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Review Article

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The Preclinical and Clinical Applications of Mesenchymal Stem Cells in Stroke

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ABSTRACT

Stroke is a kind of sudden onset of cerebral circulatory disorders with transient or permanent symptoms and signs of brain dysfunction clinically, it has a high incidence rate and a high mortality rate and is one of the severe cerebrovascular diseases that threaten human life and disability seriously and bring the heavy economic burden to patients and their families, there was no ideal treatment for it. Mesenchymal stem cells (MSCs) are a type of primitive undifferentiated cells with multi-directional differentiation potential and self-replication ability, they have the characteristic of convenient source and can differentiate into various types of somatic cells including nerve cells, cardiomyocytes, and osteoblasts and secrete various growth factors including brain derived neurotrophic factor, nerve growth factor, and epidermal growth factor, demonstrating the enormous application potential in many kinds of diseases including stroke. In this review, we summarized the basic characteristics and sources of MSCs, recent research progression of preclinical application and clinical application of MSCs in stroke, and the application limitations and the prospect were also discussed, hope our review may provide some useful clues for the related researchers.

Key words: *MSCs, Stroke, Preclinic application, Clinic application*

INTRODUCTION

It is reported that stroke has been becoming the second leading cause of death and the third leading cause of disability in the world [1]. The number of stroke patients has been increasing significantly year by year in recent ten years, because of the characteristics of high incidence rate, high recurrence rate, high disability rate, and high mortality, almost 70-85% of stroke patients experience varying degrees of loss of life and work abilities, bringing significant economic burden for families and society [2-4]. Many kinds of risk factors could result in stroke including hypertension [5, 6], diabetes mellitus [7], high fasting blood glucose [8, 9], smoking [10, 11], and drinking [12, 13], these risk factors pose significant obstacles to the prevention and treatment of stroke. How to effectively treat and alleviate the symptoms of stroke patients is becoming a global problem in the world.

Stem cells are a kind of special cells with self-renewal ability and multi-directional differentiation potential [14], they can be divided into three types including totipotent stem cells, pluripotent stem cells, and unipotent stem cells according to the developmental potential of stem cells [15, 16]. Multiple studies have demonstrated that MSCs could differentiate into different cell types including osteoblasts [17, 18], chondrocytes [19, 20], and nerve cells [21, 22] under specific conditions; MSCs could also express, synthesize, and secrete many kinds of bioactive molecules including growth factors, cytokines, signal peptides and exosomes which regulating play the important role in cell metabolism, immunity, differentiation, and proliferation in the body [23, 34]; and they also have the

characteristic of advantageous distribution towards the site of injury [25, 26], those advantages of MSCs exhibit the enormous application potential in regenerative medicine. Moreover, many studies have demonstrated that MSCs also exhibit significant advantages in alleviating and treating stroke. For example, Smith *et al*. reported that hematopoietic stem cells could reduce infarct volume, mortality rate, and microglial activation by regulating metallothionein-1 in ischemia-reperfusion injury mice model [27]; Jiao-Jiao Peng *et al*. demonstrated that repetitive transcranial magnetic stimulation could promote the embryonic stem cells-derived human neural stem cells to differentiate into neuron-like cells and to accelerate functional recovery in middle cerebral artery occlusion rat model [28]; and Wang *et al*. found that ZL006 could improve the homing ability of neural stem cells into the ischemia-injured site and accelerate the neuronal differentiation in focal cerebral ischemic male rat [29]. In this review, we will introduce the characteristics and sources of MSCs, summarize the recent research progression of MSCs application in stroke, and the application limitations and the prospect of MSCs were also discussed, hope our review may provide some clues for the related researchers **(Figure 1)**.

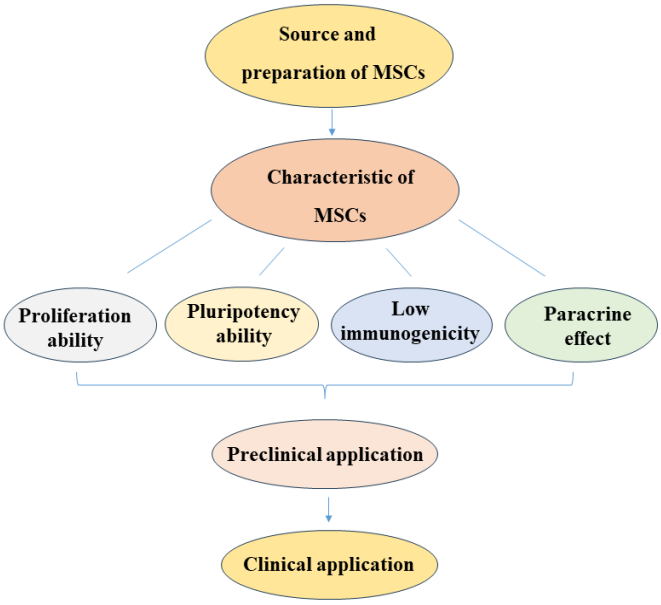

Figure 1. Graphical abstract of the review article

Characteristics and source of MSCs

Source and preparation of MSCs

MSCs are a kind of pluripotent adult stem cells with self-renewal ability derived from the mesoderm, which could differentiate into various cell types; MSCs distributed in connective tissue and interstitium of organs including adipose tissue, bone marrow, umbilical cord, dental pulp, placenta, amniotic fluid, muscle tissue, and thymus tissue [30-32]. The preparation of MSCs is relatively simple and diverse, for example, the isolation methods of bone marrow MSCs include a conventional tissue-adherent method, bone tissue digestion method, density gradient centrifugation method, immunomagnetic bead sorting method, and flow cytometry separation method [33]; the isolation methods of umbilical cord MSCs and adipose-derived stem cells include tissue block adhesion method [34], enzyme digestion method [35], adsorption column method [36] and direct centrifugation method [37]. Those different isolation methods of different kinds of MSCs have their advantages and disadvantages and could be selected according to practical requirements. We have summarized the advantages and disadvantages of the common isolation methods in **Table 1**.

