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Review

Microglia and Stem Cells for Ischemic Stroke Treatment—Mechanisms, Current Status, and Therapeutic Challenges

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Abstract

Ischemic stroke is one of the major causes of death and disability. Since the currently used treatment option of reperfusion therapy has several limitations, ongoing research is focusing on the neuroprotective effects of microglia and stem cells. By exerting the bystander effect, secreting exosomes and forming biobridges, mesenchymal stem cells (MSCs), neural stem cells (NSCs), induced pluripotent stem cells (iPSCs), and multilineage-differentiating stress-enduring cells (Muse cells) have been shown to stimulate neurogenesis, angiogenesis, cell migration, and reduce neuroinflammation. Exosome-based therapy is now being extensively researched due to its many advantageous properties over cell therapy, such as lower immunogenicity, no risk of blood vessel occlusion, and ease of storage and modification. However, although preclinical studies have shown promising therapeutic outcomes, clinical trials have been associated with several translational challenges. This review explores the therapeutic effects of preconditioned microglia as well as various factors secreted in stem cell-derived extracellular vesicles with their mechanisms of action explained. Furthermore, an overview of preclinical and clinical studies is presented, explaining the main challenges of microglia and stem cell therapies, and providing potential solutions. In particular, a highlight is the use of novel stem cell therapy of Muse cells, which bypasses many of the conventional stem cell limitations. The paper concludes with suggestions for directions in future neuroprotective research.

Keywords: ischemic stroke; stem cells; exosomes; neuroinflammation; microglia; neuroprotection; miRNA; bystander effect; extracellular vesicles; neurogenesis

1. Introduction

1.1 Stroke Statistics and Current Treatment Options

There are over 12 million stroke cases each year worldwide and over 6.5 million people die from stroke annually [1]. According to DALY (disability-adjusted life year), which is an indicator that measures the overall burden of the disease [2], more than 143 million years of healthy life are lost annually due to stroke-related death and disability [1]. As reported in the thirty-year projections of stroke incidence, prevalence, deaths, and disability-adjusted life years, there was a 27% increase in the number of people living with a stroke estimated between 2017 and 2047 in the European Union [3].

Current methods of reperfusion therapy include intravenous thrombolysis and mechanical thrombectomy. However, they are associated with several adverse effects, such as the risk of intracranial hemorrhage, allergic reactions, hypotension, risk of bleeding, acute kidney injury [4], emboli, vessel dissections, and vasospasms [5], while also having narrow therapeutic time windows [6], which means that only a small percentage of stroke patients are able to benefit from such treatment [7]. Therefore, there is a great need for the development of neuroregenerative and neuroprotective methods.

1.2 Stroke Pathophysiology

The occlusion of cerebral and precerebral arteries caused primarily by either atherosclerotic plaques in large vessels, microatheromatosis, or cardioembolism [8] leads to anoxia and activation of anaerobic metabolism, which causes the formation of lactic acid and contributes to the dysregulation of the acid–base balance and cell destruction [9]. Reduced adenosine triphosphate (ATP) production leads to the impaired function of ion pumps, the outflow of K^+ ions from cells, and the influx of Na^+ and Ca^{2+} ions into cells. Depolarization of neurons contributes to the release of glutamic acid from synaptic terminals, thereby causing excitotoxicity by increasing the influx of calcium ions into the cell, which in turn leads to the activation of enzymes that digest proteins, lipids, and nucleic acids. Oxygen free radicals that are generated as a result of lipid degradation of the cell membrane and mitochondrial dysfunction contribute to the destruction of DNA, proteins, and lipid peroxidation, thereby causing cell death [10]. The increased concentration of Na^+ ions inside the cell entails the influx of water, which leads to cell edema, increased pressure on vessels and brain tissue, increased permeability of the blood-brain barrier [11,12], and the infiltration of immune cells that release proinflammatory cytokines [13]. Microglia play an important role in the development of neuroinflammation, by, on the one hand, removing damaged

cells and, on the other, releasing cytokines and cytotoxic substances, which makes them a potential target for neuroprotective therapy [14].

1.3 Microglia Characteristics

Until the development of photon imaging, genome-wide transcription analysis, and epigenetic analysis, microglia were classified into two main phenotypes [15]: classically activated M1 with proinflammatory properties that release tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), IL-6, IL-12, IL-23, and inducible nitric oxide synthase (iNOS); alternatively activated M2 releasing anti-inflammatory cytokines: IL-4, IL-10, IL-13, transforming growth factor beta (TGF-β), and growth factors—vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), platelet-derived growth factor (PDGF), and nerve growth factor (NGF) [16]. In addition to their proangiogenic and anti-inflammatory properties, M2 microglia were associated with removing damaged neurons [17], stimulating the repair of the extracellular matrix [18], and regulating neurogenesis by modulating synapse maturation, forming dendritic spines, and producing key trophic factors for migration of new neurons [16,19]. However, such a division into M1 and M2 microglia is now considered as an oversimplified one since microglial cells were discovered to be a significantly more heterogeneous group, where every individual cell exerts a different function. Their surface markers were found to be insufficient to define their functions because different states of microglia are dynamic and depend on the changes in the local environment [20]. In the pathological stages, microglia were observed to change their molecular profile, morphology, ultrastructure, motility, and function [15,20,21]. The main purpose of microglia cell therapy in stroke treatment is to prevent their excessive activation and the production of proinflammatory molecules [22], while also inducing their protective phenotype.

1.4 Ischemia-Induced Neurogenesis

Neurogenesis involves the proliferation of neural stem cells, migration of neuroblasts, differentiation of neuroblasts into neurons, development of synaptic connections with other neurons, and survival of immature and mature neurons [7,23]. In an adult brain, neurogenesis takes place mainly in two regions: in the subgranular zone of the hippocampal dentate gyrus (SGZ) and in the subventricular zone (SVZ) along the lateral ventricles [24].

Neurogenesis can be stimulated by an ischemic episode. Unfortunately, most neurons formed in this way die within about 2 weeks of a stroke. The low survival rate of new neurons is thought to be due to a lack of trophic factors and chronic neuroinflammation [24].

One of the factors stimulating neurogenesis in an adult brain is vascular endothelial growth factor (VEGF), which inhibits apoptosis of hippocampal neurons, stimulates stem cell proliferation and migration of newly formed cells via the vascular endothelial growth factor receptor 2 (VEGFR2) pathway, and stimulates angiogenesis and the repair of damaged neurons [25]. The stroke-induced blood–brain barrier disruption facilitates the contact between adult neural stem cells (NSC) and vascular cells, including VEGF. It was shown that VEGF induces the expression of the Notch ligand Delta-like 4 (DLL4) via its receptor VEGFR2, which leads to the proliferation and differentiation of NSCs into neurons [26]. Angiopoietin-like 4 (ANGPTL4) protein, which is released by vascular cells as a result of hypoxia, has been shown to stimulate neurogenesis in the dentate gyrus of the hippocampus and subventricular zone by stimulating Akt kinase activity and it also reduces the inflammatory response and neuronal death by inhibiting Fas expression and the Fas ligand (FasL) [27]. Fibroblast growth factor 2 (FGF-2) stimulates the proliferation and differentiation of neural progenitor cells derived from the subventricular zone. It was shown that the infusion of FGF-2 into the rats' lateral ventricles increased the proliferation and migration of neurons from the subventricular zone to the olfactory bulb, and the injection of FGF-2 neutralizing antibodies contributed to the inhibition of their proliferation. Moreover, fibroblast growth factor also stimulates post-ischemic neurogenesis in classically non-neurogenic areas, such as the striatum, substantia nigra, and cerebral cortex [28]. The stromal cell-derived factor 1 and C-X-C chemokine receptor type 4 and 7 (SDF-1/CXCR4/CXCR7) signaling pathway stimulates axonal elongation and branching, remyelination, and the migration, proliferation, and differentiation of neuronal progenitor cells [29]. Moreover, insulin-like growth factor 1 (IGF-1) and brain-derived neurotrophic factor (BDNF) also enhance stem cell proliferation. Monocyte chemotactic protein (MCP-1) and matrix metalloproteinases 2, 3, and 9 (MMP) stimulate the migration of neuroblasts [30].

