Blood-brain Barrier and MSC

Subjects: Peripheral Vascular Disease
Contributor: Nikunj Satani

Ischemic stroke is a debilitating disease and one of the leading causes of long-term disability. During the early phase after ischemic stroke, the blood-brain barrier (BBB) exhibits increased permeability and disruption, leading to an influx of immune cells and inflammatory molecules that exacerbate the damage to the brain tissue. Mesenchymal stem cells have been investigated as a promising therapy to improve the recovery after ischemic stroke. The therapeutic effects imparted by MSCs are mostly paracrine.

Keywords: blood-brain barrier ; mesenchymal stem cell

1. Background

Stroke is one of the leading causes of mortality and long-term disability in the United States, accounting for approximately 1 out of every 19 deaths and 2.4% of adult disability [1][2]. Worldwide in 2019, approximately 6.6 million deaths could be attributed to stroke [1][3]. A stroke occurs when blood flow to the brain is impaired. Ischemic stroke comprises 87% of all stroke cases and is caused by an artery obstruction interrupting blood delivery [1][4]. The reduction in blood flow, which supplies nutrients to the brain, initiates a series of interconnected cascades that cause widespread tissue damage and death [5]. The blood-brain barrier (BBB) impairment is one of the main pathologies associated with ischemic stroke. BBB insult begins early, before the onset of neuronal damage and influences the extent of brain injury [6][7].

Mesenchymal stem cells (MSCs, also known as mesenchymal stromal cells) have been frequently explored as a promising potential therapy for recovery after ischemic stroke. However, since very few MSCs reach the brain, it is believed that most of the therapeutic effects of MSCs are the result of paracrine signaling [8]. One of the likely mediators for such signaling are the extracellular vesicles (EVs) released by MSCs [9][10]. EVs could mediate the transmission of molecules such as lipids, proteins, nucleic acids, cytokines, chemokines, and growth factors that may alter the behavior and phenotype of the receiving cell [11][12].

2. Changes in Blood-Brain Barrier after Stroke

The BBB is an interface that separates the central nervous system from the blood circulating in the periphery. It is a highly selective barrier and is responsible for a number of roles essential for normal brain function, including the maintenance of homeostasis, protection against pathogens and other potentially harmful substances, nutrient transport, and maintaining the optimal physiological/extracellular environment of the brain for neuronal and glial functions. The principle component of the BBB is the endothelial cells (ECs) linked together by tight junctions (TJs) that form the walls of the brain vasculature and microvasculature, however these are only one part of the broader structure that regulates the movement of molecules between the blood and brain. The whole “neurovascular unit” (NVU; or “extended BBB”) is a highly intricate and dynamic network of cellular and non-cellular interplay that regulates BBB permeability and cerebral blood flow [13]. Other components of the NVU include pericytes, neural cells, astrocytes, and microglia [13][14][15]. Deficiencies in any of the components of the NVU may result in a leaky or damaged BBB, which has severe implications on brain health. Such interconnectedness of the NVU means that in vitro models of the BBB require co-culture of multiple cell types in order to replicate even basic barrier properties [16][17].

Within minutes of stroke onset, neurons in the infarct area release massive amounts of glutamate and create an excitotoxic environment. Lack of blood flow does not allow for replenishment of oxygen and removal of cellular byproducts. This leads to the accumulation of toxic substances, such as reactive oxygen species (ROS) and NO, as well as depletion of ATP. Local immune cells such as astrocytes and microglia are activated and produce substances such as matrix metalloproteinase-9 (MMP9) that contribute to the disruption of the TJs holding the BBB together [18][19]. Energy depletion causes dysfunction of transmembrane pumps, rendering neurons unable to maintain ionic balance. Accumulation of intracellular Ca^{2+} and Na^{+} causes cellular swelling and membrane degeneration, allowing dissemination of danger signals [20]. Consequently, hematogenous immune cells and inflammatory factors that are normally blocked by

the BBB are able to permeate into the ischemic brain tissue and inflict further injury. Over the subsequent hours and days, the large scale inflammatory response in the brain, abetted by an injured BBB and vascular breakdown, leads to irreversible neuronal death in the infarct region [21][22]. Indeed, neuro-inflammation is a hallmark of stroke and several other neurological disorders, and is a key factor in BBB disruption [5][19].

