival effects. SB623 stem cells undergo *ex vivo* gene delivery to heighten their neurotrophic properties via enhanced GDNF secretion. As of now, the SB623 clinical trial has demonstrated relative safety, and preliminary reports of efficacy in chronic stroke patients [80]. Similar to the CTX cells described previously, consideration must be given to the genetically modified nature of these stem cells, and how these modifications may inadvertently affect the stemness and therapeutic properties of the cells.

3.2.2 Extraembryonic Stem Cells

Extraembryonic stem cells are a collective term for the adult-derived stem cells found in the placenta, the umbilical cord, the amnion, and Wharton's jelly [83, 87–89]. Placenta-derived MSCs, umbilical cord blood-derived MSCs (UCB-MSCs), and amnion-derived MSCs are the focus of many current investigations. Considering these stem cells' common origins, they share many therapeutic properties with BM-MSCs, such as modulating neuroinflammation, stimulating endogenous neurogenesis, releasing trophic factors, and promoting functional recovery in pre-clinical animal models of stroke [87, 90–92]. However, extraembryonic stem cells can differentiate into more cell types than BM-MSCs [89]. Recent studies have demonstrated that UCB-MSCs and placenta-derived MSCs can differentiate into neuronal cells that express markers such as Nestin or β-tubulin III—important markers of neuronal identity and function [93]. Similarly, recent studies have also reported that Wharton's jelly-derived MSCs can differentiate into various cell types such as glial, neuronal, and endothelial cell [94]. There are several advantages of extraembryonic stem cells compared to embryonic, fetal, and bone marrow-derived stem cells. These tissues are currently considered waste products, thus posing no health risk to the mother or baby, and circumventing any ethical issue related to the extraction of these extraembryonic stem cells. In the case of amnion-derived MSCs, the stem cells can be collected during amniocentesis—a safe, routine procedure during pregnancy [95]. These stem cells can then be expanded and ready to treat any disease associated with childbirth, such as hypoxia, or cryogenically preserved for future catastrophic events such as stroke or TBI [95]. However, there are also downfalls associated with extraembryonic stem cells. These extraembryonic tissues contain a

variety of cells, making it difficult to isolate a homogeneous population of stem cells. Moreover, the amount of stem cells in these tissues is limited, especially in amnion fluid, requiring more time to amplify these stem cells prior to transplantation. In addition, it is expensive and unrealistic to maintain all extraembryonic tissues for every baby. Only a small portion of the population can afford to cryogenically preserve these tissues for an extended time.

3.3 Induced Pluripotent Stem Cells: A New Horizon for Stem Cell Research

Contrary to previous dogma, recent studies have demonstrated that differentiated adult cells can be reverted back to earlier stem cell states. Through molecular manipulation, these cells can regain their stemness, especially their proliferative property [89]. These cells are termed induced pluripotent stem cells (iPSCs). One of the challenges of adult stem cells is the limited number of passages before the cells stop proliferating. iPSCs are molecularly enhanced to increase the stemness (both proliferative and differentiating capacities) of the cells and can be scaled to large quantities. Furthermore, iPSCs also bypass the ethical issues associated with harvesting embryonic or fetal stem cells. In pre-clinical studies of stroke, iPSCs have shown promising results for improving neurological deficits, decreasing neuroinflammation, promoting neurogenesis and increasing angiogenesis [96–99]. Another major advantage of iPSCs is their ability to be redirected to differentiate into various cell lineages. For example, iPSCs can be induced into neural cells such as neurons, astrocytes, microglia, and vascular endothelial cells. While iPSCs have many advantages, tumorigenesis is a major concern when using iPSCs. In most cases, cancerous genes are used to induce the iPSCs, therefore it is important to control the tumorigenic property before iPSCs can advance further into the clinic.