1.5 Stem Cell Mechanisms of Action

Although initially it was proposed that transplanted stem cells directly replace neurons in ischemic regions, currently, it is postulated that their therapeutic effect is mainly the result of their paracrine action (the "bystander effect") since most of the systemically injected stem cells are trapped in the lungs and do not reach the affected tissues [31]. The paracrine action involves the secretion of factors that stimulate endogenous neurogenesis (BDNF, FGF, angiopoietin 2), angiogenesis (VEGF, angiopoietin 2), and neuroplasticity (integrin β1) [7]. In addition, it has been shown that transplanted stem cells have immunomodulatory properties and, by modulating the levels of TNF-α, IL-1β, IL-6, and monocyte chemoattracting protein 1 (MCP-1) [32,33], they reduce the post-ischemic inflammatory response and contribute to the reduction in nerve tissue damage. The main mechanism through which mesenchymal stem cells exhibit their paracrine properties is exosome secretion [34]. Exosomes are extracellular vesicles,

which are secreted by almost every cell type and play a key role in intercellular communication [35–37]. Their applications have been extensively studied in many medical fields, including primarily oncology and cardiology, where microRNA (miRNA) can modulate angiogenesis and tumor progression [38], in addition to inhibiting inflammation in cardiac ischemic diseases [39]. They contain various proteins, including cytokines, chemokines, growth factors, and membrane receptors as well as miRNAs through which they promote neurogenesis, angiogenesis, and cell growth and reduce inflammation, oxidative stress, and cell death [35].

In 2013, a new mechanism through which transplanted stem cells exert their therapeutic functions was proposed. Exogenous cells were found to form "biobridges" between the neurogenic area (subventricular zone, SVZ) and the ischemic area, thereby facilitating the successful migration of endogenous stem cells, which is one of the key limitations in the endogenous repair system. Biobridges, consisting of metalloproteinases (MMP) and an extracellular matrix (ECM), form a pathway that helps direct the migration of endogenous stem cells to the damage zone through non-neurogenic brain areas [40]. Increased activity of MMP-9 along the formed biobridges was demonstrated, and its inhibition was shown to impair cell migration from the SVZ to the cerebral cortex, suggesting a key role of metalloproteinase 9 in ECM remodeling. Interestingly, once the exogenous stem cells form biobridges, their concentration decreases and they are replaced by endogenous cells derived from neurogenic areas of the brain; thus, making their long-term administration potentially unnecessary [41]. Moreover, an increase in endogenous cell proliferation and neural differentiation in the peri-injured cortical areas was demonstrated, which further suggests that the transplantation of exogenous stem cells and biobridge formation can facilitate endogenous repair mechanisms. As mentioned before, ischemia-induced endogenous post-stroke neurogenesis itself is insufficient because of low stem cell survival and migration rates, incomplete integration in neural circuits, and increased differentiation to glial cells [42]. However, more studies explaining the mechanisms underlying the migration pathways and their implications in stroke therapy are still needed.

The stem cells most frequently used in medical research are mesenchymal stem cells [43], which exhibit several properties that make them suitable for cell transplants in stroke therapies [44]. They are multipotent, meaning they can differentiate into more than one cell type, including mesodermal lineage adipocytes, chondrocytes, osteocytes, and ectodermal lineage cells, such as neurons and glial cells [45]. They are relatively easy to harvest because they can be obtained from various body tissues, such as adipose tissue, bone marrow, umbilical cord blood, umbilical cord tissue, dental tissue, and olfactory mucosa, while their isolation and amplification are not expensive [43]. They can be injected in several ways: intracerebrally, cerebroventricularly, intravenously, intra-arterially, or intranasally [43]. MSCs exhibit immunomodulatory properties by reducing the expression of proinflammatory cytokines, such as TNF-α, IL-1, interferon-γ (IFN-γ), and MCP-1; by reducing astrogliosis and microglia activation via atypical JAK-STAT signaling pathway [46,47]; increasing the expression of anti-inflammatory cytokines, such as IL-4, IL-10, and TNF-β [48]. Moreover, by stimulating the secretion of neurotrophic and growth factors, they promote angiogenesis (VEGF, angiogenin-1, and PDGF), cell proliferation, differentiation, migration and survival (PDGF, NGF, brain-derived growth factor, neurotrophin-3, and FGF), axonal growth (PDGF), synaptic plasticity (synaptophysin), and myelination [43]. However, their proliferation decreases over time in long-term cultures [49,50].

2. Discussion

2.1 Microglia

The current microglia research focuses on preventing the excessive activation and production of proinflammatory molecules [51]. One of the therapeutic strategies for ischemic stroke uses oxygen–glucose deprivation (OGD), whereby an optimal ischemia event is hypothesized to induce the protective phenotype in microglia [52]. Intravascular administration of OGD-preconditioned microglia in animal models was shown to promote angiogenesis, axonal outgrowth, and functional recovery. Since the main outcome of the microglial activity is considered to coincide with the result of the secreted neurotrophic factors rather than the microglial cells themselves, the effect on the neurological recovery by extracellular vesicles (EVs) derived from OGD preconditioned microglia was investigated. EVs from OGD-preconditioned microglia were found to be high in TGF-β1, which activates the Smad2/3 signaling pathway that plays a role in angiogenesis and neuronal injury repression [53]. Moreover, therapy with OGD-preconditioned peripheral blood mononuclear cells was also shown to promote angiogenesis, axonal outgrowth, and functional recovery in stroke [54]. The underlying mechanisms involved a reduction in miR-155-5p, via the hypoxia-inducible factor 1α (HIF-1α) axis [55], which increased the expression of VEGF and played a crucial role in neurovascular repair. Moreover, higher levels of anti-inflammatory cytokines, TGF-β1 and TGF-β2, and lower levels of proinflammatory cytokines, IL-1β and TNF-α, were found after OGD-preconditioning than under normoxic conditions [56]. Overall, oxygen–glucose deprivation has therapeutic potential in ischemic stroke as it was shown to promote protective phenotypic conversion and functional recovery. In addition, the usage of extracellular vesicles derived from OGD-preconditioned microglia presents several advantages over cell transplantations, such as a lack of immunogenicity, no risk of cell embolism, and lower costs. However, more research elucidating the signaling pathways mechanisms and cell-to-cell communication is still needed.

Other factors affecting microglia activation phenotypes include IL-4 and IFN-γ [57,58]. It was demonstrated that, via the PI3K-Akt pathway, the secretome of microglia induced by IL-4 promoted the proliferation, survival, and differentiation of neural stem/progenitor cells (NSPCs) into neurons and oligodendrocytes, while the induction of IFN-γ inhibited neurogenesis and oligodendrogliogenesis and led to the differentiation of NSPCs into astrocytes and induction of apoptosis. However, it remains unknown whether the induced microglia can maintain their protective phenotype after the removal of the stimulus. Recent studies [57] have demonstrated a decreased plasticity in terms of functions and phenotypic characteristics of induced microglia with time.

A variety of other factors that promote anti-inflammatory properties by microglia are being researched. Minocycline, an antibiotic from the tetracycline group was shown to increase the survival of neurons, stimulate neurogenesis, inhibit reactive gliosis, and promote functional recovery via the STAT1/STAT6 pathway in a rodent study model [59]. IL-4 was associated with improved functional recovery after stroke and a deficit in endogenous IL-4 promoted the proinflammatory phenotypic conversion [60]. Recently, tetramethylpyrazine, used in treating cerebrovascular disorders, was shown to modulate microglial polarization via the JAK2–STAT1/3 and GSK3–NFκB pathways and to stimulate the expression of anti-inflammatory cytokines, IL-10 and TGF-β, in addition to downregulating the expression of IL-6 and alleviating axonal and myelinated sheath injuries [61]. Following the development of proteomics, RNA sequencing [62], epigenetics, cell-targeted deletion [63], and an increased understanding of the inflammatory and immunological processes occurring during stroke, new therapeutic targets can be identified. However, as the role of microglia is far from being binary [21], their intercellular communication, dynamic molecular profile, and signaling pathway mechanisms still need to be elucidated to find an effective neuroprotective treatment.

