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Harnessing the anti-inflammatory properties of stem cells for transplant therapy in hemorrhagic stroke

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Abstract

Hemorrhagic stroke is a global health crisis plagued by neuroinflammation in the acute and chronic phases. Neuroinflammation approximates secondary cell death, which in turn robustly contributes to stroke pathology. Both the physiological and behavioral symptoms of stroke correlate with various inflammatory responses in animal and human studies. That slowing the secondary cell death mediated by this inflammation may attenuate stroke pathology presents a novel treatment strategy. To this end, experimental therapies employing stem cell transplants support their potential for neuroprotection and neuroregeneration after hemorrhagic stroke. In this review, we evaluate experiments using different types of stem cell transplants as treatments for stroke-induced neuroinflammation. We also update this emerging area by examining recent preclinical and clinical trials that have deployed these therapies. While further investigations are warranted to solidify their therapeutic profile, the reviewed studies largely posit stem cells as safe and potent biologics for stroke, specifically owing to their mode of action for sequestering neuroinflammation and promoting neuroregenerative processes.

1. Introduction

Treatment with autologous and allogeneic stem cell transplants stands as a valuable tool for regenerative medicine in stroke by harnessing of their capacity for secreting therapeutic molecules and replacing cells.[1-4] Endogenous and exogenous stem cells promote amelioration in brain tissues damage by stroke through their enhancement of angiogenesis, neurogenesis, and synaptogenesis.[2-8] Stem cell transplants may moderate the secondary cell death caused by stroke-induced neuroinflammation, thus stem cells with anti-inflammatory qualities are especially attractive.[2-4,9] Below, we present and evaluate the current state of different stem cell types and their potential in dampening the neuroinflammation caused by stroke.

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Conflict of interest

Cesar V Borlongan holds patents and patent applications on stem cell biology and their therapeutic applications, and receives royalties and consultancy fees from stem cell companies.

2. Stem cell therapy improves stroke outcomes

Stroke imposes an immense health and economic burden worldwide.[188] Stroke can be classified as ischemic or hemorrhagic, with the ischemic case composing 87 percent of stroke incidences, while the remainder of patients who suffer hemorrhagic are smaller in number, the prognosis is much more devastating with high mortality.[10-12,16] Despite these poor clinical outcomes, treatment options for stroke remain scarce. The thrombolytic agent tissue plasminogen activator (tPA) is only effective in the first 4.5 hours post-stroke, limiting its use to the acute phase and thus excludes the majority of stroke patients.[3,17] For hemorrhagic stroke, limited treatments are available, which focus on controlling the bleeding and reducing the pressure caused by the bleeding immediately after stroke onset.[13-15] Cell therapy has emerged as a compelling biomedical field in the search to identify a treatment that may extend stroke's currently restricted therapeutic window. Specifically, stem cell therapy has the potential to sequester the neuroinflammation that engenders secondary cell death and is thus indicated as a possible subacute- or chronic-phase treatment.[18]

A stroke therapy that is effective acutely with long-term functional benefits would improve the outcomes of many stroke patients. Stroke moves from the acute to the subacute phase after a few hours, then progressing to the chronic phase at a few days post-stroke. The emission of chemokines, cytokines, matrix metalloproteinases (MMPs), and cellular adhesion molecules (CAMs) in the stroke penumbra and surrounding tissues creates the neuroinflammation that plagues the subacute phase of stroke.[19-22] Dysfunction of the blood brain barrier (BBB) may lead to secondary brain injury, often observed in stroke, and this process may stem from these aforementioned inflammatory elements, most prominently the MMPs.[19-21] This deleterious process may be further exacerbated by CAMs, which lure harmful neutrophils, leukocytes, macrophages, and microglia to the damaged cerebral vessels, even though they may also recruit beneficial cells, including stem cells.[19-21] Taken together, these inflammatory substances impair BBB integrity and exacerbate lasting inflammation.[22-25] These symptoms mediate the gradual secondary cell death that characterizes a large portion of stroke pathology, and thus stem cell transplantation may be ideal due to their distinctive subacute and chronic efficacy.[10,23] A multi-pronged approach may be considered in regard to transplantation timing, comprising first a delivery of stem cells in the subacute phase to protect against secondary cell death followed by a second administration of stem cells during the chronic phase to prompt and support native host brain repair processes.[3,22,152] Experimental stroke models recapitulate this booster treatment approach, in which stem cells transplants bestow these anticipated early neuroprotective and delayed neuroregenerative effects.[9,26-28] Between these two, neuroregeneration may be the favored target because, compared to neuroprotection, it is more dependably beneficial in subacute and chronic stroke.

3. Establishing the ideal stem cell type for transplantation

Preclinical and clinical trials have advanced several types of stem cells as options for transplantation.[29,30,32,31,33,25,34,35] The long-standing safety profile of bone-marrow derived stem cells supports their use in the majority of these trials. Concerns regarding the ideal cell transplant timing, delivery route, and dosing for each cell type remain to optimally sequester

the neuroinflammation caused by stroke without deleterious side effects.[3] Here, we review the preclinical and, where applicable, clinical results of treatments with these stem cell types.