Whether the increased BBB permeability after stroke is caused by loss of intracellular junctions or by increased transcellular transport through ECs via caveolae and vacuoles is debated, although the variations in these findings may be due to time point differences. Nahirney et al. used electron microscopy to view the peri-infarct endothelium at 3 and 72 h after stroke [23]. They found a significant increase in the number of vacuoles at both time points and very few disrupted TJs, although the number of disruptions did increase with time [23]. Krueger et al. found that TJs appeared intact 25 h after stroke, despite the evidence of BBB leakage [24]. On the other hand, there is ample evidence for the breakdown of junction proteins caused by cerebral ischemia. The phosphorylation of occludin, claudin-5, and ZO-1 is mediated by increased activation of nPKC-θ and aPKC-ζ caused by hypoxia-reoxygenation stress [25]. Pro-inflammatory cytokines and activation of NAPDH oxidase are known to further disrupt the association between ZO-1 and occludin [26]. There is reduction in transcription or increased degradation of TJ proteins after stroke [27]. Additionally, hemorrhagic transformation may be due to TJ disruption. Taken together, the evidence points to the notion that TJ protein degradation contributes to BBB disruption.

Studies point towards a biphasic pattern of BBB permeability and disruption caused by different mechanisms. The first phase occurs approximately 6 h after reperfusion and involves increased permeability through the caveolae-mediated transcytosis of non-selective molecules across ECs [28]. This increase in caveolae activity may be caused by pericyte migration away from the BBB [28][29]. In the second phase, about 48–60 h after reperfusion, the protein junctions between ECs are broken down and remodeled [28]. Studies do not always consider that there may be different phases of BBB permeability, but it is an important aspect of stroke pathology that should be taken into account when determining appropriate therapies.

3. Current Stroke Management and Its Limitations

Currently there are only two FDA-approved therapies for stroke, and both require immediate medical care. Intravenously administered tissue plasminogen activator (tPA), a thrombolytic agent, acts to dissolve any blood clots to resume normal blood flow. However, tPA increases the risk of hemorrhagic transformation by enhancing the activity of MMPs that dissolve basal lamina supporting the integrity of the vasculature [30][31]. The short window for which tPA's therapeutic benefits outweigh the potential negative effects is only about 4 h after symptom onset, a window which is reliant on the integrity of the BBB [31][32][33]. Endovascular mechanical thrombectomy is a therapy that extends the time window for therapeutic intervention after symptom onset and increases rates of successful reperfusion. It is used for severe strokes caused by a large vessel occlusion, but it is not always successful in preventing further damage [34].

Restoring blood flow to the injured area is vital, however the reperfusion of blood after ischemic damage causes inflammatory cascades that may add insult to the area. Reperfusion stimulates leucocyte adhesion, oxidative stress, mitochondrial dysfunction, and inflammation that further aggravates brain tissue and vasculature [19][35][36][37]. Despite this, restoration of blood flow is vital, as late perfusions may exacerbate the damage [30][38][39]. Furthermore, blood vessels—especially microvessels—are at risk of collapse when they lack sufficient blood flow, a phenomenon known as "no-reflow", where reperfusion fails to occur in individual vessels despite blood supply being restored to the area [40][41]. A key factor in the no-reflow phenomenon is astrocytic damage during the initial phases of ischemia [42][43]. Due to the unbalanced cerebral environment, astrocytes swell, causing their end-feet processes to compress the microvasculature even after cerebral flood flow is restored [42][43]. It has been suggested that the administration of osmotherapeutics targeted at reducing the swelling right before or after thrombolysis may help alleviate secondary focal infarction [42]. Restoring blood flow too early, before the vasculature is sufficiently stabilized, increases the risk of hemorrhagic transformation, which may lead to death [44]. Despite the risks, early reperfusion is associated with reduced mortality and greater functional recovery [45].