2.2 Stem Cells

Stem cell-based therapies for ischemic stroke using mesenchymal stem cells (MSCs), neural stem cells (NSCs), induced pluripotent stem cells (iPSCs), and multilineage-differentiating stress-enduring cells (Muse cells) have been extensively researched recently. Indeed, stem cells can exert immunomodulatory, proangiogenic, and proneurogenic functions through their paracrine action, exosome secretion, and biobridge formations.

Because the main mechanism through which mesenchymal stem cells exhibit their paracrine properties is exosome secretion [34] and extracellular vesicles (EVs) derived from MSCs have been associated with promising therapeutic results in rodent stroke models [64–71], current research has been focusing on elucidating the mechanisms of their action. Exosomes contain various proteins, including cytokines, chemokines, growth factors, and membrane receptors as well as miRNAs through which they promote neurogenesis, angiogenesis, and cell growth and reduce inflammation, oxidative stress, and cell death [72–75]. Different miRNAs are carried in MSC-derived exosomes and target different mechanisms involved in stroke. It has been demonstrated that exosomes enriched with miRNA-17–92 increased the proliferation of neural and oligodendrocyte progenitor cells and increased neural plasticity via the PI3K/Akt/mTOR/GSK-3β signaling pathway [76,77]. Indeed, miRNA can inhibit an inflammatory response by inducing the microglia protective phenotype by either inhibiting cysteinyl leukotriene receptor 2 (CysLT2R) (miRNA-223) [78], suppressing the IRAK1/TEAF6 pathway (miRNA-146a) [79], regulating toll-like receptor 4 (TLR4) (miRNA-542) [80], or inhibiting the iron transporter—lipocalin-2 (LCN2) (miRNA-221) [81]. Moreover, they can promote angiogenesis by increasing the expression of VEGF through miR-210 [82,83] and miRNA-21-5p [84] and by targeting the transient receptor potential melastatin 7 (TRPM7) (miRNA-181b) [85]. Furthermore, they were also shown to promote cell growth by modulating the KDM6B/BMP2/BMF axis [86] and inhibiting the apoptotic pathway [87]. It was demonstrated that serum-derived exosomes helped maintain the integrity of the blood–brain barrier by inhibiting apoptosis of endothelial cells via the upregulation of B-cell lymphoma 2 (Bcl2) and the inhibition of caspase-3 activation. Moreover, by inhibiting MMP-9 and microtubule-associated protein 1 light chain 3B (LC3B)-mediated autophagy, they help maintain tight junction proteins—zonula occludens-1 (ZO-1) and claudin 5 [88]. In addition, it is worth emphasizing that exosomes may also be used as biomarkers: their miRNA content varies in relation to the progress of stroke, they elude degradation due to the vesicular structure, and they can be found in all bodily fluids, including blood plasma, which makes them easy to isolate [89–91]. Their small size, lack of immunogenicity [92], the ability to pass through the blood–brain barrier [93], and escape phagocytosis and lysosome degradation [32] make them excellent candidates for stroke therapy. In addition, using exosomes has some important advantages over any therapy that uses mesenchymal stem cells, such as no risk of blood vessel occlusion and the ease of storage and modification [94,95] (Fig. 1). However, although various mechanisms of action by the MSC-derived exosomes have been researched, exosome-based therapy still presents several translational challenges. Preclinical studies have been performed mainly on healthy animals [32]; however, stroke patients usually present many comorbidities [96], whose effects on stroke treatment should also be considered. Similarly, not enough studies have been performed on old animal stroke models [97]. Moreover, the appropriate administration methods, dosage, and time windows have not yet been established

[98], while purification, large-scale production [99], and targeting [34] remain the challenges to overcome. However, potential solutions include applying microfluidic technologies for exosome isolation [100], myelocytomatosis oncogene (MYC) transformation for large-scale production [101], and peptide conjugation on the exosome surface to improve their targeting [102].

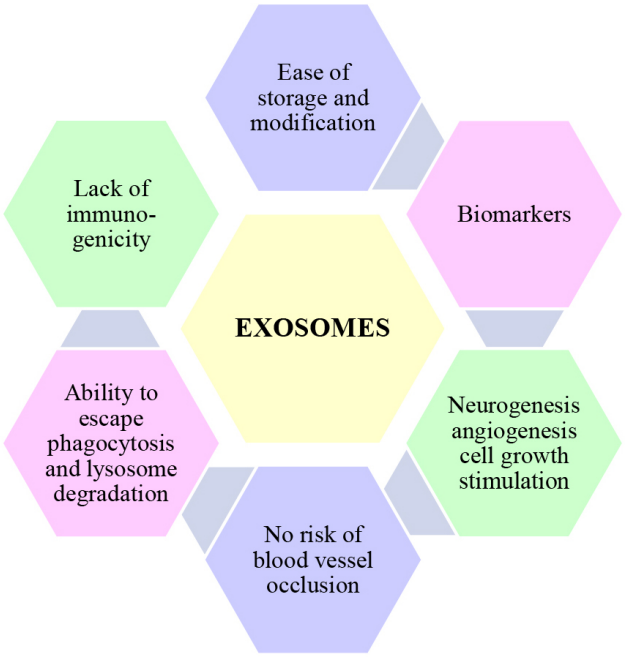

Fig. 1. Exosome characteristics. Exosomes contain various proteins, including cytokines, chemokines, growth factors, and membrane receptors as well as microRNAs through which they promote neurogenesis, angiogenesis, and cell growth and reduce inflammation, oxidative stress, and cell death. They have the ability to pass through the blood–brain barrier and escape phagocytosis and lysosome degradation. Their advantages over cell therapies include ease of storage and modification and no risk of blood vessel occlusion. As their miRNA content varies in relation to the progression of stroke, they can be used as biomarkers.

While stem cell therapies were assessed to be relatively safe, with low risks of tumorigenesis [103] and the association of only minor adverse effects [104], the results in terms of their efficacy are mixed. Several studies did not show any significant difference between the treatment and control group [105–107]. Moreover, although many clinical trials demonstrated improvements in neurological functions [108–112], the studies yielding positive results presented high risks of selection, performance, and publication bias and had small sample sizes [113]. In addition, in most cases, they lacked an effective study design, including randomization, blinding, and statistical comparison [104]. Interestingly, the unsatisfactory results for efficacy can be explained by differences between the preclinical and clinical protocols [114]. In several trials, doses below the efficacious dose previously established in preclinical studies were used [106,115]. Taking into consideration the above limitations of recent clinical trials, more studies with larger sample sizes, longer follow-ups, improved methodological designs, and better adherence to preclinical outcomes are needed.

In addition to mesenchymal stem cells, neural stem cells (NSCs) are also used in stroke research. However, their harvesting methods are problematic. Their sources are limited and transplantation from the adult brain would require complicated surgery. Derivation from neuroectoderm of the fetal tissue or from embryonic stem cells (ESCs) raises major ethical concerns and such transplantations may result in tumor formation [116,117]. Generating NSCs from induced pluripotent stem cells (iPSCs) [118] or via direct neuronal reprogramming by omitting the PSC stage would eliminate any ethical issues and the risk of immune rejection because they can be obtained from the patient's own cells [119,120]. However, the reprogramming process is long and time-consuming and iPSC transplantations in animal models have been associated with tumor formation [121]; thus, improving their safety remains the primary issue [122]. Moreover, as their engraftment efficiency is low [123], their therapeutic effects are achieved mainly by the paracrine action, including secreting factors enhancing neurogenesis, angiogenesis, and reducing inflammatory responses [119]. Thus, the current research involving neural stem cells focuses primarily on the extracellular vesicles derived from NSCs that present lower tumorigenicity, improved blood–brain barrier (BBB) permeability, and biodistribution. The comparison of extracellular vesicles (EV) derived from neural stem cells and mesenchymal stem cells (both derived from the same pluripotent stem cell line) in animal stroke models demonstrated a higher effectiveness of NSC treatment. Therapy using NSC EVs resulted in a larger reduction in infarct size, greater improvement in somatosensory function, and smaller neurological deficits than after treatment with MSC EVs. Moreover, therapy using NSC EVs was associated with an increase in macrophages with protective phenotypes and a decrease in proinflammatory T helper 17 cells (Th17) [124]. Although the initial results are encouraging, more research studies elucidating the downregulation of inflammatory responses are still needed.