3.4 Challenges in Translating Stem Cell Therapies to the Clinic

Finally, it is worth noting that there are many other logistic challenges that must be considered before any of the stem cells discussed can successfully advance into, through, and beyond clinical trials. These challenges include reaching a consensus on ideal cell type, dosage, number of transplants, timing, and route of administration. Indeed, the current clinical trials mentioned above (NCT01151124, NCT01287936) are being carefully analyzed and scrutinized for sub-optimal design and small patient pools.

The ideal timing and route of the administration depend on the intended purpose of the stem cell transplantation. Within the context of stroke, the distinct acute and

chronic pathological phases must be considered. Intracranial (IC) transplantation is preferable in the acute and subacute phase of stroke. In these time frames, the presence of stem cells at the penumbra dampens the hostile environment and reduces the spread of the infarct core. Conversely, in the chronic phase, the inflammation both in the brain and the periphery is the main concern. Therefore intravenous (IV) or intra-arterial (IA) injection of stem cells may pose as better alternatives. In addition, if there is a need for multiple transplantations or injections, IV and IA are much more desirable choices. Of note, during the IV and IA injection, the majority of stem cells are trapped in the peripheral organs such as lung and spleen. However, the route of administration does not have to be mutually exclusive; an appealing option may be first transplanting via IC injection followed by IV booster shots for maximizing effectiveness.

The growing number of unique stem cell types begs the question of which is the best candidate stem cell type for clinical application. As discussed previously, each of the various stem cell types has their specific strengths and weaknesses. A well-designed preclinical research effort geared toward evaluating the safety, efficacy and mechanism of action of each stem cell type may reveal the optimal transplantation regimen of cell therapy for clinical trials. In particular, determining the appropriate stem cell dosage, timing, and route of delivery in animals with direct human application will be critical in advancing cell therapy to the clinic.

Stem cell therapies for stroke are at a pivotal point currently. Preclinical evidence has continued to accumulate for the past four decades which indicates that transplantation of stem cells offers significant amelioration of stroke-induced deficits, both when delivered acutely as well as chronically. Furthermore, IV and IC administration have displayed unique benefits and practical advantages which broaden the applicability of stem cell transplantation and heighten their far-reaching potential. The issues described above, however, have crippled the advancement of this therapy, resulting in limited clinical trials with inconsistent measures of efficacy. Careful evaluation of the six most recent clinical trials of BM-derived stem cell therapies in stroke—four within the subacute phase of stroke (NCT01716481, NCT00859014, NCT01501773, NCT00535197), and two within the chronic phase (NCT01151124, NCT01287936)—confirms the disconnect between lab and clinic, and reveals the gaps which still exist in our knowledge of stem cell therapies. As additional clinical trials proceed with enlisting larger cohorts of patients, pursuing long-term follow-up, and thoroughly assessing the status of the transplanted cells, we will be able to further evaluate the safety, efficacy, and mechanisms of action of stem cell therapy for stroke. Indeed, the mechanisms of action by which stem cell confer their therapeutic benefits in stroke are yet to be fully understood. How stem cells achieve this regenerative process stands as the primary challenge for stem cell researchers within the field, and is a vital step in designing more successful clinical trials [68, 100, 101]. The following section will discuss the canonical mechanisms of action for stem cells, as well as explore the concept of the biobridge and how it advances our understanding of the host-transplant interactions which mediate stem cells' therapeutic effects.

4 Stem Cell Therapy: Moving Beyond the Cell Replacement Paradigm

Given the multifaceted pathology of stroke, therapies targeting only a single pathology are unlikely to resurrect the motor and cognitive deficits caused by stroke, particularly at the chronic stages. Stem cell therapy is unique in its potential to be beneficial over a wide therapeutic window and its capacity to mitigate the diverse pathological processes observed after stroke [102]. The two known and widely-accepted mechanisms by which stem cells elicit neuroprotective and neuroregenerative effects after stroke are cell replacement and bystander effects [103, 104].