Stroke presents a notoriously complex pathology, and different therapies may produce different outcomes, depending on when they are administered throughout the progression of the disease. The goals of an ideal stroke therapy would be to restore blood flow to the hypoperfused penumbral region, reduce the size of the infarct, boost regeneration in the afflicted area, and promote functional recovery through reorganization of vascular and neural networks. Thrombolysis and thrombectomy achieve the first goal. Much research has been focused on therapies targeted towards protection and regeneration of parenchymal neurons and glia, with less attention on the recovery of the vital supportive cells and tissues

upon which the functional parenchymal cells rely. Indeed, proper neurological function is intimately coupled with NVU health and repair [45]. Hence, we discuss the repair and restoration of the NVU evoked by MSC therapy and their extracellular vesicles (MSC-EVs).

4. Extracellular Vesicles from Mesenchymal Stromal Cells

MSCs have been frequently explored as a promising potential therapy for ischemic stroke. Large and readily harvested quantities of MSCs can be derived from bone marrow and adipose tissue, though they can be derived from other tissues as well [46][47]. Very few MSCs administered after stroke actually reach the infarct area. Instead, it is believed that most of the therapeutic effects of MSCs are the result of paracrine signaling [8]. Paracrine signaling can be mediated by releasing trophic factors via extracellular vesicles (EVs) [8][9][10]. Cargo carried by EVs such as lipids, proteins, nucleic acids, cytokines, chemokines, and growth factors can alter the behavior and phenotype of the receiving cell [11][12].

EVs are classified into four types, based on their size and genesis, though all arise from the cell's plasma membrane: Exosomes, Microvesicles (MVs), Apoptotic bodies, and Oncosomes. The smallest of the EVs are called exosomes. They are 50nm–150nm in diameter and are formed when multivesicular endosomes (MVEs) in the interior of a cell fuse with the outer plasma membrane and dump their cargo into the extracellular space [11][48][49][50]. Microvesicles (MVs) typically range from 50nm–500nm but can be as large as 1000 nm in diameter. These EVs are shed by the outward budding and subsequent cleavage of the cell membrane [11][48][49][50]. Apoptotic bodies are formed by the membrane blebbing of an injured cell as it undergoes apoptosis. They range from 100nm–5um [50][51]. Oncosomes are formed from the irregular membrane protrusions on malignant tumors, and may range from 1um–10um in size [11][50]. Only exosomes and MVs are relevant to the therapeutic effects imparted by MSC-EVs. A summary of different characteristics for subtypes of EVs are described in Table 1 below [11][48][50][51][52][53][54][55][56].

Table 1. Subtypes and Properties of Extracellular Vesicles.

Exosomes	Microvesicles	Apoptotic Bodies	Oncosomes	
Alternative Names	**Small EVs**	**Ectosomes, Shedding vesicles, Microparticles, Exovesicles**		**Large Oncosomes**
Intracellular Origin	**Multivesicular Endosome**	**Plasma Membrane**	**Membrane blebbing during cell death**	**Non-apoptotic tumor-cell membrane blebbing**
Size	**50–150 nm**	**150–1000 nm**	**100 nm–5 um**	**1 um–10 um**
Differential Ultracentrifugation	**100,000× *g* for 90 min**	**10,000× *g* for 30 min**	**800× *g* for 10 min; then 16,000× *g* for 20 min**	**Alternate 8000× *g* for 30 sec and 0.2 µm filtration; Oncosomes are caught by filter**
Enriched Protein Pathways	**Extracellular matrix; Heparin-binding; receptors; Immune response; Cell adhesion**	**Endoplasmic reticulum; Proteasome; Mitochondria**	**Heterogeneous**	**Extracellular matrix degradation; Angiogenesis; Cancer metabolism**
Enriched Lipid Contents	**Glycolipids, Free fatty acids, Phosphatidylserines**	**Ceramides and Sphingomyelins**		
Structural Plasma Membrane Lipids	**Phosphatidylserine enrichment; Phosphatidylcholine, Phosphatidylglycerol, Phosphatidylinositol, and Phosphatidylethanolamine depletions**	**Dependent upon cellular origin; Most have phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine depletions**	**Phosphatidylserine enrichment**	**Phospholipid and phosphatidylserine enrichment**
Contents	**Proteins, Lipids, RNAs**	**Organelles, Proteins, Lipids, RNAs**	**Organelles, Histones, DNAs, RNAs, Nuclear fractions**	**Proteins, RNAs**