Interestingly, mesenchymal stem cells used in combination with neural stem cells have been proven more effective in animal models than the use of individual therapies [125], while the co-transplantation of MSCs and NCSs in stroke patients has been shown to be a safe and feasible method [112], making it a potential new therapy for ischemic stroke patients [126]. Furthermore, stem cell therapy has also been shown to be effective in combination with gene therapy [127,128], tissue engineering scaffolds [129–132], and reperfusion therapy [114].

Table 1. Main advantages and disadvantages of using different stem cell types (MSCs, NSCs, ESCs, iPSCs, and Muse cells).

Stem cells	Advantages	Disadvantages
Mesenchymal (MSCs)	Easy cell harvesting Inexpensive isolation and amplification Low risk of tumorigenesis	Decreased proliferation over time
Neural (NSCs)	Differentiates into all neural lineages	Limited sources
Embryonic (ESCs)	Differentiates into three germ layers	Ethical issues Risk of tumorigenesis
Induced Pluripotent (iPSCs)	Renewable source for stem cell therapy	Long reprogramming process; risk of tumorigenesis
Multilineage-differentiating stress-enduring cells (Muse cells)	Differentiate into three germ layers Non-tumorigenic High homing capacity Immune-privileged	Few original papers published

Cell therapy using multilineage-differentiating stress-enduring cells (Muse cells) appears to overcome a large number of limitations by MSCs, NSCs, iPSCs, and ESCs (Table 1). Muse cells, first reported in 2010 [133], are found in a variety of tissues, such as bone marrow, peripheral blood, connective tissue, and the umbilical cord [134]. They can differentiate into three germ layers, including spontaneous *in vivo* differentiation into neuronal cells [135] and they can integrate into the neural network [136]. Moreover, they exhibit paracrine functions by secreting a variety of neurotrophic, proangiogenic, anti-inflammatory, and antiapoptotic factors. They are immune-privileged, meaning they inhibit the inflammatory immune response by, as suggested, expressing human leukocyte antigen G (HLA-G) molecules, meaning HLA-matching or immunosuppressant treatment is not required [134]. Due to the high expression of sphingosine-1-phosphate receptor 2 (S1PR2), as part of the S1P-S1PR2 axis, they can selectively migrate to the damaged site [137]. In addition, owing to their high capacity for DNA repair and lower telomerase activity and gene expression of tumorigenic factors than in ESCs and iPSCs, Muse cells are considered non-tumorigenic [134]. Muse cells have been researched in several animal stroke models [135,138–141] and were shown to differentiate into neuron cells, integrate into the cortex, improve motor functions and survival rates, and be assessed to be a safe treatment option. Moreover, in-human transplantations of allogenic Muse cells demonstrated safety and efficacy in clinical trials on myocardial infarction and dystrophic epidermolysis bullosa [142,143]. However, there are several challenges associated with culturing Muse cells, whereby expanding their small populations is time-consuming and their culture cost is higher than that of other stem cell types. Moreover, golden standards with regard to cell sources, sorting methods, and donor age still have to be established [137] alongside more preclinical and clinical studies that investigate and illustrate their mechanisms of action.

3. Conclusion

Stroke remains one of the leading causes of death and disability. The administration of stem cells and preconditioned microglia has been associated with various neuroprotective and immunomodulatory effects in preclinical studies. However, many challenges remain in cell therapy that need to be overcome.

The underlying mechanisms of their action are still not fully understood and various signaling pathways and phenotypic cell markers still need to be researched alongside their therapeutic implications established.

Moreover, cell therapy presents several important translational challenges. Preclinical studies performed *in vitro* cannot accurately mirror intricate brain environments and cellular interactions with various factors in an ischemic brain. In addition, cell sources should be also considered—for example—mouse microglia used in animal model studies present differences from human microglia. Importantly, golden standards in clinical trials regarding the dosage, administration route, time window after a stroke, cell source, and adverse event management systems should be established along with larger samples, control groups, longer follow-ups, and improved methodological designs to further study the safety and efficacy of cell therapy. Furthermore, more research on the combined use of stem cells with reperfusion methods, gene therapy, tissue scaffolds, and different types of stem cells should be conducted.

Although an overall cure for ischemic stroke has still not been found, recent advances in cell therapies and a growing understanding of their underlying mechanisms represent a promising start to achieving an effective neuroregenerative and neuroprotective treatment.

Author Contributions

AM, Ideation, literature search and writing the manuscript; DK and SS, work revision and suggestions to "stroke pathophysiology" chapter. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. 2022. Available at: https://www.world-stroke.org/assets/downloads/WSO_Global_Stroke_Fact_Sheet.pdf (Accessed: 11 May 2023).

[2] WHO. Disability-adjusted life years (DALYs). 1993 Available at: https://www.who.int/data/gho/indicator-metadata-registry/imr-details/158 (Accessed: 11 May 2023).

[3] Wafa HA, Wolfe CDA, Emmett E, Roth GA, Johnson CO, Wang Y. Burden of Stroke in Europe: Thirty-Year Projections of Incidence, Prevalence, Deaths, and Disability-Adjusted Life Years. Stroke. 2020; 51: 2418–2427.

[4] Baig MU, Bodle J. Thrombolytic Therapy. In StatPearls [Internet]. StatPearls Publishing LLC: Treasure Island (FL). 2023.

[5] Behme D, Gondecki L, Fiethen S, Kowoll A, Mpotsaris A, Weber W. Complications of mechanical thrombectomy for acute ischemic stroke-a retrospective single-center study of 176 consecutive cases. Neuroradiology. 2014; 56: 467–476.

[6] Scheldeman L, Wouters A, Lemmens R. Imaging selection for reperfusion therapy in acute ischemic stroke beyond the conventional time window. Journal of neurology. 2022; 269: 1715–1723.

[7] Xie F, Liu H, Liu Y. Adult Neurogenesis Following Ischemic Stroke and Implications for Cell-Based Therapeutic Approaches. World Neurosurgery. 2020; 138: 474–480.

[8] Donkor ES. Stroke in the 21(st) Century: A Snapshot of the Burden, Epidemiology, and Quality of Life. Stroke Research and Treatment. 2018; 2018: 3238165.

[9] Koh SH, Park HH. Neurogenesis in Stroke Recovery. Translational Stroke Research. 2017; 8: 3–13.

[10] Smith W, English J, Johnston S. Cerebrovascular diseases. In Hauser SL (ed.) Harrison's Neurology in Clinical Medicine (pp. 257–258). 3rd edn. McGraw Hill Education: New York, USA. 2013.

[11] Loh KY, Wang Z, Liao P. Oncotic Cell Death in Stroke. Reviews of Physiology, Biochemistry and Pharmacology. 2019; 176: 37–64.

[12] Orellana-Urzúa S, Rojas I, Líbano L, Rodrigo R. Pathophysiology of Ischemic Stroke: Role of Oxidative Stress. Current Pharmaceutical Design. 2020; 26: 4246–4260.

[13] Qiu YM, Zhang CL, Chen AQ, Wang HL, Zhou YF, Li YN, *et al.* Immune Cells in the BBB Disruption After Acute Ischemic Stroke: Targets for Immune Therapy? Frontiers in Immunology. 2021; 12: 678744.

[14] Liu J, Liu L, Wang X, Jiang R, Bai Q, Wang G. Microglia: A Double-Edged Sword in Intracerebral Hemorrhage From Basic Mechanisms to Clinical Research. Frontiers in Immunology. 2021; 12: 675660.