Initially, it was proposed that transplanted stem cells would serve the same function as they do within the body—generating new cells and replacing dead or damaged tissue. Transplanted stem cells were predicted to differentiate and directly replace loss cells, however, studies have demonstrated that within the injured brain, this notion is at best partially correct due to various factors [105, 106]. First, the majority of transplanted stem cells do not survive even when immunogenicity is accounted for through autologous transplant or Immunosuppressants [107]. Second, while many stem cells have demonstrated that they can differentiate into neuronal cells *in vitro* under highly-controlled conditions, they failed to do so in large numbers within *in vivo* model [108, 109]. One explanation for both issues is that transplanted stem cells enter a hostile microenvironment which is not conducive to long-term survival, differentiation and maturation. Thus, merely increasing the number of transplanted cells would not solve the problem. Furthermore, even with the small number of differentiated and living cells, there is little evidence to support that these cells integrate into neural networks to a significant extent, hence cell replacement is not considered a primary mechanism of action of stem cells.

Instead, evidence supports that the therapeutic capacity of stem cells lies largely within its bystander effects in which the stem cells secrete trophic factors and anti-inflammatory cytokines [110]. Stem cells secrete a cocktail of vital growth factors and, as mentioned previously, a reduction in growth factors is a key player in secondary cell death [111, 112]. For example, in animal studies, BM-MSCs secrete a variety of trophic factors which stimulate the neuroregeneration process [113]. Some of the notable trophic factors are VEGF, BDNF, NGF, insulin growth factor-1, and hepatocyte growth factor [113]. Similarly, several growth factors such as VEGF and BDNF were elevated after the transplantation of UBC-MSCs or placenta derived-MSCs [114]. In addition to growth factors, stem cells secrete anti-inflammatory molecules that mitigate neuroinflammation [115]. Stem cells secrete microvesicles and exosomes known to contain growth factors, proteins, anti-inflammatory cytokines such as IL-10 and IL-4 [9, 74, 116, 117], microRNA and lncRNA such as nuclear enriched abundant transcript 1 (NEAT1) and metastasis associated adenocarcinoma transcript 1 (MALAT1) which play key roles in inflammation, gene expression, and cell survival [74]. When transplanted after stroke, not only do stem cells have the capacity to sequester inflammation at the ischemic source, but also throughout the periphery. Intravenous administration of human

bone marrow stromal cells (hBMSCs) in rats following stroke resulted in the preferential migration of stem cells to the spleen compared to the brain [9]. Treated animals presented with lower infarct volumes, and reduced cerebral and splenic inflammation [9]. Interestingly, this study reported that a greater number of hBMSCs observed in the spleen correlated to decreased infarct and peri-infarct volume, as well as lower TNF-α density in the spleen [9]. Viewed holistically, these results indicate that peripheral implantation of stem cells may afford neuroprotection indirectly by moderating the overactive and global inflammatory response following stroke by similar anti-inflammatory mechanisms as observed in IC injection.

Mounting evidence has shifted the consensus respecting the primary mechanism of action from cell replacement paradigm toward bystander effect [118]. Indeed, stem cells are now well known for their therapeutic trophic mechanisms that contribute to neuroprotection. However, even combined, both mechanisms do not fully explain the endogenous recovery effect observed after transplantation. While the trophic factors can stimulate endogenous stem cells to proliferate and differentiate, it is unclear how these endogenous stem cells can then migrate to the injured brain regions [119]. Migration is a challenging and complex process, especially in a mature adult brain. Without external support and guidance, inflammatory cytokines are not enough to attract the endogenous stem cells over long distances. To this end, we propose a third mechanism of action for stem cell transplants that our lab has revealed—the formation of a stem cell-paved biobridge—which furthered our understanding of how endogenous stem cells achieve migration from deep neurogenic niches to distal injured regions of the brain.

5 The Biobridge: Exogenous Stem Cells Guide Endogenous NSCs Towards Repair

For the past five decades, the scientific community has been aware of the neurogenic capacity of the adult mammalian brain [120], however, the precise role and regulation of neural stem cells (NSCs) remains an active area of research. Evidence contradicts the original assumption that the primary role of endogenous NSCs is to regenerate damaged tissue after brain injury. Instead, NSCs take part in brain plasticity by both direct and indirect mechanisms which are crucial for certain types of hippocampal and/or olfactory bulb-dependent learning and memory [121]. Unfortunately, NSCs' capacity for tissue regeneration after brain injury is extremely limited despite an increase in activation following such injurious events. Poor cell survival and proliferation, lack of commitment to neuronal lineage, and limited migration are all challenges that prevent these endogenous NSCs from facilitating significant regeneration after brain injury [21].