Angiogenesis is part of the brain's endogenous repair process after ischemic injury. Recovery of the cerebral vasculature and neuronal recovery are tightly coupled [57][58]. Ischemic stroke patients with greater angiogenesis and vasculogenesis have longer survival times, while older patients with reduced new vessel formation fare worse [59][60]. In addition, post-stroke dementia may be related to lower cerebral perfusion and impairments of the NVU [61][62]. Research suggests that administration of MSCs and MSC-EVs is able to boost the brain's regenerative potential [63][64][65][66][67]. The therapeutic effects of MSC-EV administration yield functionally equivalent benefits to MSC administration, including angiogenesis, neuroprotection, neurogenesis, and functional recovery [63][64][65]. Critically, MSC-EVs are able to go one step farther than MSCs; they can cross the BBB. [68].

MSC-EV administration attenuates post-ischemia immunosuppression, resulting in an environment favorable to neuronal recovery [63]. In a rat traumatic brain injury (TBI) model, MSC-derived exosomes did not affect lesion volume; however; it did improve functional recovery, increase vascular density, increase the number of new neuroblasts, reduce inflammation, and increase angiogenesis [69]. Administration of MSC-EVs during the subacute phase of neonatal hypoxic-ischemic (HI) brain injury resulted in increased proliferation of endothelial cells, as well as a reduction in pro-inflammatory astroglia and microglia activations [66]. These studies show that MSC-EVs exert positive regenerative effects on the ruptured BBB.

References

1. Virani, S.S.; Alonso, A.; Aparicio, H.J.; Benjamin, E.J.; Bittencourt, M.S.; Callaway, C.W.; Carson, A.P.; Chamberlain, A.M.; Cheng, S.; Delling, F.N.; et al. Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. Circulation 2021, 143, e254–e743.
2. Prevalence and Most Common Causes of Disability Among Adults—United States, 2005. Morb. Mortal. Wkly. Rep. 2009, 58, 421–426.
3. Diseases, G.B.D.; Injuries, C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet 2020, 396, 1204–1222.
4. Collaborators, G.B.D.S. Global, regional, and national burden of stroke, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019, 18, 439–458.
5. Kim, S.Y.; Buckwalter, M.; Soreq, H.; Vezzani, A.; Kaufer, D. Blood-brain barrier dysfunction-induced inflammatory signaling in brain pathology and epileptogenesis. Epilepsia 2012, 53 (Suppl. S6), 37–44.
6. Sakadzic, S.; Lee, J.; Boas, D.A.; Ayata, C. High-resolution in vivo optical imaging of stroke injury and repair. Brain Res 2015, 1623, 174–192.
7. DiNapoli, V.A.; Huber, J.D.; Houser, K.; Li, X.; Rosen, C.L. Early disruptions of the blood-brain barrier may contribute to exacerbated neuronal damage and prolonged functional recovery following stroke in aged rats. Neurobiol. Aging 2008, 29, 753–764.
8. Satani, N.; Savitz, S.I. Is Immunomodulation a Principal Mechanism Underlying How Cell-Based Therapies Enhance Stroke Recovery? Neurotherapeutics 2016, 13, 775–782.
9. Drago, D.; Cossetti, C.; Iraci, N.; Gaude, E.; Musco, G.; Bachi, A.; Pluchino, S. The stem cell secretome and its role in brain repair. Biochimie 2013, 95, 2271–2285.
10. Maumus, M.; Jorgensen, C.; Noel, D. Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: Role of secretome and exosomes. Biochimie 2013, 95, 2229–2234.
11. Van Niel, G.; D'Angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. Nat. Rev. Mol. Cell Biol. 2018, 19, 213–228.
12. Raposo, G.; Stoorvogel, W. Extracellular vesicles: Exosomes, microvesicles, and friends. J. Cell Biol. 2013, 200, 373–383.
13. Obermeier, B.; Daneman, R.; Ransohoff, R.M. Development, maintenance and disruption of the blood-brain barrier. Nat. Med. 2013, 19, 1584–1596.
14. Jiang, X.; Andjelkovic, A.V.; Zhu, L.; Yang, T.; Bennett, M.V.L.; Chen, J.; Keep, R.F.; Shi, Y. Blood-brain barrier dysfunction and recovery after ischemic stroke. Prog. Neurobiol. 2018, 163–164, 144–171.
15. Keaney, J.; Campbell, M. The dynamic blood-brain barrier. FEBS J. 2015, 282, 4067–4079.
16. Xue, Q.; Liu, Y.; Qi, H.; Ma, Q.; Xu, L.; Chen, W.; Chen, G.; Xu, X. A novel brain neurovascular unit model with neurons, astrocytes and microvascular endothelial cells of rat. Int. J. Biol. Sci. 2013, 9, 174–189.
17. Canfield, S.G.; Stebbins, M.J.; Faubion, M.G.; Gastfriend, B.D.; Palecek, S.P.; Shusta, E.V. An isogenic neurovascular unit model comprised of human induced pluripotent stem cell-derived brain microvascular endothelial cells, pericytes,