3.1. Embryonic stem cells

Embryonic stem (ES) cells remain the gold standard in cell-based regenerative treatments, due to their differentiation into tissues of all three germ layers and their indeterminate proliferation. These favorable properties notwithstanding, ES cells' penchant for tumor formation, related to their signature proliferation, and the ethically controversial nature of their tissue source often consign this cell type to preclinical studies. Transplanting ES cells intracerebrally amplifies cerebral angiogenesis and diminishes neuronal damage in experimental models.[34,36] In addition, transplanted embryoid body (EB) cells, which are derived from ES cells, induce behavioral benefits in animals seven days post-stroke.[36] Likewise, transplanted human embryonic stem cells (hESCs) produce similar results,[36] an effect possibly regulated by the differentiation of hESCs into neural phenotypes.[37,38] Further, genetically engineering ES cells by overexpressing neuroprotective factors has also proven to be a viable option for functional recovery in stroke animals.[8] Moreover, experiments performed with cell cultures reveal that ES cells may emit therapeutic molecules after exposure to a hypoxic microenvironment, which is reminiscent of stroke conditions.[39] These therapeutic molecules may, in turn, inhibit the apoptotic cell death normally caused by stroke.[34] Additionally, the harsh inflammatory microenvironment achieved through these hypoxic conditions also demonstrates the ability of ES cells to rescue host cells from neuroinflammation. That ES cells may secrete anti-inflammatory signals in response to hypoxic conditions advances a possible mechanism for their sequestration of neuroinflammation. However, *in vivo* results that reveal an increase in pro-inflammatory cytokines that suppress ES cells conflict with these *in vitro* findings.[40] Thus, further research into the mechanism responsible for these effects is needed to resolve these discordant verdicts and to elucidate ES cells' potential as a stroke therapy.

When considering the translation of ES cells from the bench to the clinic, their propensity for tumorigenicity mars their otherwise auspicious affinity for cell replacement due to their high proliferative power.[3,41] This proliferation actually may be enhanced by stroke conditions.[37,41] ES cells' promising aspects warrant investigation into both implementing a regulator of cell proliferation to check the cells' tumorigenic tendencies and deriving stem cells that retain the same traits as ES cells from non-embryonic sources.

Taking these tumorigenic issues together with the inherent ethical concerns, ES cells may remain mostly relegated to preclinical studies until the establishment of suitable solutions. Subsequently, due to present lack of federal funding, recent research advancing the use of ES cells as a stroke therapy is severely limited. However, previous inquiries uphold the therapeutic potential of ES cells in treating stroke-induced injury. Namely, ES cells engrafted in the cerebral tissue of stroke mice traveled to the site of injury, alleviated behavioral impairments,[42] and restored blemished synaptic connections characteristic of stroke lesions.[43,44] While encouraging, these findings clearly require further development in forthcoming investigations. Additionally, transplantation of embryonic neural stem cells in a

traumatic brain injury (TBI) model of adult mice evidently attenuated neuroinflammation by reducing microglial activation and astrogliosis, while also enhancing neurogenesis.[45] Although not completely overlapping, stroke and TBI possess similar pathologies, particularly with regard to exacerbated neuroinflammation, suggesting that the induction of anti-inflammatory effects by ES cells may be mediated through similar pathways following stroke. To this end, differentiating ES cells towards an anti-inflammation-conferring cell type may enhance their utility for stroke, such as the use of curcumin nanoformulation.[46] Nonetheless, the previously discussed ethical concerns and risk of tumorigenesis associated with ES cell transplantation limit the use of these cells, and recent advancement of other stem cell types (i.e., induced pluripotent stem cells) has shifted focus towards these novel options which circumvent many of these logistical issues.

3.2. Adult tissue-derived stem cells

Although use of ES cells in both the laboratory and clinic has been largely discontinued over the past two decades due to the aforementioned lack of federal funding by the United States government, investigation of adult tissue-derived stem cells has progressed markedly in its stead. Lacking the ethical hurdles which had plagued ES cell research, advancement of adult-tissue derived stem cells to clinical trials has gained public support. However, the use of adult stem cells continues to present a number of practical challenges, the most significant being the effective derivation of an abundant and homogeneous stem cell population. Definitively establishing a set of biomarkers to phenotypically characterize adult stem cells is a topic of ongoing debate and is further discussed below.

3.2.1. Hematopoietic stem cells—Hematopoietic stem cells (HSCs) can be identified by their expression of cell surface receptor CD34+ with surrogate markers of CD150+, CD244−, and CD48−.[47] Following cerebrovascular insult such as stroke, HSCs begin circulating in the bloodstream and are recruited by cytokines to the injured brain.[48,125] Treatment with granulocyte-colony stimulating factor (G-CSF),[48] as well as with neurotransmitters that upregulate HSC populations within the bone marrow,[49,50] can increase the mobilization of HSCs to blood circulation and, in turn, their availability within the injured brain. Indeed, HSCs may be intimately linked with stroke pathology, as the extent of HSC mobilization not only directly correlates with the severity of cerebral insult,[50] but also with the degree of functional recovery.[51]

Laboratory studies to date have explored HSCs as a therapy for stroke. However, in experimental stroke with supporting evidence from clinical studies of other diseases, HSC transplantation evidently worsened neuroinflammation, inhibited functional recovery,[52-54] and may have even caused cerebral infarct.[55] While such negative outcomes may indicate that transplanted HSCs lack viability as a stroke therapeutic, one clinical trial found that HSC transplantation in stroke patients was successfully tolerated and even mitigated functional deficits.[56] These mixed results clearly warrant further investigation of HSC transplantation for stroke therapy. Elucidating the intrinsic cellular properties of HSCs and the extrinsic microenvironment that influences their fate after transplantation may help to reveal a means of suppressing the pro-inflammatory phenotype of HSCs while optimizing their anti-inflammatory effects against secondary neuronal cell loss following stroke.