Much like peripheral inflammatory cells, transplanted stem cells are drawn towards molecular signals from the peri-infarct area. Extracellular matrix (ECM) remodeling allows these cells to move through the brain parenchyma. Interestingly,

the process of migrating exogenous stem cells benefits endogenous neural stem cells (NSCs) as well [21]. NSCs are not ubiquitous throughout the brain, but are instead restricted to neurogenic niches in two brain regions—the SVZ of the lateral ventricles and the SGZ of the dentate gyrus of the hippocampus (although quiescent NSCs have been identified in other brain regions) [121]. When brain injury occurs at sites distal to these locations, the potential for robust repair or neuroprotection afforded by endogenous NSCs is diminished due to their limited capacity for migration. In a previous study, our lab discovered that this shortcoming of NSCs may be compensated for by additional mechanisms of transplanted stem cells [21]. In this investigation, a controlled cortical impact was delivered to the frontal cortex of Sprague-Dawley (SD) rats, a common model for TBI. Intracerebral injection of Notch-induced hBMSCs (referred to as SB623, supplied by SanBio Inc.—see Sect. 3.2.1) [8, 122] was performed 7 days post TBI. Locomotor and neurological tests were completed prior to TBI, pre-transplantation, and monthly following transplantation for up to 3 months. As expected, at 1, 2 and 3 months post TBI, treated animals displayed significant improvements in motor and neurological tasks. Histological analysis at both 1 and 3 month time-points also showed reduced lesion size and improved cell survival in the peri-impact area. Notably, the engraftment rate for the transplantation was minimal, at only 0.60% at 1 month post-transplantation and 0.16% at 3 months.

While these findings were similar to other reports of stem cell transplantation after TBI, immunohistochemistry and laser capture revealed a previously unreported phenomenon in which exogenous stem cells form a cellular bridge between the neurogenic SVZ and the lesion within cortex. With the formation of this biobridge came successful endogenous stem cell migration; a pathway was observed alongside the same trajectory of the migrating injected stem cells. The pattern of endogenous stem cell migration was remarkably different between treated and untreated animals. In vehicle injected animals, endogenous cells were sparse throughout peri-impact cortical regions and newly formed neural cells within the SVZ were nearly absent. Additionally, cell proliferation and neural differentiation was stunted in non-treated animals. By contrast, in animals that received the SB623 cells, at 1 month post-transplantation, robust endogenous cell proliferation (Ki67) and immature neural differentiation (nestin) was observed in peri-impact cortical regions and the SVZ, with migrating cells (DCX) along the corpus callosum. Immunohistochemistry revealed hBMSCs localized within the impacted region, down into the cortex, across the corpus callosum and along the ventricles to the location of neurogenic niche. At 3 months post transplantation, $DCX^+/HuNu^+$ (human nuclei) cells were identified alongside the hBMSCs transplanted cells indicating that non-transplanted cells were able to navigate through the ECM that was likely recently remodeled by the migrating hBMSCs. It is important to note that the transplanted stem cells survival was largely diminished by 3 months, suggesting that even though these cells did not persist, endogenous cells were still able to utilize the same route through the ECM where they could continue to migrate through and thrive, sustaining endogenous recovery efforts despite the absence of transplanted stem cells.