astrocytes, and neurons. Fluids Barriers CNS 2019, 16, 25.

18. Underly, R.G.; Levy, M.; Hartmann, D.A.; Grant, R.I.; Watson, A.N.; Shih, A.Y. Pericytes as Inducers of Rapid, Matrix Metalloproteinase-9-Dependent Capillary Damage during Ischemia. J. Neurosci. 2017, 37, 129–140.
19. Chen, J.; Leak, R.K.; Yang, G.Y. Perspective for stroke and brain injury research: Mechanisms and potential therapeutic targets. CNS Neurosci. Ther. 2015, 21, 301–303.
20. Iadecola, C.; Anrather, J. The immunology of stroke: From mechanisms to translation. Nat. Med. 2011, 17, 796–808.
21. Lai, T.W.; Zhang, S.; Wang, Y.T. Excitotoxicity and stroke: Identifying novel targets for neuroprotection. Prog. Neurobiol. 2014, 115, 157–188.
22. Anrather, J.; Iadecola, C. Inflammation and Stroke: An Overview. Neurotherapeutics 2016, 13, 661–670.
23. Nahirney, P.C.; Reeson, P.; Brown, C.E. Ultrastructural analysis of blood-brain barrier breakdown in the peri-infarct zone in young adult and aged mice. J. Cereb. Blood Flow. Metab. 2016, 36, 413–425.
24. Krueger, M.; Hartig, W.; Reichenbach, A.; Bechmann, I.; Michalski, D. Blood-brain barrier breakdown after embolic stroke in rats occurs without ultrastructural evidence for disrupting tight junctions. PLoS ONE 2013, 8, e56419.
25. Willis, C.L.; Meske, D.S.; Davis, T.P. Protein kinase C activation modulates reversible increase in cortical blood-brain barrier permeability and tight junction protein expression during hypoxia and posthypoxic reoxygenation. J. Cereb. Blood Flow Metab. 2010, 30, 1847–1859.
26. Rochfort, K.D.; Cummins, P.M. Cytokine-mediated dysregulation of zonula occludens-1 properties in human brain microvascular endothelium. Microvasc. Res. 2015, 100, 48–53.
27. Andjelkovic, A.V.; Xiang, J.; Stamatovic, S.M.; Hua, Y.; Xi, G.; Wang, M.M.; Keep, R.F. Endothelial Targets in Stroke: Translating Animal Models to Human. Arterioscler. Thromb. Vasc. Biol. 2019, 39, 2240–2247.
28. Knowland, D.; Arac, A.; Sekiguchi, K.J.; Hsu, M.; Lutz, S.E.; Perrino, J.; Steinberg, G.K.; Barres, B.A.; Nimmerjahn, A.; Agalliu, D. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. Neuron 2014, 82, 603–617.
29. Armulik, A.; Genove, G.; Mae, M.; Nisancioglu, M.H.; Wallgard, E.; Niaudet, C.; He, L.; Norlin, J.; Lindblom, P.; Strittmatter, K.; et al. Pericytes regulate the blood-brain barrier. Nature 2010, 468, 557–561.