Current efforts reveal the advantages and disadvantages of HSC treatment in stroke therapy. Treating mice with hematopoietic stem and progenitor cells (HSPC) after transient MCAO dampens cellular responses, including inflammation, for up to a week post-operation.[57] Additionally, HSPCs afford protection against the residual neuroinflammation.[57] That metallothionein I may serve as the mechanism behind these effects advances the importance of these findings.[57] On the other hand, damage in stroke tissue may prompt HSPC activity among bone marrow-generating myeloid cells. Continuous HSPC activation may incite long-term inflammation and inhibit the tissue recovery process. A murine hindlimb ischemia model demonstrates HSCs' bidirectional effect on inflammation. Specifically, HSPCs may exacerbate the ischemia-induced inflammation through their monocyte activation.[58] Nox 2 may suppress these adverse effects, positing it as a possible method of exploiting the otherwise anti-inflammatory and therapeutic aspects of HSPCs.[58] While there is a dearth of recent studies measuring inflammation after HSC transplants in stroke therapies, coupling these two studies still exemplifies the contradictory nature of HSCs warranting further investigations.

3.2.2. Bone marrow-derived stem cells—Bone marrow has been well established as a tissue source of numerous types of stem cells, known in general as bone marrow-derived mesenchymal stem or stromal cells (BM-MSCs). Specific subgroupings of BM-MSCs have been characterized and designated as endothelial progenitor cells (EPCs), notch-transfected mesenchymal stromal cells (SB623), multipotent adult progenitor cells (MAPCs), and multilineage-differentiating stress enduring (Muse) cells. These BM-MSCs have been previously examined in experimental stroke models and are currently being assessed in multiple clinical trials. While peripheral delivery of BM-MSCs is effective due to the cells' capacity to migrate to the injured brain,[59] a number of studies have also tested the direct delivery of BM-MSCs into the stroke-damaged brain.[60,61]

BM-MSCs can be derived and harvested not only from bone marrow, but also from practically any tissue type, and these stem cells are identified by the phenotypic markers CD29+, CD44+, CD105+, CD73+, CD90+, CD106+, CD166+, CD14−, CD34−, and CD45−.[60,93] Further, BM-MSCs display potential to differentiate into osteogenic, chondrogenic, and adipogenic cells,[62] as well as the ability to enhance functional recovery following transplantation in stroke animals.[63] BM-MSCs have been hypothesized to exert their effects via various mechanisms of action, including the secretion of neurotrophic factors,[64-66] stimulation of angiogenesis,[93,63] and promotion of endogenous neurogenesis by recruiting host stem cells from the subventricular zone (SVZ) and subgranular zone (SGZ) to the injury site.[93]

Numerous studies have thoroughly outlined the therapeutic benefits of BM-MSCs in experimental models of stroke. Stroke animals treated with transplanted BM-MSCs exhibited behavioral improvements that concurred with elevated angiogenesis, synaptogenesis, and neurogenesis.[64-68] Likewise, the potent anti-inflammatory effects of BM-MSCs have been well described in stroke models and included the modulation of T-cell proliferation rates, activation of regulatory T-cell (Treg) phenotype CD8+CD28−, and the inhibition of CD4+ and CD8+ T-cells.[69-71] Moreover, transplanted BM-MSCs upregulated expression of anti-inflammatory cytokines, such as interleukin (IL)-4, IL-10, and

transforming growth factor-β1, while downregulating levels of pro-inflammatory cytokines, such as IL-1β and IL-6, and decreasing microglial activation within the stroke brain.[69,153,72,73]

In vitro studies have provided further evidence supporting the therapeutic benefits of BM-MSCs. Specifically, BM-MSCs protected primary neural cell cultures against stroke damage by limiting apoptosis and necroptosis, decreasing expression of necroptosis-related receptor interacting protein kinase 1 and 3, and deactivating caspase-3, an enzyme which plays a key role in apoptosis.[68] Recent data also indicate that BM-MSCs are capable of secreting therapeutic exosomes.[64,66,74,75] In this regard, BM-MSCs reduced neuroinflammation by blocking the activation of microglia and lowering levels of astrogliosis in organotypic hippocampal cultures exposed to stroke insult.[76] Finally, the activity of T-cells or natural killer cells[77] and leukocyte proliferation[78,79] were both restricted in the presence of BM-MSCs as well.