To better understand the mechanism of biobridge formation, we explored metallomatrix protein (MMP) expression, specifically, MMP-9. Molecular analysis via

laser-capture revealed increased MMP-9 expression along the migratory pathway [21]. Notably, TBI-vehicle animals also displayed an increased MMP-9 expression following stroke, however, this upregulation reverted to levels comparable to sham animals at 3-months post-injury. In SB623 transplant animals, MMP-9 expression doubled that of TBI-vehicle animals at 1 month post-TBI and expression increased ninefold by month 3. This data suggest the importance of this neurovascular proteinase in the long-term neural regenerative efforts of transplanted stem cells. While these results indicate that endogenous cells alone increase MMP-9 expression after brain injury, stem cell transplantation promotes a more robust mechanism for ECM remodeling than unaided endogenous stem cells by leaving a direct pathway for the endogenous stem cells to utilize.

Complementary to these *in vivo* results, an *in vitro* study presented SB623-promoted cell migration via an ECM-mediated mechanism [21]. Primary rat cortical cells were grown by themselves or co-cultured with SB623 cells in two different conditions—with or without Cyclosporin-A, an MMP-9 inhibitor. Co-culture of SB623 cells without the presence of MMP-9 inhibitor significantly enhanced the migration of primary cortical rat cells. The migration of primary cortical rat cells into the chamber containing the SB623 cells was significantly reduced when treated with Cyclosporin-A, with no significant difference compared to the cultures without stem cells. This study further supports that stem cells, particularly SB623 cells, promote cell migration mediated largely via MMP-induced ECM remodeling.

Moreover, it is believed that migratory trophic factors released by the exogenous stem cells such as cysteine-x-cysteine motif chemokine ligand 14 (CXCL14) and monocyte chemoattractant protein 1 (MCP1) further promote endogenous stem cell migration from the neurogenic niche. It is important to note that the transplanted MSC's long-term survival was not necessary for functional improvements in this study. Instead, the therapeutic benefit was attributed to their ability to manipulate the microenvironment and stimulate endogenous stem cell migration, proliferation, and differentiation. These findings positively address some of the tumorigenic concerns mentioned in previous sections, as eventual death of transplanted stem cells and loss of stemness characteristics are increasingly regarded as important in preventing tumorigenesis.

To further investigate this novel stem cell mechanism of action, we designed a pilot study to investigate if a similar biobridge formation occurs after stem cell transplantation in the MCAO stroke model. We would like to share our promising ongoing study. Normal male SD rats (n = 10, average weight = 200 g) were subjected to MCAO surgery. Three days post stroke, the animals were split into two groups that received a one-time transplantation of human BM-MSCs by either IC (n = 5) with 1.0×10^6 cells or IV (n = 5) with 4.0×10^6 cells. The animals were sacrificed and processed for immunohistological staining at day 7 post-stroke. Similar to previous reports, we observed MSCs in the cortex (Fig. 8.2) and striatum in both IC and IV groups (Fig. 8.3), showing that the MSCs can infiltrate the brain either through IC or IV transplantation. Interestingly, the transplanted MSCs from the IC injection group mainly traveled along the corpus callosum, while the MSCs from the IV group disperse throughout the striatum and cortex. DCX^+ stain-

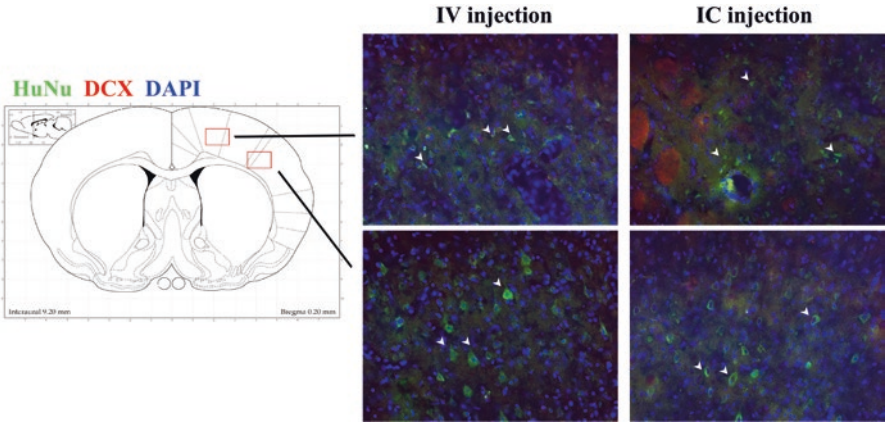

Fig. 8.2 Distribution of human BM-MSCs (HuNu$^+$) in the cortex. After transplantation, BM-MSCs (HuNu$^+$) succesfully infiltrated the ischemic brains in both IC and IV route of administration. HuNu$^+$ cells were detected, however DCX$^+$ cells were not found in the cortex