[15] Wang J, He W, Zhang J. A richer and more diverse future for microglia phenotypes. Heliyon. 2023; 9: e14713.

[16] Qin C, Zhou L, Ma X, Hu Z, Yang S, Chen M, *et al*. Dual Functions of Microglia in Ischemic Stroke. Neuroscience Bulletin. 2019; 35: 921–933.

[17] Badimon A, Strasburger HJ, Ayata P, Chen X, Nair A, Ikegami A, *et al*. Negative feedback control of neuronal activity by microglia. Nature. 2020; 586: 417–423.

[18] Yu F, Wang Y, Stetler AR, Leak RK, Hu X, Chen J. Phagocytic microglia and macrophages in brain injury and repair. CNS Neuroscience & Therapeutics. 2022; 28: 1279–1293.

[19] Xiong XY, Liu L, Yang QW. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. Progress in Neurobiology. 2016; 142: 23–44.

[20] Stratoulias V, Venero JL, Tremblay MÈ, Joseph B. Microglial subtypes: diversity within the microglial community. The EMBO Journal. 2019; 38: e101997.

[21] Paolicelli RC, Sierra A, Stevens B, Tremblay M, Aguzzi A, Ajami B, *et al*. Microglia states and nomenclature: a field at its crossroads. Neuron. 2022; 110: 3458–3483.

[22] Qiu L, Wang Y, Wang Y, Liu F, Deng S, Xue W, *et al.* Ursolic Acid Ameliorated Neuronal Damage by Restoring Microglia-Activated MMP/TIMP Imbalance in vitro. Drug Design, Development and Therapy. 2023; 17: 2481–2493.

[23] Kozareva DA, Cryan JF, Nolan YM. Born this way: Hippocampal neurogenesis across the lifespan. Aging Cell. 2019; 18: e13007.

[24] Lindvall O, Kokaia Z. Neurogenesis following Stroke Affecting the Adult Brain. Cold Spring Harbor Perspectives in Biology. 2015; 7: a019034.

[25] Han W, Jiang L, Song X, Li T, Chen H, Cheng L. VEGF Modulates Neurogenesis and Microvascular Remodeling in Epileptogenesis After Status Epilepticus in Immature Rats. Frontiers in Neurology. 2021; 12: 808568.

[26] Lin R, Cai J, Kenyon L, Iozzo R, Rosenwasser R, Iacovitti L. Systemic Factors Trigger Vasculature Cells to Drive Notch Signaling and Neurogenesis in Neural Stem Cells in the Adult Brain. Stem Cells. 2019; 37: 395–406.

[27] Qiu Z, Yang J, Deng G, Li D, Zhang S. Angiopoietin-like 4 promotes angiogenesis and neurogenesis in a mouse model of acute ischemic stroke. Brain Research Bulletin. 2021; 168: 156–164.

[28] Mudò G, Bonomo A, Di Liberto V, Frinchi M, Fuxe K, Belluardo N. The FGF-2/FGFRs neurotrophic system promotes neurogenesis in the adult brain. Journal of Neural Transmission. 2009; 116: 995–1005.

[29] Cheng X, Wang H, Zhang X, Zhao S, Zhou Z, Mu X, *et al.* The Role of SDF-1/CXCR4/CXCR7 in Neuronal Regeneration after Cerebral Ischemia. Frontiers in Neuroscience. 2017; 11: 590.

[30] Dillen Y, Kemps H, Gervois P, Wolfs E, Bronckaers A. Adult Neurogenesis in the Subventricular Zone and its Regulation after Ischemic Stroke: Implications for Therapeutic Approaches. Translational Stroke Research. 2020; 11: 60–79.

[31] Cunningham CJ, Redondo-Castro E, Allan SM. The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. Journal of Cerebral Blood Flow & Metabolism. 2018; 38: 1276–1292.

[32] Xiong Y, Song J, Huang X, Pan Z, Goldbrunner R, Stavrinou L, *et al.* Exosomes Derived From Mesenchymal Stem Cells: Novel Effects in the Treatment of Ischemic Stroke. Frontiers in Neuroscience. 2022; 16: 899887.

[33] Cun Y, Jin Y, Wu D, Zhou L, Zhang C, Zhang S, *et al*. Exosome in Crosstalk between Inflammation and Angiogenesis: a Potential Therapeutic Strategy for Stroke. Mediators of Inflammation. 2022; 2022: 7006281.

[34] Hong S, Yang H, Manaenko A, Lu J, Mei Q, Hu Q. Potential of Exosomes for the Treatment of Stroke. Cell Transplantation. 2019; 28: 662–670.