30. Simpkins, A.N.; Dias, C.; Leigh, R.; National Institutes of Health Natural History of Stroke Investigators. Identification of Reversible Disruption of the Human Blood-Brain Barrier Following Acute Ischemia. Stroke 2016, 47, 2405–2408.
31. Wang, C.X.; Shuaib, A. Critical role of microvasculature basal lamina in ischemic brain injury. Prog. Neurobiol. 2007, 83, 140–148.
32. Rosell, A.; Foerch, C.; Murata, Y.; Lo, E.H. Mechanisms and markers for hemorrhagic transformation after stroke. Acta Neurochir. Suppl. 2008, 105, 173–178.
33. Pfefferkorn, T.; Rosenberg, G.A. Closure of the blood-brain barrier by matrix metalloproteinase inhibition reduces rtPA-mediated mortality in cerebral ischemia with delayed reperfusion. Stroke 2003, 34, 2025–2030.
34. De Meyer, S.F.; Denorme, F.; Langhauser, F.; Geuss, E.; Fluri, F.; Kleinschnitz, C. Thromboinflammation in Stroke Brain Damage. Stroke 2016, 47, 1165–1172.
35. Eltzschig, H.K.; Collard, C.D. Vascular ischaemia and reperfusion injury. Br. Med. Bull. 2004, 70, 71–86.
36. Wang, Z.; Wang, Y.; Ye, J.; Lu, X.; Cheng, Y.; Xiang, L.; Chen, L.; Feng, W.; Shi, H.; Yu, X.; et al. bFGF attenuates endoplasmic reticulum stress and mitochondrial injury on myocardial ischaemia/reperfusion via activation of PI3K/Akt/ERK1/2 pathway. J. Cell Mol. Med. 2015, 19, 595–607.
37. Yu, H.C.; Qin, H.Y.; He, F.; Wang, L.; Fu, W.; Liu, D.; Guo, F.C.; Liang, L.; Dou, K.F.; Han, H. Canonical notch pathway protects hepatocytes from ischemia/reperfusion injury in mice by repressing reactive oxygen species production through JAK2/STAT3 signaling. Hepatology 2011, 54, 979–988.
38. Yang, G.Y.; Betz, A.L. Reperfusion-induced injury to the blood-brain barrier after middle cerebral artery occlusion in rats. Stroke 1994, 25, 1658–1664.
39. Yin, K.J.; Hamblin, M.; Chen, Y.E. Angiogenesis-regulating microRNAs and Ischemic Stroke. Curr. Vasc. Pharmacol. 2015, 13, 352–365.
40. Ames, A., 3rd; Wright, R.L.; Kowada, M.; Thurston, J.M.; Majno, G. Cerebral ischemia. II. The no-reflow phenomenon. Am. J. Pathol. 1968, 52, 437–453.
41. Reffelmann, T.; Hale, S.L.; Dow, J.S.; Kloner, R.A. No-reflow phenomenon persists long-term after ischemia/reperfusion in the rat and predicts infarct expansion. Circulation 2003, 108, 2911–2917.