Table 1. The advantages and disadvantages of the common isolation methods

Isolation method	Advantages	Disadvantages
Adherent screening method	simple operation, time-saving, less pollution	low cell purity

Density gradient centrifugation method	high quality and higher purity	strict and complex operations, low cell activity
Tissue digestion method	simple operation, low cost	collagenase could affect the cell activity and proliferation ability
The immunomagnetic bead sorting method	simple and efficient, and high purity	magnetic separator required, high cost, complex operations
Flow cytometry separation method	high purity	complex operations, high cost and time-consuming

Proliferation ability of MSCs

MSCs have the advantage of being capable of large-scale amplification and a strong proliferation ability in vitro. In theory, MSCs have high physiological activity and could proliferate infinitely. Some evidence suggested that MSCs could still maintain pluripotency and immunogenicity after many passages. Qinjun Zhao *et al*. compared the biological properties of human umbilical cord MSCs at P3, P6 and P15, found that human umbilical cord MSCs at P3, P6 and P15 had the higher consistency in morphology, expression of biomarkers and cytokines [38]; Urszula Kozlowska *et al*. compared the biological activity of bone marrow MSCs, adipose tissue-derived MSCs, skeletal muscles-derived MSCs and skin MSCs which cultured for ten weeks, found that four different types of long term cultured MSCs exhibited the basic relatively consistent phenotypes of MSCs, especially, long term cultured bone marrow MSCs and long term cultured adipose tissue-derived MSCs could keep the expression of Sox2 and Oct4 and multilineage differentiation ability [39]; Shannon S Connard *et al*. compared the immunomodulatory properties changes of P3 equine bone marrow MSCs, P6 equine bone marrow MSCs, and P9 equine bone marrow MSCs, found that there were no significant differences in promoting cytokine synthesis and expression of nterleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) in three different passage of bone marrow MSCs [40]; Mengbo Yang *et al*. demonstrated that P2 human umbilical cord MSCs and P8 umbilical cord MSCs had the same cell morphology and same expression of surface markers [41]; Mahnaz Babaahmadi *et al.* provided the evidences that bone marrow MSCs with long-term passages and the early passages of WJ-MSCs and BM-hMSCs had the similar therapeutic effects in collagen-induced arthritis rat [42], reflecting the same application advantages of long-term cultured MSCs and short-term cultured MSCs with a large amount of amplification.

Pluripotency ability of MSCs

Evidence demonstrated that MSCs could differentiate into three different germinal layers including neural cells [43], cardiomyocytes [44], kidney cells [45], and skin cells [46]; MSCs could also express the pluripotency markers NANOG, OCT-4, and SSEA-4; MSCs exhibit the excellent pluripotency ability. Aleksandra Musiał-Wysocka *et al*. isolated the MSCs from Wharton's jelly of the umbilical cord and confirmed that expression of NANOG, OCT-4, and SSEA-4 in Wharton's jelly of umbilical cord-derived MSCs was lower than in iPSCs, and hypoxia could improve the expression of NANOG, OCT-4, and SSEA-4 and increase its pluripotency [47]. Interestingly, the pluripotency ability of MSCs could be enhanced through various physical and chemical pathways. For example, Ana Borojević *et al*. reported that Vitamin D3 could increase the expression of pluripotency markers in human Bone Marrow MSCs through SIRT1 signaling [48]; Mekhemar *et al*. found that Thymoquinone from Nigella sativa could improve the expression of TLR3 and NANOG in Gingival MSCs [49]; Gitika Thakur *et al*. demonstrated that the special 3D culture could provide the cell microenvironments to enhance the expression of pluripotency markers in Wharton's jelly MSCs [50]. Therefore, MSCs exhibit enormous clinical application potential including neurological diseases and kidney diseases because of the pluripotent characteristics of MSCs.

Low immunogenicity of MSCs

Low immunogenicity is another important characteristic of MSCs for clinical application. It is reported that MSCs do not express major histocompatibility complex class II (MHC-II) molecules, MSCs could exhibit low immunogenicity in their immune characteristics and do not actively release their identity information when entering the host; and MSCs could evade the host's immune response through immune privilege during their participation in the immune process [51-53].

Paracrine effect of MSCs

MSCs could express, synthesize, and secrete many kinds of bioactive molecules including growth factors, cytokines, transcription factors, and signal peptides to regulate metabolism, cell differentiation, proliferation, migration, and apoptosis to balance the internal homeostasis of the body, the paracrine effect of MSCs plays the important role in tissue regeneration and organ repair [54, 55].