[35] Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone

L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney International. 2010; 78: 838–848.
[36] Nazimek K, Bryniarski K, Santocki M, Ptak W. Exosomes as mediators of intercellular communication: clinical implications. Polish Archives of Internal Medicine. 2015; 125: 370–380.
[37] Chen J, Chopp M. Exosome Therapy for Stroke. Stroke. 2018; 49: 1083–1090.
[38] Jo H, Shim K, Jeoung D. Exosomes: Diagnostic and Therapeutic Implications in Cancer. Pharmaceutics. 2023; 15: 1465.
[39] Pan Y, Wu W, Jiang X, Liu Y. Mesenchymal stem cell-derived exosomes in cardiovascular and cerebrovascular diseases: from mechanisms to therapy. Biomedicine & Pharmacotherapy. 2023; 163: 114817.
[40] Tajiri N, Kaneko Y, Shinozuka K, Ishikawa H, Yankee E, McGrogan M, *et al.* Stem cell recruitment of newly formed host cells via a successful seduction? Filling the gap between neurogenic niche and injured brain site. PLoS ONE. 2013; 8: e74857.
[41] Sullivan R, Duncan K, Dailey T, Kaneko Y, Tajiri N, Borlongan CV. A possible new focus for stroke treatment – migrating stem cells. Expert Opinion on Biological Therapy. 2015; 15: 949–958.
[42] Ejma M, Madetko N, Brzecka A, Alster P, Budrewicz S, Koszewicz M, *et al*. The Role of Stem Cells in the Therapy of Stroke. Current Neuropharmacology. 2022; 20: 630–647.
[43] Zhang Y, Dong N, Hong H, Qi J, Zhang S, Wang J. Mesenchymal Stem Cells: Therapeutic Mechanisms for Stroke. International Journal of Molecular Sciences. 2022; 23: 2550.
[44] Li J, Zhang Q, Wang W, Lin F, Wang S, Zhao J. Mesenchymal stem cell therapy for ischemic stroke: a look into treatment mechanism and therapeutic potential. Journal of Neurology. 2021; 268: 4095–4107.
[45] Liu J, Gao J, Liang Z, Gao C, Niu Q, Wu F, *et al.* Mesenchymal stem cells and their microenvironment. Stem Cell Research & Therapy. 2022; 13: 429.
[46] Jain M, Singh MK, Shyam H, Mishra A, Kumar S, Kumar A, *et al*. Role of JAK/STAT in the Neuroinflammation and its Association with Neurological Disorders. Annals of Neurosciences. 2021; 28: 191–200.
[47] McGuckin CP, Jurga M, Miller A, Sarnowska A, Wiedner M, Boyle NT, *et al*. Ischemic brain injury: a consortium analysis of key factors involved in mesenchymal stem cell-mediated inflammatory reduction. Archives of Biochemistry and Biophysics. 2013; 534: 88–97.
[48] Wang F, Tang H, Zhu J, Zhang JH. Transplanting Mesenchymal Stem Cells for Treatment of Ischemic Stroke. Cell Transplantation. 2018; 27: 1825–1834.
[49] Bortolotti F, Ukovich L, Razban V, Martinelli V, Ruozi G, Pelos B, *et al*. *In Vivo* Therapeutic Potential of Mesenchymal Stromal Cells Depends on the Source and the Isolation Procedure. Stem Cell Reports. 2015; 4: 332–339.
[50] Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, *et al*. Challenges and advances in clinical applications of mesenchymal stromal cells. Journal of Hematology & Oncology. 2021; 14: 24.
[51] Zhou Y, Bhatt H, Mojica CA, Xin H, Pessina MA, Rosene DL, *et al*. Mesenchymal-derived extracellular vesicles enhance microglia-mediated synapse remodeling after cortical injury in aging Rhesus monkeys. Journal of neuroinflammation. 2023; 20: 201.
[52] Kanazawa M, Miura M, Toriyabe M, Koyama M, Hatakeyama M, Ishikawa M, *et al.* Microglia preconditioned by oxygen-glucose deprivation promote functional recovery in ischemic rats. Scientific Reports. 2017; 7: 42582.
[53] Zhang L, Wei W, Ai X, Kilic E, Hermann DM, Venkataramani V, *et al.* Extracellular vesicles from hypoxia-preconditioned microglia promote angiogenesis and repress apoptosis in stroke mice via the TGF-β/Smad2/3 pathway. Cell Death & Disease. 2021; 12: 1068.
[54] Hatakeyama M, Kanazawa M, Ninomiya I, Omae K, Kimura Y, Takahashi T, *et al.* A novel therapeutic approach using peripheral blood mononuclear cells preconditioned by oxygen-glucose deprivation. Scientific Reports. 2019; 9: 16819.
[55] Otsu Y, Hatakeyama M, Kanayama T, Akiyama N, Ninomiya I, Omae K, *et al*. Oxygen–Glucose Deprived Peripheral Blood Mononuclear Cells Protect against Ischemic Stroke. Neurotherapeutics. 2023; 20: 1369–1387.
[56] Moon S, Chang MS, Koh SH, Choi YK. Repair Mechanisms of the Neurovascular Unit after Ischemic Stroke with a Focus on VEGF. International Journal of Molecular Sciences. 2021; 22: 8543.
[57] Jiang X, Yi S, Liu Q, Zhang J. The secretome of microglia induced by IL-4 of IFN-γ differently regulate proliferation, differentiation and survival of adult neural stem/progenitor cell by targeting the PI3K-Akt pathway. Cytotechnology. 2022; 74: 407–420.
[58] Zhang J, Rong P, Zhang L, He H, Zhou T, Fan Y, *et al*. IL4-driven microglia modulate stress resilience through BDNF-dependent neurogenesis. Science Advances. 2021; 7: eabb9888.
[59] Lu Y, Zhou M, Li Y, Li Y, Hua Y, Fan Y. Minocycline promotes functional recovery in ischemic stroke by modulating microglia polarization through STAT1/STAT6 pathways. Biochemical Pharmacology. 2021; 186: 114464.
[60] Liu X, Liu J, Zhao S, Zhang H, Cai W, Cai M, *et al*. Interleukin-4 is Essential for Microglia/Macrophage M2 Polarization and Long-Term Recovery after Cerebral Ischemia. Stroke. 2016; 47: 498–504.
[61] Feng X, Li M, Lin Z, Lu Y, Zhuang Y, Lei J, *et al*. Tetramethylpyrazine promotes axonal remodeling and modulates microglial polarization via JAK2-STAT1/3 and GSK3-NFκB pathways in ischemic stroke. Neurochemistry International. 2023; 170: 105607.
[62] Thapa K, Shivam K, Khan H, Kaur A, Dua K, Singh S, *et al*. Emerging Targets for Modulation of Immune Response and Inflammation in Stroke. Neurochemical Research. 2023; 48: 1663–1690.
[63] Wang Y, Leak RK, Cao G. Microglia-mediated neuroinflammation and neuroplasticity after stroke. Frontiers in Cellular Neuroscience. 2022; 16: 980722.
[64] Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic Administration of Exosomes Released from Mesenchymal Stromal Cells Promote Functional Recovery and Neurovascular Plasticity after Stroke in Rats. Journal of Cerebral Blood Flow & Metabolism. 2013; 33: 1711–1715.
[65] Doeppner TR, Herz J, Görgens A, Schlechter J, Ludwig A, Radtke S, *et al*. Extracellular Vesicles Improve Post-Stroke Neuroregeneration and Prevent Postischemic Immunosuppression. Stem Cells Translational Medicine. 2015; 4: 1131–1143.
[66] Lee JY, Kim E, Choi SM, Kim DW, Kim KP, Lee I, *et al.* Microvesicles from brain-extract-treated mesenchymal stem cells improve neurological functions in a rat model of ischemic stroke. Scientific Reports. 2016; 6: 33038.
[67] Otero-Ortega L, Laso-García F, Gómez-de Frutos MD, Rodríguez-Frutos B, Pascual-Guerra J, Fuentes B, *et al.* White Matter Repair After Extracellular Vesicles Administration in an Experimental Animal Model of Subcortical Stroke. Scientific Reports. 2017; 7: 44433.
[68] Huang X, Ding J, Li Y, Liu W, Ji J, Wang H, *et al*. Exosomes derived from PEDF modified adipose-derived mesenchymal stem cells ameliorate cerebral ischemia-reperfusion injury by regulation of autophagy and apoptosis. Experimental Cell Research. 2018; 371: 269–277.
[69] Liu Y, Fu N, Su J, Wang X, Li X. Rapid Enkephalin Delivery Using Exosomes to Promote Neurons Recovery in Ischemic Stroke