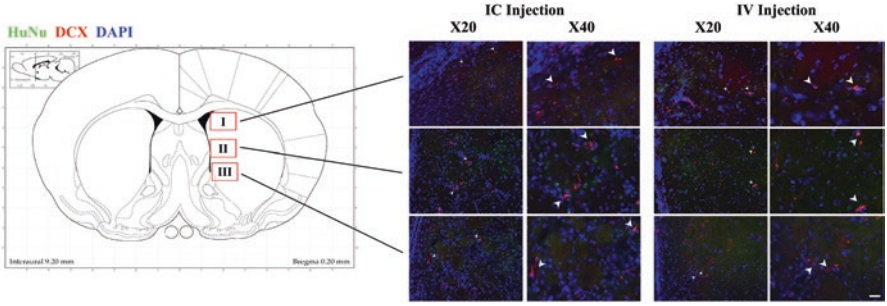

Fig. 8.3 Distribution of human BM-MSCs (HuNu$^+$) and immature neurons (DCX$^+$) in the striatum. HuNu$^+$ cells from the IC group traveled along the corpus callosum, whereas HuNu$^+$ cells from the IV group dispersed throughout. Transplanted human BM-MSCs were found around DCX$^+$ cells, paving the way for these immature neurons to migrate toward the penumbra

ing, a marker for cell migration, revealed that DCX$^+$ cells from the IV group traveled along the corpus callosum and the ventricle wall (Fig. 8.3). The DCX$^+$ cells found in the IC group traveled more laterally. However, it is worth noting that the DCX$^+$ cells from the IV group traveled further into the striatum compared to the IC group. The migration pattern of the immature neurons is summarized in Fig. 8.4. To further validate our findings, we performed another set of staining for proliferating neuronal cells (Ki67$^+$/Nestin$^+$). Similar to the DCX staining results, Ki67$^+$/Nestin$^+$ cells were found along the corpus callosum and ventricle wall. In addition, fewer Ki67$^+$/Nestin$^+$ cells were found further into the striatum (Fig. 8.5). In conclusion, we have demonstrated in this pilot study that a similar phenomenon