The preclinical application of MSCs in stroke

Due to the rapid development of stem cell technology, MSCs have been widely used and proven to alleviate and treat stroke in many kinds of animal models. The preclinical application of MSCs is mainly based on the different characteristics of MSCs including proliferation and pluripotency ability, low immunogenicity, and paracrine effect acting on animal models to explore their therapeutic effects and underlying mechanisms. For example, Fenjun Jiang *et al*. obtained a kind of neurotrophic factor-secreting MSCs from the bone marrow MSCs, found that this kind of neurotrophic factor-secreting MSCs could secrete highly level of neurotrophic factors including glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) compared with the bone marrow MSCs, and could reduce the infarct volume and increase the functional recovery in ischemic stroke rats [56]; Oh Young Bang *et al*. compared the effects of normal MSCs and CXCR4 overexpression of MSCs on rat stroke model, found that the migration of CXCR4 overexpression of MSCs was better than normal MSCs, and CXCR4 overexpression of MSCs could promote the behavioral recovery significantly compared with normal MSCs [57]; and Sanghun Lee *et al*. demonstrated that CCL2-overexpressing human umbilical cord MSCs could improve the functional recovery and decrease the stroke volume in middle cerebral arterial occlusion rats compared with normal human umbilical cord MSCs [58]. We summarized the recent preclinical application of MSCs in stroke in **Table 1**. In summary, cell resources mainly include humans, mice, and rats; stem cell type includes neural stem cells, adipose-derived MSCs, bone marrow MSCs, umbilical cord blood MSCs, and hair follicle stem cells; the main stroke modeling method is middle cerebral artery occlusion; the administration dosage focus on 1.0×10^5 cells, the details have been shown in **Table 2**.

Table 2. The recent preclinical application of MSCs in stroke

Cell resource	Stem cell type	Model	Dosage	Administration method	Therapeutic effect	Mechanism	References
Human	NSCs	Middle cerebral artery occlusion rat	5×10^5/20uL	Stereotactic injection	reduced brain tissue atrophy and memory functional loss	neuroprotective and pro-angiogenic paracrine activities by JAK2/STAT3	[59]
Mouse	ASCs	middle cerebral artery occlusion mice	2×10^6/20uL	intraperitoneal injection	infarct size was reduced, and neurological recovery was increased	EVs containing miR-25-3p regulate autophagy	[60]
Human	BMMSCs	distal middle cerebral artery occlusion mice	1×10^6	intraperitoneal injection	increased peri-infarct blood flow and vascular density and reduced infarct volume	upregulation of Rabep2	[61]
Mouse	NSCs	transient middle cerebral artery occlusion mice	/	intraperitoneal injection	reduction in neurological deficit along with reduced infarct area	Jak2/Stat3 pathway	[62]
Rat	BMMSCs	middle cerebral artery occlusion rat	5×10^6 cells/kg	intraperitoneal injection	reductions in infarct size and inhibition of microglial activation	upregulated neuron-glia antigen 2	[63]
Rat	MSCs	middle cerebral artery occlusion rat	/	intraperitoneal injection	improving the mitochondrial activity and functional recovery	mitochondria transfer	[64]

Human	BMMSCs	transient middle cerebral artery occlusion mice	1×10^6/50uL	intraperitoneal injection	improving functional recovery	activation of PI3K/Akt pathway	[65]
Human	NSCs	middle cerebral artery occlusion (MCAO) and oxygen-glucose deprivation rat	/	intraperitoneal injection	improving the spatial memory performance	promoting β-catenin nuclear translocation	[66]
Mouse	MSCs	permanent focal cerebral ischemia mice	5×10^5	intraperitoneal injections	improving functional recovery	gap junction-mediated cell-cell interaction	[67]
Human	BMMSCs	1 hour middle cerebral artery occlusion rat	3×10^5 cell/9μL	intraperitoneal injections	brain-to-periphery migration	lymphatic and inflammation pathways	[68]
Mouse	NSCs	middle cerebral artery occlusion mouse	1×10^5cell /μL	intraperitoneal injection	neurological function improvement	inhibited p53-mediated Proapoptotic Pathway	[69]
Mouse	NSCs	injecting endothelin in the right pons	0.5×10^6 /μL	intraperitoneal injection	improving neurological function	overexpressed BDNF and Dlx2	[70]
Human	UCBMSCs	Middle cerebral Artery Occlusion rat	5×10^5	intraperitoneal injection	improve neuroprotection, decrease inflammation, and increase angiogenesis	release cytokines and decrease inflammation	[71]
Human	UCBMSCs	Middle cerebral Artery Occlusion rat	2.5×10^5	intraperitoneal injection	improved the recovery of sensory and motor function	/	[72]
Mouse	BMMSCs	bilateral common carotid artery occlusion mouse	2×10^6	intraperitoneal injection	improved neuroprotective property	attenuating the host cell response	[73]
Rat	HFSCs	Middle cerebral Artery Occlusion rat	1×10^6 cells/mL	intraperitoneal injection	reduced the infarct volume and promoted neurological recovery	/	[74]

Abbreviations: MSCs: Mesenchymal stem cells; NSCs: Neural stem cells; ASCs : Adipose-derived MSCs; BMMSCs: Bone marrow mesenchymal stem cells; UCB-MSCs: Umbilical cord blood mesenchymal stem cells; HFSCs: Hair follicle stem cells.

The clinical application of MSCs in stroke

It is essential to conduct systematic studies on patients or healthy volunteers to confirm the efficacy and safety of the investigational drug for final validation. Similarly, to verify the therapeutic effect of MSCs in stroke, numerous clinical trials have been conducted around the world in the past ten years. The results demonstrated that there were 16 clinic trials (phase I stage or phase II stage) of MSCs application in stroke had been reported from 2014-2023; stem cell type mainly included neural stem cells (5 clinic trials), bone marrow MSCs (4 clinic trials) and adipose MSCs (3 clinic trials); the dosage of MSCs were variant from 0.5×10^6 to 3.0×10^8 cells; intraperitoneal injection was the main administration; injection period was from 3 months to 30 months; there were 6 clinic trials reported that intraperitoneal injection of MSCs had the adverse effect in patients, the ratio was 37.5% (6/16); the main countries for conducting clinical trials were China (5 clinic trials), Japan (2 clinic trials) and Spain (2 clinic trials). The details are summarized in **Table 3**.