by Inhibiting Neuronal p53/Caspase-3. BioMed Research International. 2019; 2019: 4273290.
[70] Moon GJ, Sung JH, Kim DH, Kim EH, Cho YH, Son JP, *et al*. Application of Mesenchymal Stem Cell-Derived Extracellular Vesicles for Stroke: Biodistribution and MicroRNA Study. Translational Stroke Research. 2019; 10: 509–521.
[71] Geng W, Tang H, Luo S, Lv Y, Liang D, Kang X, *et al.* Exosomes from miRNA-126-modified ADSCs promotes functional recovery after stroke in rats by improving neurogenesis and suppressing microglia activation. American Journal of Translational Research. 2019; 11: 780–792.
[72] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology. 2007; 9: 654–659.
[73] Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, *et al.* Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nature Communications. 2015; 6: 8472.
[74] Sen CK. MicroRNAs as new maestro conducting the expanding symphony orchestra of regenerative and reparative medicine. Physiological Genomics. 2011; 43: 517–520.
[75] Jiang L, Chen W, Ye J, Wang Y. Potential Role of Exosomes in Ischemic Stroke Treatment. Biomolecules. 2022; 12: 115.
[76] Xin H, Liu Z, Buller B, Li Y, Golembieski W, Gan X, *et al*. MiR-17-92 enriched exosomes derived from multipotent mesenchymal stromal cells enhance axon-myelin remodeling and motor electrophysiological recovery after stroke. Journal of Cerebral Blood Flow & Metabolism. 2021; 41: 1131–1144.
[77] Xin H, Katakowski M, Wang F, Qian JY, Liu XS, Ali MM, *et al.* MicroRNA cluster miR-17-92 Cluster in Exosomes Enhance Neuroplasticity and Functional Recovery After Stroke in Rats. Stroke. 2017; 48: 747–753.
[78] Zhao Y, Gan Y, Xu G, Hua K, Liu D. Exosomes from MSCs overexpressing microRNA-223-3p attenuate cerebral ischemia through inhibiting microglial M1 polarization mediated inflammation. Life Sciences. 2020; 260: 118403.
[79] Zhang Z, Zou X, Zhang R, Xie Y, Feng Z, Li F, *et al*. Human umbilical cord mesenchymal stem cell-derived exosomal miR-146a-5p reduces microglial-mediated neuroinflammation via suppression of the IRAK1/TRAF6 signaling pathway after ischemic stroke. Aging. 2021; 13: 3060–3079.
[80] Haupt M, Zheng X, Kuang Y, Lieschke S, Janssen L, Bosche B, *et al*. Lithium modulates miR-1906 levels of mesenchymal stem cell-derived extracellular vesicles contributing to post-stroke neuroprotection by toll-like receptor 4 regulation. Stem Cells Translational Medicine. 2021; 10: 357–373.
[81] Deng Y, Chen D, Gao F, Lv H, Zhang G, Sun X, *et al*. Exosomes derived from microRNA-138-5p-overexpressing bone marrow-derived mesenchymal stem cells confer neuroprotection to astrocytes following ischemic stroke via inhibition of LCN2. Journal of Biological Engineering. 2019; 13: 71.
[82] Meng ZY, Kang HL, Duan W, Zheng J, Li QN, Zhou ZJ. MicroRNA-210 Promotes Accumulation of Neural Precursor Cells Around Ischemic Foci After Cerebral Ischemia by Regulating the SOCS1-STAT3-VEGF-C Pathway. Journal of the American Heart Association. 2018; 7: e005052.
[83] Lou Y, Guo F, Liu F, Gao F, Zhang P, Niu X, *et al*. MiR-210 activates notch signaling pathway in angiogenesis induced by cerebral ischemia. Molecular and Cellular Biochemistry. 2012; 370: 45–51.
[84] Hu H, Hu X, Li L, Fang Y, Yang Y, Gu J, *et al.* Exosomes Derived from Bone Marrow Mesenchymal Stem Cells Promote Angiogenesis in Ischemic Stroke Mice via Upregulation of MiR-21-5p. Biomolecules. 2022; 12: 883.
[85] Yang Y, Cai Y, Zhang Y, Liu J, Xu Z. Exosomes Secreted by Adipose-Derived Stem Cells Contribute to Angiogenesis of Brain Microvascular Endothelial Cells Following Oxygen–Glucose Deprivation in Vitro through MicroRNA-181b/TRPM7 Axis. Journal of Molecular Neuroscience. 2018; 65: 74–83.
[86] Zhang Y, Liu J, Su M, Wang X, Xie C. Exosomal microRNA-22-3p alleviates cerebral ischemic injury by modulating KDM6B/BMP2/BMF axis. Stem Cell Research & Therapy. 2021; 12: 111.
[87] Sun Y, Gui H, Li Q, Luo Z, Zheng M, Duan J, *et al*. MicroRNA-124 Protects Neurons against Apoptosis in Cerebral Ischemic Stroke. CNS Neuroscience & Therapeutics. 2013; 19: 813–819.
[88] Huang L-Y, Song J-X, Cai H, Wang P-P, Yin Q-L, Zhang Y-D, *et al.* Healthy Serum-Derived Exosomes Improve Neurological Outcomes and Protect Blood–Brain Barrier by Inhibiting Endothelial Cell Apoptosis and Reversing Autophagy-Mediated Tight Junction Protein Reduction in Rat Stroke Model. Frontiers in Cellular Neuroscience. 2022; 16: 841544.
[89] Forró T, Bajkó Z, Bălașa A, Bălașa R. Dysfunction of the Neurovascular Unit in Ischemic Stroke: Highlights on microRNAs and Exosomes as Potential Biomarkers and Therapy. International Journal of Molecular Sciences. 2021; 22: 5621.
[90] Lin J, Li J, Huang B, Liu J, Chen X, Chen X, *et al*. Exosomes: Novel Biomarkers for Clinical Diagnosis. The Scientific World Journal. 2015; 2015: 657086.
[91] Console L, Scalise M, Indiveri C. Exosomes in inflammation and role as biomarkers. Clinica Chimica Acta. 2019; 488: 165–171.
[92] EL Andaloussi S, Mäger I, Breakefield XO, Wood MJA. Extracellular vesicles: biology and emerging therapeutic opportunities. Nature Reviews Drug Discovery. 2013; 12: 347–357.
[93] Chen CC, Liu L, Ma F, Wong CW, Guo XE, Chacko JV, *et al*. Elucidation of Exosome Migration across the Blood–Brain Barrier Model *in Vitro*. Cellular and Molecular Bioengineering. 2016; 9: 509–529.
[94] Xin H, Li Y, Chopp M. Exosomes/miRNAs as mediating cell-based therapy of stroke. Frontiers in Cellular Neuroscience. 2014; 8: 377.
[95] Li Y, Cheng Q, Hu G, Deng T, Wang Q, Zhou J, *et al.* Extracellular vesicles in mesenchymal stromal cells: A novel therapeutic strategy for stroke. Experimental and Therapeutic Medicine. 2018; 15: 4067–4079.
[96] Przykaza Ł. Understanding the Connection Between Common Stroke Comorbidities, Their Associated Inflammation, and the Course of the Cerebral Ischemia/Reperfusion Cascade. Frontiers in Immunology. 2021; 12: 782569.
[97] Li J, Li Q, Sheng R. The role and therapeutic potential of exosomes in ischemic stroke. Neurochemistry International. 2021; 151: 105194.
[98] Yang L, Qian J, Yang B, He Q, Wang J, Weng Q. Challenges and Improvements of Novel Therapies for Ischemic Stroke. Frontiers in Pharmacology. 2021; 12: 721156.
[99] Nikfarjam S, Rezaie J, Zolbanin NM, Jafari R. Mesenchymal stem cell derived-exosomes: a modern approach in translational medicine. Journal of Translational Medicine. 2020; 18: 449.
[100] Le MN, Fan ZH. Exosome isolation using nanostructures and microfluidic devices. Biomedical Materials. 2021; 16: 022005.
[101] Chen TS, Arslan F, Yin Y, Tan SS, Lai RC, Choo ABH, *et al*. Enabling a robust scalable manufacturing process for therapeutic exosomes through oncogenic immortalization of human ESC-derived MSCs. Journal of Translational Medicine. 2011; 9: 47.
[102] Tian T, Zhang H, He C, Fan S, Zhu Y, Qi C, *et al*. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. Biomaterials. 2018; 150: 137–149.
[103] Stonesifer C, Corey S, Ghanekar S, Diamandis Z, Acosta SA, Borlongan CV. Stem cell therapy for abrogating stroke-induced neuroinflammation and relevant secondary cell death mecha-