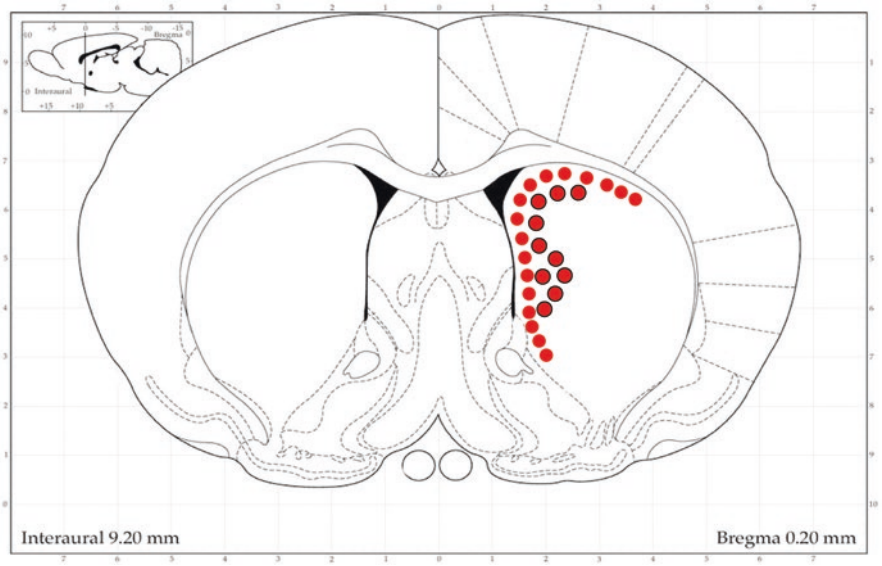

Fig. 8.4 Schematic of the distribution of DCX$^+$ cells between the IV and IC group. DCX$^+$ cells from the IV group travel along the corpus callosum and the ventricle wall. DCX$^+$ cells from the IC group travel more horizontally compared to IV group. However, more DCX$^+$ cells from IV group travel further into the striatum compared to IC group

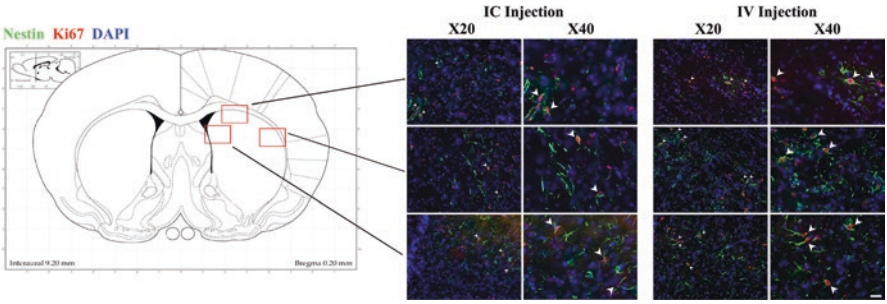

Fig. 8.5 Distribution of proliferating neuronal cells (Nestin$^+$Ki67$^+$) in the striatum. Nestin$^+$Ki67$^+$ cells have similar migration pattern compared to the DCX$^+$ immature neurons. In both IC and IV groups, Nestin$^+$Ki67$^+$ cells were found along the wall of the ventricle and the corpus callosum

reported in TBI, whereby transplanted BM-MSCs modify the environment to facilitate the migration of proliferating and immature neurons, also occurs in stroke. Interestingly, with the help of transplanted stem cells, both DCX$^+$ and Nestin$^+$Ki67$^+$ cells utilized the corpus callosum as a highway to travel further into the penumbra.

6 Future Directions for Advancing the Biobridge Concept

Although the biobridge concept has now been demonstrated in TBI and preliminarily in stroke, barriers still exist to translating these findings into being clinically relevant. A more complete understanding of the cellular and molecular processes which define the biobridge formation and how the assisted migration of endogenous stem cells can be optimized by exogenous transplantation must be unveiled before patients can benefit from these findings. Future studies should aim to more fully characterize the underlying molecular changes that produce the biobridge. Our group has revealed the role of MMP-9 in extracellular matrix remodeling in vitro, yet this single protein is unlikely to account for the totality of the extensive remodeling seen within the biobridge region. Conditional MMP-9 knock-out animals could be valuable in further illustrating the role this protein has in vivo [123–125]. Moreover, transplanted stem cells modified to overexpress MMP-9 and other remodeling factors may reveal a target for heightening the graft-host cell interactions, providing an avenue by which this new mechanism could be utilized to improve clinical outcomes. Importantly, data on the global effects of MMP-9 after stroke are inconclusive, and thus exploring the biobridge formation in MMP-9 knockout mice could help characterize the complex roles which MMP-9 has after brain injury, perhaps playing protective and detrimental roles in different capacities.

Future research efforts should investigate the molecular interactions and crosstalk of the transplant and host stem cells. Here, we describe the remodeling processes observed in brain regions where host stem cells overlap with transplant stem cells. Importantly, transplanted MSCs have been shown to secrete factors which not only promote the survival of host neurons, but are also likely to promote survival of the endogenous stem cells which they come into close contact with. The vast prosurvival secretion profile of transplanted hMSCs, such as wnt3a, VEGF, and BDNF, among others [110], could mean that endogenous stem cells are both guided, and nurtured, by transplanted cells, thereby heightening their regenerative capacity upon arrival to the peri-injured regions. Additionally, factors such as wtn3a and VEGF have been shown to inhibit the quiescent state of host stem cells, wherein their migratory and regenerative properties are stagnated [121]. Beyond the ECM remodeling discussed extensively above, exploring how transplanted MSCs enhance the therapeutic capabilities of host stem cells through cell-to-cell interactions will further enhance our understanding of the robust benefits offered by stem cell transplantation.