Table 3. The clinical application of MSCs in stroke from 2014-2023

Clinic trial type	Stem cell type	Stroke type	Dosage	Administration method	Period	Adverse effect	Country	References
open-label intervention study	BMMSCs	perinatal arterial ischaemic stroke	45-50×10^6	intraperitoneal injection	3 months	a mild transient fever of 38°C without the need for clinical intervention	Netherlands	[75]
prospective randomized controlled trial	MSCs	chronic major stroke	5×10^6 cells/mL	infused via the antecubital vein	3 months	unknown	South Korea	[76]
a prospective, multicentre, single-arm, open-label study	NSCs	ischaemic stroke	2×10^6 cells	stereotaxic injection	6-12 months	no cell-related adverse events	United Kingdom	[77]
single-center, open-label, randomized controlled trials	BMMSCs	moderate-severe ischemic carotid stroke	unknown	intravenous injection	6-24 months	yes	France	[78]
randomized, double-blind, placebo-controlled, multicentre, phase 1/2 clinical trial	DPSCs s	acute ischaemic stroke	1-3×10^8	intravenous administration	30 months	unknown	Japan	[79]
Phase I/II Study	BMMSCs	chronic stroke	3.6-12.4×10^6 cells/kg	Intravenous transfusion	12 months	15 serious adverse events including infections, vascular disorders, and pain syndromes	USA	[80]
single-centre, randomized, double-blinded, parallel-controlled trial	NSCs	ischaemic stroke	2.5×10^6 cells/100uL	intranasal administration	12 months	unknown	China	[81]
a phase IIb, multicentre, randomized, double-blind, placebo-controlled clinical trial	ASCs	ischaemic stroke	1.0×10^6 cells/mL	intranasal administration	24 months	unknown	Spain	[82]
phase I open-label clinical trial	ASCs	chronic ischemic stroke	1×10^8	intracerebral transplantation	6 months	no adverse events	China	[83]
an open-label, single-site, dose-escalation trial	NSCs	chronic ischaemic stroke	2.0-20×10^6 cells	stereotactic ipsilateral putamen injection	29 months	no adverse events	USA	[84]
a Phase I/II randomized, placebo-controlled trial	allogeneic MSCs	Chronic Stroke	0.5-1.5×10^6 cells/kg	intracerebral transplantation	12 months	15 serious adverse events	Canada	[85]
a single-site, phase I trial	NSCs	ischemic stroke	1.2-7.2×10^7 cells	intracerebral microinjection	24 months	unknown	China	[86]

a Phase I/II randomized, placebo-controlled trial	NSCs, MSCs	ischemic stroke	0.5-6.0×10^6 cells/kg	intravenous injection	24 months	low fever (<38.5°C)	China	[87]
a Phase II single-arm, open-label trial	autologous MSCs	stroke	0.5-2.0×10^8 cells	intravenous injection	6 months	unknown	Japan	[88]
A phase II, randomized, double-blind, placebo-controlled, single-center, pilot clinical trial	ASCs	acute ischemic stroke	1.0×10^6 cells/kg	intravenous injection	24 months	1 case of serious adverse event was found	Spain	[89]
a randomized controlled observer-blinded trial	bone marrow MSCs	severe ischemic stroke	1.0×10^6 cells/kg	intravenous injection	12 months	unknown	China	[90]

Abbreviations: MSCs: Mesenchymal stem cells; NSCs: Neural stem cells; ASCs : Adipose-derived MSCs; BMMSCs: Bone marrow mesenchymal stem cells; UCB-MSCs: Umbilical cord blood mesenchymal stem cells; HFSCs: Hair follicle stem cells; dental pulp stem cells: DPSCs.

CONCLUSION

Stem cell therapy is becoming one of the most promising treatment methods for difficult diseases including stroke because of its convenient preparation of MSCs, excellent differentiation, renewal, and repair capabilities, and low immunogenicity. While stem cell technology is becoming increasingly mature and has good development prospects, there are still many urgent problems that need to be solved for applications of MSCs. (1) Heterogeneity of MSCs. The main manifestation is heterogeneous cell populations with unclear definitions, varying sizes and shapes, and different epigenetic imprints of cells from different tissue sources [91, 92]; how to obtain a homogeneous population of MSCs with the same epigenetic imprints is an urgent issue to be solved. Isolation and seek for suitable subpopulations of MSCs seems to be an ideal method. For example, Hongwei Chen *et al*. identified the BAMBI^{high}MFGE8^{high} C1 subgroup by scRNA-seq technology, this kind of special umbilical cord MSCs had a unique phenotype and distinct transcriptomic profile, which exhibited excellent clinic application perspectives [93]; and Penghong Chen *et al*. identified four different types of subpopulations of Wharton's jelly MSCs including proliferative Wharton's jelly MSCs, niche-supporting Wharton's jelly MSCs, metabolism-related Wharton's jelly MSCs and biofunctional-type Wharton's jelly MSCs by single cell and spatial transcriptome sequencing technology; Subsequently, they isolated a special S100A9$^+$CD29$^+$ CD142$^+$ subpopulation from biofunctional-type MSCs, found that this kind of S100A9$^+$CD29$^+$CD142$^+$subpopulation had the better effect on wound healing that traditional Wharton's jelly MSCs [94]. This evidence may provide a new solution method for the heterogeneity of MSCs. (2) The standardization of methods for stem cell isolation, amplification, and differentiation. There are many kinds of MSCs including bone marrow MSCs, umbilical cord MSCs, adipose MSCs, and hematopoietic stem cells, and has been reported, that there were many kinds of isolation methods and culture methods for MSCs, the inconsistency and non-standardization of isolation methods and culture methods resulted in the huge barriers of MSCs applications. Establishing standardized quality control standards for MSCs may be one of the ways to solve the problem. (3) further verification for the safety and effectiveness of MSC therapy is still needed. At present, the application of MSCs is mostly limited to animal experiments, and there is a huge gap between animal experiments and clinical trials. How to obtain more reliable clinical trial data to support the MSCs therapy is also a big barrier. (4) Establishment and implementation of MSCs policies. The policies related to MSCs directly determine whether MSCs can enter the clinical stage from animal experiments to marketing approval, improving relevant regulations can provide a basis for the clinical application of MSCs and have a targeted approach.