nisms. Progress in Neurobiology. 2017; 158: 94–131.
[104] Gautam J, Alaref A, Hassan A, Sharma Kandel R, Mishra R, Jahan N. Safety and Efficacy of Stem Cell Therapy in Patients with Ischemic Stroke. Cureus. 2020; 12: e9917.
[105] Savitz SI, Yavagal D, Rappard G, Likosky W, Rutledge N, Graffagnino C, *et al*. A Phase 2 Randomized, Sham-Controlled Trial of Internal Carotid Artery Infusion of Autologous Bone Marrow–Derived ALD-401 Cells in Patients with Recent Stable Ischemic Stroke (RECOVER-Stroke) Circulation. 2019; 139: 192–205.
[106] Prasad K, Sharma A, Garg A, Mohanty S, Bhatnagar S, Johri S, *et al*. Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial. Stroke. 2014; 45: 3618–3624.
[107] Sprigg N, O'Connor R, Woodhouse L, Krishnan K, England TJ, Connell LA, *et al.* Granulocyte Colony Stimulating Factor and Physiotherapy after Stroke: Results of a Feasibility Randomised Controlled Trial: Stem Cell Trial of Recovery EnhanceMent after Stroke-3 (STEMS-3 ISRCTN16714730). PLoS ONE. 2016; 11: e0161359.
[108] Steinberg GK, Kondziolka D, Wechsler LR, Lunsford LD, Kim AS, Johnson JN, *et al*. Two-year safety and clinical outcomes in chronic ischemic stroke patients after implantation of modified bone marrow–derived mesenchymal stem cells (SB623): a phase 1/2a study. Journal of Neurosurgery. 2018; 1–11.
[109] Levy ML, Crawford JR, Dib N, Verkh L, Tankovich N, Cramer SC. Phase I/II Study of Safety and Preliminary Efficacy of Intravenous Allogeneic Mesenchymal Stem Cells in Chronic Stroke. Stroke. 2019; 50: 2835–2841.
[110] Laskowitz DT, Bennett ER, Durham RJ, Volpi JJ, Wiese JR, Frankel M, *et al*. Allogeneic Umbilical Cord Blood Infusion for Adults with Ischemic Stroke: Clinical Outcomes from a Phase I Safety Study. Stem Cells Translational Medicine. 2018; 7: 521–529.
[111] Kalladka D, Sinden J, Pollock K, Haig C, McLean J, Smith W, *et al*. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. The Lancet. 2016; 388: 787–796.
[112] Qiao L, Huang F, Zhao M, Xie J, Shi J, Wang J, *et al*. A Two-Year Follow-up Study of Cotransplantation with Neural Stem/Progenitor Cells and Mesenchymal Stromal Cells in Ischemic Stroke Patients. Cell Transplantation. 2014; 23: S65–S72.
[113] Boncoraglio GB, Ranieri M, Bersano A, Parati EA, Del Giovane C. Stem cell transplantation for ischemic stroke. Cochrane Database of Systematic Reviews. 2019; 5: Cd007231.
[114] Borlongan CV. Concise Review: Stem Cell Therapy for Stroke Patients: are we there yet? Stem Cells Translational Medicine. 2019; 8: 983–988.
[115] Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Annals of Neurology. 2005; 57: 874–882.
[116] Tuazon JP, Castelli V, Lee J, Desideri GB, Stuppia L, Cimini AM, *et al*. Neural Stem Cells. Advances in Experimental Medicine and Biology. 2019; 8: 79–91.
[117] Li Y, Fang B. Neural stem cell-derived extracellular vesicles: the light of central nervous system diseases. Biomedicine & Pharmacotherapy. 2023; 165: 115092.
[118] Glicksman MA. Induced Pluripotent Stem Cells: the most Versatile Source for Stem Cell Therapy. Clinical Therapeutics. 2018; 40: 1060–1065.
[119] Hamblin MH, Lee JP. Neural Stem Cells for Early Ischemic Stroke. International Journal of Molecular Sciences. 2021; 22: 7703.
[120] Huang L, Zhang L. Neural stem cell therapies and hypoxic-ischemic brain injury. Progress in Neurobiology. 2019; 173: 1–17.
[121] Kawai H, Yamashita T, Ohta Y, Deguchi K, Nagotani S, Zhang X, *et al*. Tridermal Tumorigenesis of Induced Pluripotent Stem Cells Transplanted in Ischemic Brain. Journal of Cerebral Blood Flow & Metabolism. 2010; 30: 1487–1493.
[122] Suman S, Domingues A, Ratajczak J, Ratajczak MZ. Potential Clinical Applications of Stem Cells in Regenerative Medicine. Advances in Experimental Medicine and Biology. 2019; 1201: 1–22.
[123] Zhang G, Zhu Z, Wang Y. Neural stem cell transplantation therapy for brain ischemic stroke: Review and perspectives. World Journal of Stem Cells. 2019; 11: 817–830.
[124] Webb RL, Kaiser EE, Scoville SL, Thompson TA, Fatima S, Pandya C, *et al*. Human Neural Stem Cell Extracellular Vesicles Improve Tissue and Functional Recovery in the Murine Thromboembolic Stroke Model. Translational Stroke Research. 2018; 9: 530–539.
[125] Hosseini SM, Farahmandnia M, Razi Z, Delavari S, Shakibajahromi B, Sarvestani FS, *et al*. Combination Cell Therapy with Mesenchymal Stem Cells and Neural Stem Cells for Brain Stroke in Rats. International Journal of Stem Cells. 2015; 8: 99–105.
[126] Kaminska A, Radoszkiewicz K, Rybkowska P, Wedzinska A, Sarnowska A. Interaction of Neural Stem Cells (NSCs) and Mesenchymal Stem Cells (MSCs) as a Promising Approach in Brain Study and Nerve Regeneration. Cells. 2022; 11: 1464.
[127] Nourbakhsh A, Colbert BM, Nisenbaum E, El-Amraoui A, Dykxhoorn DM, Koehler KR, *et al*. Stem Cells and Gene Therapy in Progressive Hearing Loss: the State of the Art. Journal of the Association for Research in Otolaryngology. 2021; 22: 95–105.
[128] De Rosa L, Latella MC, Secone Seconetti A, Cattelani C, Bauer JW, Bondanza S, *et al*. Toward Combined Cell and Gene Therapy for Genodermatoses. Cold Spring Harbor Perspectives in Biology. 2020; 12: a035667.
[129] Chen S, Ugwu F, Li W, Caplice NM, Petcu E, Yip SP, *et al*. Vascular Tissue Engineering: Advanced Techniques and Gene Editing in Stem Cells for Graft Generation. Tissue Engineering Part B: Reviews. 2021; 27: 14–28.
[130] Tevlin R, Walmsley GG, Marecic O, Hu MS, Wan DC, Longaker MT. Stem and progenitor cells: advancing bone tissue engineering. Drug Delivery and Translational Research. 2016; 6: 159–173.
[131] Zhang Z, Gupte MJ, Ma PX. Biomaterials and stem cells for tissue engineering. Expert Opinion on Biological Therapy. 2013; 13: 527–540.
[132] Zhao C, Tan A, Pastorin G, Ho HK. Nanomaterial scaffolds for stem cell proliferation and differentiation in tissue engineering. Biotechnology Advances. 2013; 31: 654–668.
[133] Kuroda Y, Kitada M, Wakao S, Nishikawa K, Tanimura Y, Makinoshima H, *et al*. Unique multipotent cells in adult human mesenchymal cell populations. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107: 8639–8643.
[134] Yamashita T, Kushida Y, Abe K, Dezawa M. Non-Tumorigenic Pluripotent Reparative Muse Cells Provide a New Therapeutic Approach for Neurologic Diseases. Cells. 2021; 10: 961.
[135] Uchida H, Morita T, Niizuma K, Kushida Y, Kuroda Y, Wakao S, *et al*. Transplantation of Unique Subpopulation of Fibroblasts, Muse Cells, Ameliorates Experimental Stroke Possibly via Robust Neuronal Differentiation. Stem Cells. 2016; 34: 160–173.
[136] Niizuma K, Borlongan CV, Tominaga T. Application of Muse Cell Therapy to Stroke. Advances in Experimental Medicine and Biology. 2018; 29: 167–186.
[137] Li H, Wei J, Liu X, Zhang P, Lin J. Muse cells: ushering in a new era of stem cell-based therapy for stroke. Stem Cell Re-

search & Therapy. 2022; 13: 421.

[138] Yamauchi T, Kuroda Y, Morita T, Shichinohe H, Houkin K, Dezawa M, *et al.* Therapeutic effects of human multilineage-differentiating stress enduring (MUSE) cell transplantation into infarct brain of mice. PLoS ONE. 2015; 10: e0116009.

[139] Uchida H, Niizuma K, Kushida Y, Wakao S, Tominaga T, Borlongan CV, *et al*. Human Muse Cells Reconstruct Neuronal Circuitry in Subacute Lacunar Stroke Model. Stroke. 2017; 48: 428–435.

[140] Abe T, Aburakawa D, Niizuma K, Iwabuchi N, Kajitani T, Wakao S, *et al*. Intravenously Transplanted Human Multilineage-Differentiating Stress-Enduring Cells Afford Brain Repair in a Mouse Lacunar Stroke Model. Stroke. 2020; 51: 601–611.

[141] Shimamura N, Kakuta K, Wang L, Naraoka M, Uchida H, Wakao S, *et al*. Neuro-regeneration therapy using human Muse cells is highly effective in a mouse intracerebral hemorrhage model. Experimental Brain Research. 2017; 235: 565–572.

[142] Noda T, Nishigaki K, Minatoguchi S. Safety and Efficacy of Human Muse Cell-Based Product for Acute Myocardial Infarction in a first-in-Human Trial. Circulation Journal. 2020; 84: 1189–1192.

[143] Fujita Y, Nohara T, Takashima S, Natsuga K, Adachi M, Yoshida K, *et al.* Intravenous allogeneic multilineage-differentiating stress-enduring cells in adults with dystrophic epidermolysis bullosa: a phase 1/2 open-label study. Journal of the European Academy of Dermatology and Venereology. 2021; 35: e528–e531.