Although generally considered safe, use of BM-MSCs in some animal models increases the risk of tumor development.[80] which may be linked to a tumor-inducing factor secreted by these cells.[81,82] In fact, the tissue substrate from which BM-MSCs are grown and derived may determine their tumorigenicity.[83,84] Thoroughly clarifying the therapeutic mechanism mediated by BM-MSCs may illuminate a solution to the tumorigenic risks of BM-MSC transplantation. Furthermore, direct investigation of the host microenvironment, particularly its genetic composition, may provide critical insight into refining BM-MSC transplantation as a stroke therapy. Additionally, preconditioning BM-MSCs in a hypoxic environment may improve their proliferation, angiogenic effects, and migration capacity.[85,86] In similar fashion, altering the host microenvironment via hyperbaric oxygen therapy could enhance BM-MSC migration and their anti-inflammatory effects.[87,88] Genetically engineering BM-MSCs may improve their post-stroke benefits as well.[89-93]

Recent laboratory investigations provide further evidence of the anti-inflammatory properties of BM-MSCs in stroke. Intraventricular infusion of BM-MSCs in hemorrhagic stroke rats suppresses inflammation by decreasing mean expression levels of pro-inflammatory cytokines IL-1α, IL-6, and IFN-γ, suggesting that transplanted BM-MSCs attenuate inflammation by altering cytokine expression.[94] Furthermore, combined treatment of BM-MSCs and peroxisome proliferator-activated receptor gamma (PPARγ) agonist pioglitazone (PGZ) advances that PGZ-treated rats' increased PPARγ expression confers anti-inflammatory effects mediated by its mobilization of transplanted allogeneic BM-MSCs, indicating further that BM-MSCs reduce inflammation.[95] Additionally, male MCAO rats display decreased numbers of inflammatory M1-like macrophages and significantly downregulated expression of inflammatory IL-6 and caspase-3, though not of inflammatory IL-1β and tumor necrosis factor-α (TNF-α) after combined treatment.[95] Echoing this robust anti-inflammatory action of the cells, rats treated with extracellular regulating kinase 1/2 (ERK1/2)-overexpressing BM-MSCs demonstrate better neurological and functional outcomes and reduced levels of CD68, signifying suppression of microglial activation.[96] However, the number of reactive astrocytes and levels of TNF-α were elevated in the ERK/BM-MSC treatment group, demonstrating that ERK1/2 overexpression may differentially modulate inflammatory factors.[96] Further research concerning the pro- and

anti-inflammatory mechanisms of ERK1/2 and BM-MSCs is needed to clarify the connection between increased astrocyte activation and upregulation of TNF-α, as well as the aforementioned unchanged levels of IL-1β and TNF-α. In addition, native populations of Tregs have been identified in BM-MSC samples.[97] This finding suggests a possible mechanism of action for BM-MSCs' capacity to reduce neuroinflammation. Overall, BM-MSCs demonstrate promising therapeutic potential as mediators of anti-inflammation in animal models of stroke.

3.2.3. Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are characterized by their expression of CD34 or CD133 and endothelial cell markers, such as protein vascular endothelial growth factor receptor 2 (VEGRF2), Von Willebrand factor, vascular endothelial cadherin (VE-cadherin or CD144), CD31, Tie2, c-kit/CD117E, and CD62E (E-selectin).[98] EPCs can be classified as early EPCs or late EPCs,[99,100] with the former being harvested after culturing mononuclear cells from peripheral blood for 4–10 days, and the latter, also known as endothelial colony forming cells (ECFCs), being harvested after culturing mononuclear cells for >14 days. Late EPCs highly express VE-cadherin and kinase insert domain receptors, while early EPCs secrete angiogenic growth factors.[99] In the context of stroke, major vascular dysfunction may be treatable via EPC transplantation, as EPCs facilitate neovascularization[98,101,102] and thus promote angiogenesis following stroke.[101] Additionally, the quantity of circulating EPCs may correlate with the extent of post-stroke recovery for large-artery atherosclerosis and small-vessel disease etiologic subtypes.[103]

Possibly through mechanisms of vasculogenesis and angiogenesis,[98,104-106,114] EPCs may ameliorate cerebral microvascular density, regional cortical blood flow, and behavioral recovery for stroke animals.[107,108,109] Although EPCs may be inactive during homeostasis, chemoattractant signaling pathways, such as stromal-derived factor 1[110] or granulocyte-stimulating factor, guide EPCs to the stroke-damaged brain.[98] Cerebral infrastructure repair, an EPC-mediated process, seems to be linked to anti-inflammatory effects,[111,112] as transplanted EPCs preserve mitochondrial morphology in endothelial cells of the stroke brain, reduce perivascular edema, promote therapeutic pinocytotic activity, and reinforce the integrity of microvessels, including those of the BBB.[113] When EPCs are supplemented with p38 mitogen-activated protein kinase inhibitor, neuroinflammation decreases as well.[114] However, EPCs may paradoxically worsen neuroinflammation through the release of pro-inflammatory molecules.[98,99,115,116] Certain *in vitro* models have suggested mechanisms by which EPCs mediate neuroinflammation, including downregulation of tumor necrosis factor-α-induced apoptosis and caspase-3 activity.[117] Nonetheless, further examination is necessary to determine whether EPCs suppress or induce neuroinflammation following stroke insults *in vivo*.[98,99,115,116]