Anyway, with the rapid development of MSC technology and solving the above problems step by step, MSC applications in stroke will make breakthrough progress in the future.

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REFERENCES

1. Saini V, Guada L, Yavagal DR. Global epidemiology of stroke and access to acute ischemic stroke interventions. Neurology. 2021;97(20 Suppl 2):S6-16.
2. Wang YJ, Li ZX, Gu HQ, Zhai Y, Zhou Q, Jiang Y, et al. China stroke statistics: An update on the 2019 report from the national center for healthcare quality management in neurological diseases, China national clinical research center for neurological diseases, the Chinese stroke association, national center for chronic and non-communicable disease control and prevention, Chinese center for disease control and prevention and institute for global neuroscience and stroke collaborations. Stroke Vasc Neurol. 2022,7(5):415-50.
3. Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, et al. World stroke organization (WSO): Global stroke fact sheet 2022. Int J Stroke. 2022;17(1):18-29.
4. Tu WJ, Zhao Z, Yin P, Cao L, Zeng J, Chen H, et al. Estimated burden of stroke in China in 2020. JAMA Netw Open. 2023;6(3):e231455.
5. Yang MJ, Zhang Z, Wang YJ, Li JC, Guo QL, Chen X, et al. Association of nap frequency with hypertension or ischemic stroke supported by prospective cohort data and mendelian randomization in predominantly middle-aged European subjects. Hypertension. 2022;79(9):1962-70.
6. Turana Y, Tengkawan J, Chia YC, Nathaniel M, Wang JG, Sukonthasarn A, et al. Hypertension and stroke in Asia: A comprehensive review from HOPE Asia. J Clin Hypertens (Greenwich). 2021;23(3):513-21.
7. van Sloten TT, Sedaghat S, Carnethon MR, Launer LJ, Stehouwer CD. Cerebral microvascular complications of type 2 diabetes: Stroke, cognitive dysfunction, and depression. Lancet Diabetes Endocrinol. 2020;8(4):325-36.
8. Shi H, Ge Y, Wang H, Zhang Y, Teng W, Tian L. Fasting blood glucose and risk of Stroke: A dose-response meta-analysis. Clin Nutr. 2021;40(5):3296-304.
9. Liu Y, Wang W, Huang X, Zhang X, Lin L, Qin JJ, et al. Global disease burden of stroke attributable to high fasting plasma glucose in 204 countries and territories from 1990 to 2019: An analysis of the global burden of disease study. J Diabetes. 2022;14(8):495-513.
10. Larsson SC, Burgess S, Michaëlsson K. Smoking and stroke: A mendelian randomization study. Ann Neurol. 2019;86(3):468-71.
11. Harshfield EL, Georgakis MK, Malik R, Dichgans M, Markus HS. Modifiable lifestyle factors and risk of stroke: A mendelian randomization analysis. Stroke. 2021;52(3):931-6.
12. Yang W, Kang DW, Ha SY, Lee SH. Drinking patterns and risk of ischemic stroke in middle-aged adults: Do beneficial drinking habits indeed exist? Stroke. 2021;52(1):164-71.
13. Helte E, Säve-Söderbergh M, Larsson SC, Åkesson A. Calcium and magnesium in drinking water and risk of myocardial infarction and stroke-A population-based cohort study. Am J Clin Nutr. 2022;116(4):1091-100.
14. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: Past, present, and future. Stem Cell Res Ther. 2019;10(1):68.
15. Yamanaka S. Pluripotent stem cell-based cell therapy-promise and challenges. Cell Stem Cell. 2020;27(4):523-31.
16. Sarkar A, Saha S, Paul A, Maji A, Roy P, Maity TK. Understanding stem cells and its pivotal role in regenerative medicine. Life Sci. 2021;273:119270.
17. Chen L, Shi K, Ditzel N, Qiu W, Figeac F, Nielsen LH, et al. KIAA1199 deficiency enhances skeletal stem cell differentiation to osteoblasts and promotes bone regeneration. Nat Commun. 2023;14(1):2016.
18. Lin Z, He H, Wang M, Liang J. MicroRNA-130a controls bone marrow mesenchymal stem cell differentiation towards the osteoblastic and adipogenic fate. Cell Prolif. 2019;52(6):e12688.
19. Chen Y, Ouyang X, Wu Y, Guo S, Xie Y, Wang G. Co-culture and mechanical stimulation on mesenchymal stem cells and chondrocytes for cartilage tissue engineering. Curr Stem Cell Res Ther. 2020;15(1):54-60.