The chronological characteristics of the biobridge also deserve additional evaluation—both with regards to its structure and composition over time, as well as how its development varies with different transplant time points. To date, our group has only investigated the progress of the biobridge formation out to 3 days in stroke, making it imperative for additional studies which investigate the biobridge structure and formation through the sub-acute and chronic phases. Understanding how ongoing molecular changes encourage the migration of endogenous stem cells could

provide indications for the effects which acute biobridge formation, and sub-acute progression, have in ameliorating chronic deficits. Discrepancies in the ideal time point for stem cell transplantation post-injury already exist, so careful consideration must be given in determining the transplant time which not only augments the biobridge formation but also gives equal consideration to the various other therapeutic mechanisms occurring concurrently.

Finally, the prevalence of the biobridge concept in other neurological disorders should also be explored. That this process has been demonstrated in two different disease models indicates that this graft-host cell interaction is a more general mechanism of stem cell therapies, and not specific to the pathologies of a single disease. Indeed, this therapeutic mechanism may have far-reaching implications in other neurological diseases amenable to cell transplantation, although the intricacies of its formation may vary greatly between diseases with and without focal damage. This was partly demonstrated in our TBI versus stroke comparison, with TBI brains showing a more unidirectional biobridge and stroke brains displaying a three-dimensional, multi-directional biobridge. How this biobridge concept manifests in neurological disease without focal lesions—i.e. amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease, transient global ischemic events, and neonatal hypoxic—will need to be further explored.

7 Conclusion

Tailoring the use of stem cell therapies in stroke, TBI, and other neurological disorders is an ongoing scientific effort. The unique pathology of neurodegenerative diseases poses a challenge seemingly too large for traditional pharmaceuticals to compensate for, and thus alternative therapeutic options—namely stem cells and regenerative medicine—have received increased attention. Stroke, in particular, has received significant attention as a possible beneficiary of stem cell transplantations. The various pathological processes which accompany stroke appear highly compatible with the dynamic therapeutic profile of stem cells. Transplanted stem cells' ability to secrete anti-inflammatory factors, pro-survival/anti-apoptotic molecules, and to integrate into the host parenchyma contribute to the benefits which they confer. The therapeutic capacity of stem cells in stroke has been demonstrated repeatedly in pre-clinical investigations, yet translating this promise into widely-available clinical treatment options has been slow. This is in no small part to the inherent complications which accompany non-traditional pharmaceuticals, including issues of dose, timing, route of administration, and stem cell source.

The shortcomings of clinical trials of cell transplantation have resulted in a renewed effort to explore the basic science mechanisms of stem cell therapies. The path to successful clinical trials will likely be paved by basic science discoveries concerning the complex therapeutic mechanisms of stem cells. Here, we described a novel therapeutic mechanism of stem cells, the biobridge, which works in conjunction with the

established mechanisms to produce the functional improvements observed following stroke, as well as TBI. The discovery of this mechanism has both basic science, as well as translational, Implications; that exogenous stem cells interact with and encourage the movement of endogenous host stem cells to regions of damage aids in explaining the seemingly paradoxically-robust functional recovery seen in stem cell transplantations despite minimal graft survival rates. Moreover, understanding the extracellular matrix remodeling capacity of transplanted stem cells provides a novel bioengineering target for genetically enhancing stem cells. These findings, in the context of the larger scientific effort to better understand the details of stem cell therapeutic modalities, assist in providing the preclinical basis for more effective clinical trials, bringing stem cell therapies closer to positively impacting stroke patient recovery.

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