Accumulating evidence suggests that EPCs may aid in combating endothelial dysfunction, a major component inherently linked with inflammation in stroke pathology, to preserve vascular homeostasis and enhance neurological and functional recovery.[118] Recent studies investigating the effects of EPCs on the inflammatory stroke vasculature reveal an elevation of inflammation-associated genes IκB, Foxf1, ITIH-5, and BRM with OGD, yet co-culture

of EPCs impeded this upregulation, suggesting the potential of EPCs to modulate this rampant vascular inflammation *in vitro*.[119] Further, intracerebral administration of EPCs *in vivo* to the striatum and cortex 4 hours following stroke revealed substantial behavioral improvement and rehabilitation in comparison to vehicle-treated stroke animals.[119] Seven days following transplantation, a fluorescent staining procedure uncovered a considerable increase in endothelial marker RECA1 and a declining amount of specific inflammation-related vasculome genes in the ipsilateral cortex and striatum of EPC-administered stroke animals, further suggesting key inflammatory-based mechanisms underlying EPCs effects in stroke.[119] Moreover, emerging data document heightened amounts of early EPCs in acute stroke patients.[120] The negative impact of inflammatory factors on EPC continuity and differentiation was displayed by the contradictory relationship between inflammatory parameters and EPCs,[120] advancing a vital mechanism connecting inflammation and stroke pathology. Moreover, current laboratory investigations report a novel development of regeneration-associated cells (RACs) for transplantation in stroke animals, which not only promote EPC differentiation, but also induce anti-inflammatory monocytes/macrophages,[121] thus indicating potential EPC-mediated environmental regulation involving inflammatory mechanisms. Recent efforts aimed to elucidate the effects of EPCs on neuroinflammation in stroke also suggest that levels of alpha-2-macroglobulin (a2MG), a key protein mediating the inflammatory state in stroke, may be correlated with EPC numbers[122] Altogether, this emerging data implicate that EPC therapy may largely exert therapeutic effects by modulating the robust inflammatory response to stroke.

3.2.4. Very small embryonic-like stem cells—Very small embryonic-like stem cells (VSELs) uniquely express Sca-1+, CD45−, and pluripotent stem cell markers SSEA-1, Oct-4, Nanog, and Rex-1.[123,124] Similar to embryonic stem cells, VSELs possess a large nucleus-to-cytoplasm ratio and can be distinguished by the single type of chromatin (euchromatin) within their nuclei.[124,125] In the aftermath of an stroke event, VSELs are mobilized from adult tissues to circulation in peripheral blood, mirroring the migratory behavior of endogenous HSCs.[125] Considered epiblast-derived pluripotent stem cells, VSELs enter dormancy after embryonic development, yet during aging and pathological conditions, they can function as a reservoir of therapeutic stem cells.[125] Furthermore, VSELs may be transplantable for stroke therapy,[126] as they exhibit the capacity to differentiate into neurons, oligodendrocytes, and microglia.[127] Relative to HSCs, VSELs were highly resilient to radiation damage,[125] and further data suggest that VSELs are mobilized to blood circulation after tissue injury, including hypoxia.[128,129] Moreover, in one mouse model, exogenous VSELs transplanted after myocardial infarction enhanced ventricular function and cardiac remodeling.[130] In an animal model in which cerebral injury was induced by the neurotoxin kainic acid, VSELs were recruited from the bone marrow.[126] Notably, upregulation of circulating VSELs has been observed following stroke, indicating their potential as minimally invasive stroke therapeutics via migration from the periphery to the injury site within the brain.[131] Additionally, cultured VSELs exhibited a strong chemotactic attraction to HGF, SDF-1, and leukemia inhibitory factor,[128] thereby emphasizing the cells' migratory potential to reach stroke brain sites from their initial peripheral location.[124] Evidently, the early mobilization of VSELs into circulation reflects the cells' prominent function in disease onset and progression, particularly with regard to

neuroinflammation. Although VSELs may secrete pro-inflammatory cytokines, such as IL-6, IL-8 and chemokine (C–C motif) ligand 5,[132] they may also hold an immunoregulatory role, a discrepancy which requires future studies to clarify VSELs' true potential as a treatment for stroke-induced neuroinflammation.

Recent laboratory investigations evaluating the transplantation of VSELs to target stroke-induced neuroinflammation remain limited, but current data provide some insights on the relationships between VSELs and inflammation, in that stimulation of inflammasome NIrp3 in an ATP-dependent method coordinates the mobilization of VSELs from the bone marrow into the peripheral blood,[133] suggesting an inflammatory-based mechanism that may link stroke-induced tissue damage with VSEL recruitment. Interestingly, recent evidence also reveals that patients with IgA nephropathy possess high levels of circulating VSELs and classic anti-inflammatory M2-like monocytes, further advancing a connection between VSEL mobilization in response to inflammatory activation.[134] VSEL transplantation therapy targeting the inflammatory-plagued response may thus represent a novel avenue for stroke therapy, yet the role of this stem cell type in immunoregulation warrants much future investigation before extending this therapy to the clinic.