20. De Kinderen P, Meester J, Loeys B, Peeters S, Gouze E, Woods S, et al. Differentiation of induced pluripotent stem cells into chondrocytes: Methods and applications for disease modeling and drug discovery. J Bone Miner Res. 2022;37(3):397-410.
21. Corti S, Bonjean R, Legier T, Rattier D, Melon C, Salin P, et al. Enhanced differentiation of human induced pluripotent stem cells toward the midbrain dopaminergic neuron lineage through GLYPICAN-4 downregulation. Stem Cells Transl Med. 2021;10(5):725-42.
22. Lin HC, He Z, Ebert S, Schörnig M, Santel M, Nikolova MT, et al. NGN2 induces diverse neuron types from human pluripotency. Stem Cell Rep. 2021;16(9):2118-27.
23. Amidzadeh Z, Yasami-Khiabani S, Rahimi H, Bonakdar S, Shams D, Habibi-Anbouhi M, et al. Enhancement of keratinocyte growth factor potential in inducing adipose-derived stem cell differentiation into keratinocytes by collagen-targeting. J Cell Mol Med. 2022;26(23):5929-42.
24. Ma J, Yan X, Lin Y, Tan Q. Hepatocyte growth factor secreted from human adipose-derived stem cells inhibits fibrosis in hypertrophic scar fibroblasts. Curr Mol Med. 2020;20(7):558-71.
25. Chen L, Luo W, Wang Y, Song X, Li S, Wu J, et al. Directional homing of glycosylation-modified bone marrow mesenchymal stem cells for bone defect repair. J Nanobiotechnology. 2021;19(1):228.
26. Ullah M, Liu DD, Thakor AS. Mesenchymal stromal cell homing: Mechanisms and strategies for improvement. iScience. 2019;15:421-38.
27. Smith HK, Omura S, Vital SA, Becker F, Senchenkova EY, Kaur G, et al. Metallothionein I as a direct link between therapeutic hematopoietic stem/progenitor cells and cerebral protection in stroke. FASEB J. 2018;32(5):2381-94.
28. Peng JJ, Sha R, Li MX, Chen LT, Han XH, Guo F, et al. Repetitive transcranial magnetic stimulation promotes functional recovery and differentiation of human neural stem cells in rats after ischemic stroke. Exp Neurol. 2019;313:1-9.
29. Wang DL, Qian XD, Lin YH, Tian BB, Liang HY, Chang L, et al. ZL006 promotes migration and differentiation of transplanted neural stem cells in male rats after stroke. J Neurosci Res. 2017;95(12):2409-19.
30. Bunnell BA. Adipose tissue-derived mesenchymal stem cells. Cells. 2021;10(12):3433.
31. Heo JS, Choi Y, Kim HS, Kim HO. Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta, and adipose tissue. Int J Mol Med. 2016;37(1):115-25.
32. Wang S, Mundada L, Johnson S, Wong J, Witt R, Ohye RG, et al. Characterization and angiogenic potential of human neonatal and infant thymus mesenchymal stromal cells. Stem Cells Transl Med. 2015;4(4):339-50.
33. Huang S, Xu L, Sun Y, Wu T, Wang K, Li G. An improved protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. J Orthop Translat. 2014;3(1):26-33.
34. Wang ZG, He ZY, Liang S, Yang Q, Cheng P, Chen AM. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. Stem Cell Res Ther. 2020;11(1):511.
35. Si Z, Wang X, Sun C, Kang Y, Xu J, Wang X, et al. Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. Biomed Pharmacother. 2019;114:108765.
36. Doi K, Kuno S, Kobayashi A, Hamabuchi T, Kato H, Kinoshita K, et al. Enrichment isolation of adipose-derived stem/stromal cells from the liquid portion of liposuction aspirates with the use of an adherent column. Cytotherapy. 2014;16(3):381-91.
37. Shah FS, Li J, Zanata F, Curley JL, Martin EC, Wu X, et al. The relative functionality of freshly isolated and cryopreserved human adipose-derived stromal/stem cells. Cells Tissues Organs. 2015-2016;201(6):436-44.
38. Zhao Q, Zhang L, Wei Y, Yu H, Zou L, Huo J, et al. Systematic comparison of hUC-MSCs at various passages reveals the variations of signatures and therapeutic effects on acute graft-versus-host disease. Stem Cell Res Ther. 2019;10(1):354.
39. Kozlowska U, Krawczenko A, Futoma K, Jurek T, Rorat M, Patrzalek D, et al. Similarities and differences between mesenchymal stem/progenitor cells derived from various human tissues. World J Stem Cells. 2019;11(6):347-74.