42. Ito, U.; Hakamata, Y.; Kawakami, E.; Oyanagi, K. Temporary [corrected] cerebral ischemia results in swollen astrocytic end-feet that compress microvessels and lead to delayed [corrected] focal cortical infarction. J. Cereb. Blood Flow Metab. 2011, 31, 328–338.

43. Ito, U.; Hakamata, Y.; Watabe, K.; Oyanagi, K. Astrocytic involvement in the maturation phenomenon after temporary cerebral ischemia. Acta Neurochir. Suppl. 2013, 118, 23–29.

44. Leigh, R.; Jen, S.S.; Hillis, A.E.; Krakauer, J.W.; Barker, P.B.; Albers, G.W.; Davis, S.M.; Donnan, G.A.; Fisher, M.; Furlan, A.J.; et al. Pretreatment blood-brain barrier damage and post-treatment intracranial hemorrhage in patients receiving intravenous tissue-type plasminogen activator. Stroke 2014, 45, 2030–2035.

45. Nadareishvili, Z.; Simpkins, A.N.; Hitomi, E.; Reyes, D.; Leigh, R. Post-Stroke Blood-Brain Barrier Disruption and Poor Functional Outcome in Patients Receiving Thrombolytic Therapy. Cerebrovasc. Dis. 2019, 47, 135–142.

46. Wei, X.; Yang, X.; Han, Z.P.; Qu, F.F.; Shao, L.; Shi, Y.F. Mesenchymal stem cells: A new trend for cell therapy. Acta Pharmacol. Sin. 2013, 34, 747–754.

47. Kehl, D.; Generali, M.; Mallone, A.; Heller, M.; Uldry, A.C.; Cheng, P.; Gantenbein, B.; Hoerstrup, S.P.; Weber, B. Proteomic analysis of human mesenchymal stromal cell secretomes: A systematic comparison of the angiogenic potential. NPJ Regen. Med. 2019, 4, 8.

48. Colombo, M.; Raposo, G.; Thery, C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell Dev. Biol. 2014, 30, 255–289.

49. Veziroglu, E.M.; Mias, G.I. Characterizing Extracellular Vesicles and Their Diverse RNA Contents. Front. Genet. 2020, 11, 700.

50. Zaborowski, M.P.; Balaj, L.; Breakefield, X.O.; Lai, C.P. Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. Bioscience 2015, 65, 783–797.

51. Hristov, M.; Erl, W.; Linder, S.; Weber, P.C. Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells in vitro. Blood 2004, 104, 2761–2766.

52. Heijnen, H.F.G.; Schiel, A.E.; Fijnheer, R.; Geuze, H.J.; Sixma, J.J. Activated Platelets Release Two Types of Membrane Vesicles: Microvesicles by Surface Shedding and Exosomes Derived From Exocytosis of Multivesicular Bodies and α-Granules. Blood 1999, 94, 3791–3799.

53. Purvinsh, L.; Gorshkov, A.; Brodskaia, A.; Vasin, A. Extracellular Vesicles in Viral Pathogenesis: A Case of Dr. Jekyll and Mr. Hyde. Life 2021, 11, 45.

54. Haraszti, R.A.; Didiot, M.C.; Sapp, E.; Leszyk, J.; Shaffer, S.A.; Rockwell, H.E.; Gao, F.; Narain, N.R.; DiFiglia, M.; Kiebish, M.A.; et al. High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources. J. Extracell Vesicles 2016, 5, 32570.

55. Witwer, K.W.; Van Balkom, B.W.M.; Bruno, S.; Choo, A.; Dominici, M.; Gimona, M.; Hill, A.F.; De Kleijn, D.; Koh, M.; Lai, R.C.; et al. Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. J. Extracell Vesicles 2019, 8, 1609206.

56. Minciacchi, V.R.; You, S.; Spinelli, C.; Morley, S.; Zandian, M.; Aspuria, P.J.; Cavallini, L.; Ciardiello, C.; Reis Sobreiro, M.; Morello, M.; et al. Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles. Oncotarget 2015, 6, 11327–11341.