3.3. Neural stem cells

Neural stem cells (NSCs), whether derived from embryonic, fetal, or adult tissues, are a class of multipotent cells that can give rise to various neural phenotypes.[135] Thus, their differentiation capacity makes them an intriguing transplantable cell type for stroke therapy with the disease pathology linked to a defective neurovascular unit. Displayed by their upregulation after stroke insult,[136] SGZ and SVZ serve as major brain tissue sources of NSCs.[137] Although NSCs are initially grown in culture with basic fibroblast growth factor and epidermal growth factor,[137,138] removal of these cytokines allows NSCs to differentiate into neurons, astrocytes, and oligodendrocytes.[135] Concerning their therapeutic benefits, NSCs induce angiogenesis[139] and neurogenesis[138] in stroke models. Based on previous studies, overexpression of miR-381, nuclear factor-kappa B (NF-κB), Nox4-generated superoxide levels,[135,140,141] and ephrinB2 and Jagged1[142] may influence the activity of NSCs. Also important to note, NSC-associated exosomes can regulate inflammation by releasing neuroprotective compounds such as VEGF, NGF, BDNF, and neurotrophins.[143] Assuredly, further study is required to elucidate the interaction between NSC exosomes and secreted growth factors, as well as the mechanism by which this interaction mediates anti-inflammatory effects. While NSC differentiation was evidently altered by pro-inflammatory T-cells,[144] intravenous transplantation of NSCs in stroke animals reduced cerebral and splenic expression of TNF-α, IL-6, and NF-κB while also reducing levels of OX-42+microglia and MPO+ infiltration into the injury site.[145] Furthermore, an acute anti-inflammatory effect resulted from injection of NSCs into the ipsilesional hippocampus, as this infusion downregulated expression of pro-inflammatory factors (TNF-α, IL-6, IL-1β, MCP-1, MIP-1α), diminished numbers of adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1), attenuated microglial activation, and ameliorated BBB permeability.[146] Future research on NSCs is needed to fully illuminate their anti-inflammatory properties in the context of stroke. Moreover, safety concerns regarding the use of NSCs in stroke primarily center upon their possible tumorigenicity.[8]

Thus, future clarification of the cells' mechanisms of action will lead the way towards safe and effective use of NSC transplantation as a treatment for stroke.

Emerging evidence provides further support for NSCs' anti-inflammatory role. Alongside other functional improvements, NSC treatment successfully lowers inflammation by controlling levels of anti-inflammatory cytokine TIMP-1 and pro-inflammatory cytokines leptin, L-selectin, MCP-1, and TNF-α in a permanent MCAO rodent model of stroke.[147] Another rat model produced similar results, observing that NSC treatment increases expression of anti-inflammatory cytokines IL-10 and TGF-β1 and decreases expression of pro-inflammatory cytokines IL-1β, IL-6, IFN-γ and TNF-α.[148,149] Interestingly, this comes on the heels of the revelation that low doses of IFN-γ can produce greater neuroregenerative effects when co-injected with NSCs than NSCs alone.[148] That the combined treatment activated the IFN-γ/Stat1 signaling pathway provides a possible mechanism for these benefits, with IFN-γ enhancing the potency of this "help me" signal.[148,148] NSC extracellular vesicles (EV) may also confer similar effects on neuroinflammation. In a middle-aged murine thromboembolic stroke model, NSC EV treatment inhibited pro-inflammatory Th17 cells through its upregulation of Tregs and encouraged macrophages to adopt their anti-inflammatory M2 phenotype.[150] While more research is needed, these findings depict a multifaceted effect of NSCs on stroke-induced neuroinflammation.

3.4. Extraembryonic stem cells

Umbilical cord blood-derived MSCs (UCB-MSCs) and placenta-derived MSCs predominantly comprise the extraembryonic stem cells types. Investigated in experimental models of stroke, these extraembryonic MSCs exert therapeutic benefits involving the substitution of damaged cells and alterations of the stroke environment[151-154] by improving neurogenesis, enhancing vascular structure, and secreting growth factors.[152,155] Moreover, these extraembryonic MSCs may modulate the inflammatory response, owing to their immunosuppressive effects within the innate immune system,[151,156] and specifically via secretion of leukemia inhibitory factor (LIF).[157]

UCB-MSCs have demonstrated remarkable potential thus far in autologous and allogeneic transplantation models, as well as in clinical trials.[158] UCB-MSCs remain an attractive option due to their immunological immaturity[156] and their capacity to dampen the immune response via reduction of pro-inflammatory factors following transplantation.[159,160] Transplantation of umbilical cord blood in stroke animals reduces infarct volume, enhances motor function, and increases neurotrophic factor expression.[155,161-164] UCB-MSCs may prevent the mobilization of immune cells to the damaged region.[164] Likewise, placenta-derived MSCs may attenuate neuroinflammation by improving microglial survival.[136,162] UCB-MSCs may also possess similar anti-inflammatory properties[165] as evidenced by reduced proliferation of B-cells and T-cells and modulated monocytes with the co-culture of UCB-MSCs.[165,166] Along this line, placenta-derived MSCs dampened allogenic T-cell proliferation.[151] Altogether, these findings suggest the potential of extraembryonic stem cells to attenuate the rampant inflammatory response induced by stroke.