40. Connard SS, Linardi RL, Even KM, Berglund AK, Schnabel LV, Ortved KF. Effects of continuous passage on the immunomodulatory properties of equine bone marrow-derived mesenchymal stem cells in vitro. Vet Immunol Immunopathol. 2021;234:110203.
41. Yang M, Lin J, Tang J, Chen Z, Qian X, Gao WQ, et al. Decreased immunomodulatory and secretory capability of aging human umbilical cord mesenchymal stem cells in vitro. Biochem Biophys Res Commun. 2020;525(3):633-8.
42. Babaahmadi M, Tayebi B, Gholipour NM, Bendele P, Pheneger J, Kheimeh A, et al. Long-term passages of human clonal mesenchymal stromal cells can alleviate the disease in the rat model of collagen-induced arthritis resembling early passages of different heterogeneous cells. J Tissue Eng Regen Med. 2022;16(12):1261-75.
43. Park S, Kim JY, Myung S, Jung N, Choi Y, Jung SC. Differentiation of motor neuron-like cells from tonsil-derived mesenchymal stem cells and their possible application to neuromuscular junction formation. Int J Mol Sci. 2019;20(11):2702.
44. Xu H, Zhou Q, Yi Q, Tan B, Tian J, Chen X, et al. Islet-1 synergizes with Gcn5 to promote MSC differentiation into cardiomyocytes. Sci Rep. 2020;10(1):1817.
45. Tsuji K, Kitamura S, Wada J. Immunomodulatory and regenerative effects of mesenchymal stem cell-derived extracellular vesicles in renal diseases. Int J Mol Sci. 2020;21(3):756.
46. Bian D, Wu Y, Song G, Azizi R, Zamani A. The application of mesenchymal stromal cells (MSCs) and their derivative exosome in skin wound healing: A comprehensive review. Stem Cell Res Ther. 2022;13(1):24.
47. Musiał-Wysocka A, Kot M, Sułkowski M, Badyra B, Majka M. Molecular and functional verification of wharton's jelly mesenchymal stem cells (WJ-MSCs) pluripotency. Int J Mol Sci. 2019;20(8):1807.
48. Borojević A, Jauković A, Kukolj T, Mojsilović S, Obradović H, Trivanović D, et al. Vitamin D3 stimulates proliferation capacity, expression of pluripotency markers, and osteogenesis of human bone marrow mesenchymal stromal/stem cells, partly through SIRT1 signaling. Biomolecules. 2022;12(2):323.
49. Mekhemar M, Tölle J, Hassan Y, Dörfer C, El-Sayed KF. Thymoquinone-mediated modulation of toll-like receptors and pluripotency factors in gingival mesenchymal stem/progenitor cells. Cells. 2022;11(9):1452.
50. Thakur G, Bok EY, Kim SB, Jo CH, Oh SJ, Baek JC, et al. Scaffold-free 3D culturing enhances pluripotency, immunomodulatory factors, and differentiation potential of Wharton's jelly-mesenchymal stem cells. Eur J Cell Biol. 2022;101(3):151245.
51. Machado C, de V, Telles PD, da S, Nascimento ILO. Immunological characteristics of mesenchymal stem cells. Rev Bras Hematol E Hemoter. 2013;35:62-7.
52. Guo Y, Yu Y, Hu S, Chen Y, Shen Z. The therapeutic potential of mesenchymal stem cells for cardiovascular diseases. Cell Death Dis. 2020;11(5):349.
53. De Witte SF, Merino AM, Franquesa M, Strini T, Van Zoggel JA, Korevaar SS, et al. Cytokine treatment optimizes the immunotherapeutic effects of umbilical cord-derived MSC for treatment of inflammatory liver disease. Stem Cell Res Ther. 2017;8(1):140.
54. Guillamat-Prats R. The role of msc in wound healing, scarring and regeneration. Cells. 2021;10(7):1729.
55. Lei F, Li M, Lin T, Zhou H, Wang F, Su X. Treatment of inflammatory bone loss in periodontitis by stem cell-derived exosomes. Acta Biomater. 2022l;141:333-43.
56. Jiang F, Zhou H, Cheng Y, He Z, Meng P, Sun K, et al. Various detailed characteristics of a new enhanced neurotrophic factor secreting rat-derived bone marrow mesenchymal stem cells and its preliminary application in rat models of ischemic stroke. Exp Cell Res. 2022;416(1):113140.
57. Bang OY, Jin KS, Hwang MN, Kang HY, Kim BJ, et al. The effect of CXCR4 overexpression on mesenchymal stem cell transplantation in ischemic stroke. Cell Med. 2012;4(2):65-76.
58. Lee S, Kim OJ, Lee KO, Jung H, Oh SH, Kim NK. Enhancing the therapeutic potential of CCL 2-overexpressing mesenchymal stem cells in acute stroke. Int J Mol Sci. 2020;21(20):7795.
59. Ha GH, Kim EJ, Park JS, Kim JE, Nam H, Yeon JY, et al. JAK2/STAT3 pathway mediates neuroprotective and pro-angiogenic treatment effects of adult human neural stem cells in middle cerebral artery occlusion stroke animal models. Aging (Albany NY). 2022;14(22):8944-69.
60. Kuang Y, Zheng X, Zhang L, Ai X, Venkataramani V, Kilic E, et al. Adipose-derived mesenchymal stem cells reduce autophagy in stroke mice by extracellular vesicle transfer of miR-25. J Extracell Vesicles. 2020;10(1):e12024.