57. Hatakeyama, M.; Ninomiya, I.; Kanazawa, M. Angiogenesis and neuronal remodeling after ischemic stroke. Neural. Regen. Res. 2020, 15, 16–19.

58. Teng, H.; Zhang, Z.G.; Wang, L.; Zhang, R.L.; Zhang, L.; Morris, D.; Gregg, S.R.; Wu, Z.; Jiang, A.; Lu, M.; et al. Coupling of angiogenesis and neurogenesis in cultured endothelial cells and neural progenitor cells after stroke. J. Cereb. Blood Flow Metab. 2008, 28, 764–771.

59. Szpak, G.M.; Lechowicz, W.; Lewandowska, E.; Bertrand, E.; Wierzba-Bobrowicz, T.; Dymecki, J. Border zone neovascularization in cerebral ischemic infarct. Folia Neuropathol. 1999, 37, 264–268.

60. Krupinski, J.; Kaluza, J.; Kumar, P.; Kumar, S.; Wang, J.M. Role of angiogenesis in patients with cerebral ischemic stroke. Stroke 1994, 25, 1794–1798.

61. Ding, R.; Hase, Y.; Ameen-Ali, K.E.; Ndung'u, M.; Stevenson, W.; Barsby, J.; Gourlay, R.; Akinyemi, T.; Akinyemi, R.; Uemura, M.T.; et al. Loss of capillary pericytes and the blood-brain barrier in white matter in poststroke and vascular dementias and Alzheimer's disease. Brain Pathol. 2020, 30, 1087–1101.

62. Schmidt, R.; Schmidt, H.; Fazekas, F. Vascular risk factors in dementia. J. Neurol. 2000, 247, 81–87.

63. Doeppner, T.R.; Herz, J.; Gorgens, A.; Schlechter, J.; Ludwig, A.K.; Radtke, S.; de Miroschedji, K.; Horn, P.A.; Giebel, B.; Hermann, D.M. Extracellular Vesicles Improve Post-Stroke Neuroregeneration and Prevent Postischemic

Immunosuppression. Stem Cells Transl. Med. 2015, 4, 1131–1143.

64. Xin, H.; Li, Y.; Cui, Y.; Yang, J.J.; Zhang, Z.G.; Chopp, M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J. Cereb. Blood Flow Metab. 2013, 33, 1711–1715.
65. Zhang, Y.; Zhang, Y.; Chopp, M.; Zhang, Z.G.; Mahmood, A.; Xiong, Y. Mesenchymal Stem Cell-Derived Exosomes Improve Functional Recovery in Rats After Traumatic Brain Injury: A Dose-Response and Therapeutic Window Study. Neurorehabilit. Neural. Repair. 2020, 34, 616–626.
66. Kaminski, N.; Koster, C.; Mouloud, Y.; Borger, V.; Felderhoff-Muser, U.; Bendix, I.; Giebel, B.; Herz, J. Mesenchymal Stromal Cell-Derived Extracellular Vesicles Reduce Neuroinflammation, Promote Neural Cell Proliferation and Improve Oligodendrocyte Maturation in Neonatal Hypoxic-Ischemic Brain Injury. Front. Cell Neurosci. 2020, 14, 601176.
67. Doeppner, T.R.; Bahr, M.; Giebel, B.; Hermann, D.M. Immunological and non-immunological effects of stem cell-derived extracellular vesicles on the ischaemic brain. Ther. Adv. Neurol. Disord. 2018, 11, 1756286418789326.
68. Xin, H.; Li, Y.; Liu, Z.; Wang, X.; Shang, X.; Cui, Y.; Zhang, Z.G.; Chopp, M. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. Stem Cells 2013, 31, 2737–2746.
69. Zhang, Y.; Chopp, M.; Meng, Y.; Katakowski, M.; Xin, H.; Mahmood, A.; Xiong, Y. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. J. Neurosurg. 2015, 122, 856–867.