The transplantation of placenta-derived MSCs has been explored in experimental models of stroke, yet only placental MSCs derived from the maternal line has exhibited significant

improvements.[167] Future direction should thus investigate the restrictions of maternally derived placental MSCs effects, and if these findings apply to UCB-MSCs as well. Recognizing the therapeutic benefits of UCB for stroke mediated by estradiol[162] also provides grounds for further studies examining combination therapies with extraembryonic stem cells. Moreover, thoroughly investigating the capacity of extraembryonic stem cells to dampen neuroinflammation is necessary to advance their application in stroke.

Promisingly, a recent Phase 1 safety clinical trial has reported that intravenous administration of unrelated allogeneic UCB cells, matched for blood group antigens but not for human leukocyte antigen, was not only well-tolerated by stroke patients, but also improved their neurological and functional performance at 3 months post-treatment.[168] Novel laboratory investigations utilizing extraembryonic stem cells have also expanded upon past rodent models in a variety of ways. Using four animal models of stroke including non-human primates, a preclinical study determined that human amnion epithelial cells, a subset of placenta-derived stem cells, were selectively recruited to the spleen and injured brain, where they promoted functional recovery by reducing apoptosis, inflammation, and infiltration of peripheral immune cells into the brain.[169] On another note, brain transcriptome profiling analysis in rats revealed that, of the 275 genes differentially expressed following ischemia, 220 returned to normal expression levels following UCB-MSC treatment and primarily involved downregulation of post-stroke immune response activation.[170] Studies using UCB-MSCs in combination therapies have also produced notable results. Although effective as alone treatments, combined administration of UCB-MSCs and erythropoietin in MCAO rats resulted in the most significant behavioral improvements, most elevated levels of neurogenesis and angiogenesis, and most diminished levels astrogliosis.[171] Similarly, stroke mice treated with UCB-MSCs in conjunction with nitrogen-doped carbon nanocages exhibit synergistic benefits with regard to the reduction of infarct volume, behavioral improvements, and attenuation of inflammation, as well as downregulation of TNF-α expression, upregulation of IL-10 expression, and elevation of TNF-α stimulated gene/protein 6 and prostaglandin 2 levels in lipopolysaccharide-treated microglia.[172] Collectively, these findings suggest that extraembryonic stem cells, whether alone or in combination, may represent an optimal approach to treating stroke due to their potent anti-inflammatory and immunomodulatory effects.

3.5. Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are a type of pluripotent stem cell which can be generated from fibroblasts via retrograde manipulation to be reprogrammed back into an embryonic-like pluripotent state enabling the generation of an unlimited source of any type of human cell needed for therapeutic purposes.[173] This property has then been applied to umbilical cord blood cells, placental mesenchymal stromal cells, adipose-derived stem cells, and neural stem cells.[174,175]

The improved proliferative function of these precursor cells over adult cells marks a key appealing feature of retrograde conversion.[173] Transplantation of iPSCs in stroke animals reduces infarction and enhances functional improvements, likely via observed downregulation of pro-inflammatory factors and upregulation of anti-inflammatory factors.

[176,177] That iPSCs possess anti-inflammatory characteristics is bolstered by findings of reduced activated microglia, decreased inflammatory cytokines (TNF-α, IL-6, IL-1β, MCP-1, MIP-1α), and enhanced BBB integrity in stroke.[178] Moreover, cultured iPSCs can differentiate into microglia[179] as well as antigen-specific Tregs,[180] both key components of immunoregulation in the brain. These findings suggest that iPSCs may exert therapeutic benefits in stroke by differentiating into these anti-inflammatory cells. This utility may be extended to exploit the secretion of anti-inflammatory factors to further enhance stroke recovery. iPSCs thus hold remarkable potential in their ability to dampen inflammation following stroke.

The risk of tumorigenesis remains a valid issue when contemplating the use of iPSCs for stroke. Oncogenic transcription factors are involved in the transfection procedure to generate iPSCs[3] indicating a high potential of oncogenesis. High tumorigenicity exists with undifferentiated iPSCs compared to other known cells transplanted to the stroke brain,[181] and iPSCs may promote teratoma formation in the stroke tissue.[41,181] Another concern involving transplantation of these cells is immunogenicity. iPSCs may activate an immune response, suggesting an increased risk of rejection.[182] Elucidating the risk of both tumorigenic and immunogenic effects limiting their transplantation is necessitated to support iPSCs as a viable therapy for stroke.

While direct transplantation of iPSCs has been previously explored,[176,177] recent investigations have primarily focused on pre-differentiating iPSCs towards a neuronal phenotype prior to transplantation in experimental models of stroke.[183] Current efforts utilizing iPSCs as a source for derivation hence aim to prevent tumorigenesis and maximize neuronal differentiation by exploring adjunctive therapies. Preconditioning human iPSC-derived neural stem cells (hiPSC-NSCs) with FDA-approved drug Metformin prior to transplantation reveals enhanced differentiation, improved graft survival, decreased infarct size, and attenuated motor dysfunction.[184] In addition to combination drug therapies, selective excitation of transplanted cells using optochemogenetic fusion protein luminopsin 3 (LMO3) promotes neural network connectivity and functional recovery following stroke.[185] While the majority of these studies examining iPSCs for stroke have employed hiPSCs,[183] innovative studies recognizing the unique cellular preservation of hibernating mammals support the use of ground squirrel iPSCs (GS-iPSCs). These newly reported GS-iPSCs possess cold-adaptive properties such as microtubule stability to relieve mitochondrial stress and limit reactive oxygen specific (ROS) production,[186] which may broaden the application of novel combination therapies for stroke involving stem cells and hypothermia for neuroinflammation. The inflammatory properties of iPSCs have been further explored in recent laboratory investigations implicating a role of apolipoprotein allele epsilon 4 (ApoE4), a prominent genetic risk factor for stroke, with ApoE4-positive iPSCs acquiring a pro-inflammatory state.[187] Further elucidating the inflammatory components underlying transplantation of iPSCs, as well as exploration into adjunctive therapies, will likely promote the safety and efficacy of iPSCs for stroke.

4. Conclusion

While basic science and clinical research have improved our grasp on stroke pathology, this disease possesses limited treatment options and presents a major health and economic burden globally.[188] Neuroinflammation represents a key component in the secondary cell death cascade associated with both acute and chronic stroke, but, in turn, marks a potential strategy for therapeutic efforts.[3,10,12,18,23] Dampening the rampant inflammatory response to stroke may thus be a key to improving functional outcomes. Stem cells present novel therapeutic potential in this regard due to their regenerative nature, wide administrative window, and anti-inflammatory capabilities demonstrated in *in vitro* and *in vivo* models (Fig. 1). Indeed, some stem cell lines have advanced to clinical trials for stroke.[56,189-195] Despite their potential to sequester inflammation and promote regeneration, future investigations are warranted to resolve moderate efficacy and safety issues such as tumorigenesis and immunogenicity to advance these stem cells as a viable treatment option in the clinic. Revealing the full potential of stem cell therapy for CNS diseases such as stroke must adhere to the Stem cell Therapeutics as an Emerging Paradigm for Stroke or STEPS guidelines to improve translation from bench to bedsideyy.[31,196,197] Altogether, stem cell therapy targeting the neuroinflammatory cascade presents remarkable potential as a stroke therapy.

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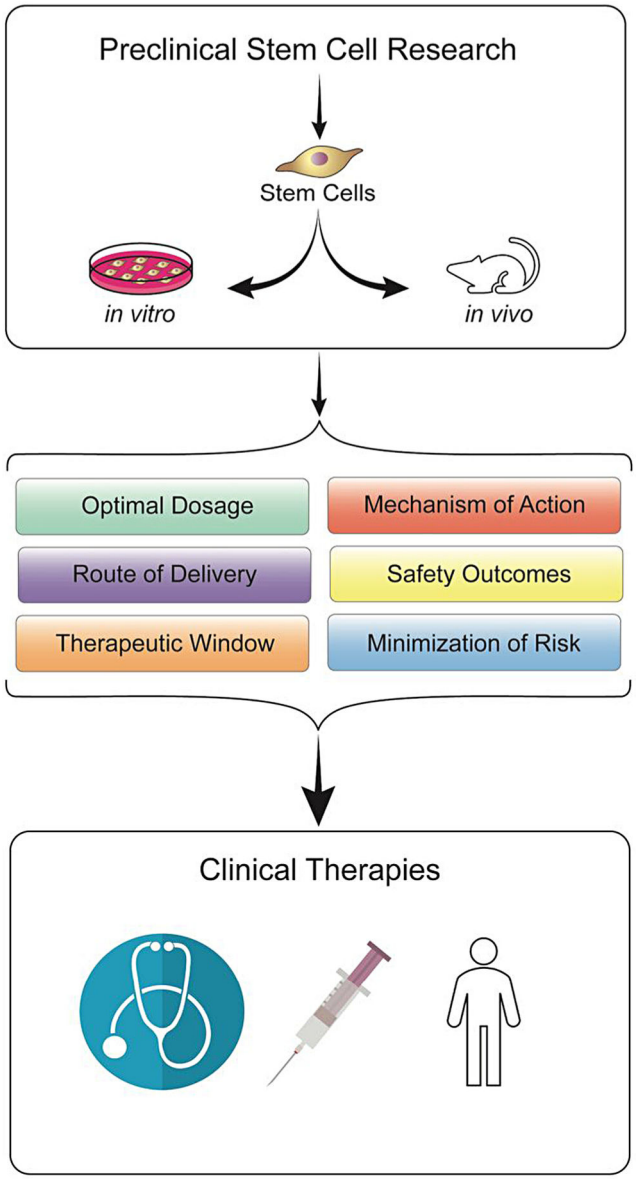

Fig. 1.

Translation of stem cell therapy from the laboratory to the clinic. Stem cell transplantation has emerged as a potent stroke therapy as evidenced by *in vitro* and *in vivo* stroke models. Translational research investigations are necessary to optimize the stem cell transplant regimen in order to enhance the efficacy and safety of its clinical application for stroke patients.