61. Tian H, Yang X, Zhao J, Liu X, Liu X, Cai Y, et al. Hypoxia-preconditioned bone marrow mesenchymal stem cells improved cerebral collateral circulation and stroke outcome in mice. Arterioscler Thromb Vasc Biol. 2023;43(7):1281-94.
62. Cheng X, Yeung PK, Zhong K, Zilundu PL, Zhou L, Chung SK. Astrocytic endothelin-1 overexpression promotes neural progenitor cell proliferation and differentiation into astrocytes via the Jak2/Stat3 pathway after stroke. J Neuroinflammation. 2019;16:227.
63. Tobin MK, Stephen TK, Lopez KL, Pergande MR, Bartholomew AM, Cologna SM, et al. Activated mesenchymal stem cells induce recovery following stroke via regulation of inflammation and oligodendrogenesis. J Am Heart Assoc. 2020;9(7):e013583.
64. Liu K, Guo L, Zhou Z, Pan M, Yan C. Mesenchymal stem cells transfer mitochondria into cerebral microvasculature and promote recovery from ischemic stroke. Microvasc Res. 2019;123:74-80.
65. Yuan X, Rosenberg JT, Liu Y, Grant SC, Ma T. Aggregation of human mesenchymal stem cells enhances survival and efficacy in stroke treatment. Cytotherapy. 2019;21(10):1033-48.
66. Zhang F, Li Q, Liang H, Zhang Y. Phosphofructokinase-1 inhibition promotes neuronal differentiation of neural stem cells and functional recovery after stroke. Neuroscience. 2021;459:27-38.
67. Kikuchi-Taura A, Okinaka Y, Saino O, Takeuchi Y, Ogawa Y, Kimura T, et al. Gap junction-mediated cell-cell interaction between transplanted mesenchymal stem cells and vascular endothelium in stroke. Stem Cell. 2021;39(7):904-12.
68. Xu K, Lee JY, Kaneko Y, Tuazon JP, Vale F, van Loveren H, et al. Human stem cells transplanted into the rat stroke brain migrate to the spleen via lymphatic and inflammation pathways. Haematologica. 2019;104(5):1062-73.
69. Xu P, Shi X, Zhang X, Liu Q, Xie Y, Hong Y, et al. Overexpression of BRCA1 in neural stem cells enhances cell survival and functional recovery after transplantation into experimental ischemic stroke. Oxid Med Cell Longev. 2019;2019:8739730.
70. Tang X, Wu L, Zhu J, Xu M, Li S, Zeng G, et al. GABAergic neurons differentiated from BDNF- and Dlx2-modified neural stem cells restore disrupted neural circuits in brainstem stroke. Stem Cell Res Ther. 2023;14:170.
71. Fu YS, Yeh CC, Chu PM, Chang WH, Lin MY, Lin YY. Xenograft of human umbilical mesenchymal stem cells promotes recovery from chronic ischemic stroke in rats. Int J Mol Sci. 2022;23(6):3149.
72. Nalamolu KR, Chelluboina B, Fornal CA, Challa SR, Pinson DM, Wang DZ, et al. Stem cell treatment improves post-stroke neurological outcomes: A comparative study in male and female rats. Stroke Vasc Neurol. 2021;6(4):519-27.
73. Wlodarek L, Alibhai FJ, Wu J, Li SH, Li RK. Stroke-induced neurological dysfunction in aged mice is attenuated by preconditioning with young Sca-1+ stem cells. Stem Cells. 2022;40(6):564-76.
74. Zhang X, Tang H, Mao S, Li B, Zhou Y, Yue H, et al. Transplanted hair follicle stem cells migrate to the penumbra and express neural markers in a rat model of cerebral ischaemia/reperfusion. Stem Cell Res Ther. 2020;11:413.
75. Baak LM, Wagenaar N, van der Aa NE, Groenendaal F, Dudink J, Tataranno ML, et al. Feasibility and safety of intranasally administered mesenchymal stromal cells after perinatal arterial ischaemic stroke in the Netherlands (PASSIoN): A first-in-human, open-label intervention study. Lancet Neurol. 2022;21(6):528-36.
76. Bang OY, Kim EH, Cho YH, Oh MJ, Chung JW, Chang WH, et al. Circulating extracellular vesicles in stroke patients treated with mesenchymal stem cells: A biomarker analysis of a randomized trial. Stroke. 2022;53(7):2276-86.
77. Muir KW, Bulters D, Willmot M, Sprigg N, Dixit A, Ward N, et al. Intracerebral implantation of human neural stem cells and motor recovery after stroke: Multicentre prospective single-arm study (PISCES-2). J Neurol Neurosurg Psychiatry. 2020;91(4):396-401.
78. Jaillard A, Hommel M, Moisan A, Zeffiro TA, Favre-Wiki IM, Barbieux-Guillot M, et al. Autologous mesenchymal stem cells improve motor recovery in subacute ischemic stroke: A randomized clinical trial. Transl Stroke Res. 2020;11(5):910-23.
79. Suda S, Nito C, Ihara M, Iguchi Y, Urabe T, Matsumaru Y, et al. Randomized placebo-controlled multicentre trial to evaluate the efficacy and safety of JTR-161, allogeneic human dental pulp stem cells, in patients with Acute Ischaemic stroke (J-REPAIR). BMJ Open. 2022;12(5):e054269.

80. Levy ML, Crawford JR, Dib N, Verkh L, Tankovich N, Cramer SC, et al. Phase I/II study of safety and preliminary efficacy of intravenous allogeneic mesenchymal stem cells in chronic stroke. Stroke. 2019;50(10):2835-41.
81. Xie C, Wang K, Peng J, Jiang X, Pan S, Wang L, et al. Efficacy and safety of human-derived neural stem cell in patients with ischaemic stroke: Study protocol for a randomized controlled trial. BMJ Open. 2022;12(11):e055108.
82. de Celis-Ruiz E, Fuentes B, Moniche F, Montaner J, Borobia AM, Gutiérrez-Fernández M, et al. Allogeneic adipose tissue-derived mesenchymal stem cells in ischaemic stroke (AMASCIS-02): A phase IIb, multicentre, double-blind, placebo-controlled clinical trial protocol. BMJ Open. 2021;11(8):e051790.
83. Chiu TL, Baskaran R, Tsai ST, Huang CY, Chuang MH, Syu WS, et al. Intracerebral transplantation of autologous adipose-derived stem cells for chronic ischemic stroke: A phase I study. J Tissue Eng Regen Med. 2022;16(1):3-13.
84. 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. Lancet. 2016;388(10046):787-96.
85. 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(10):2835-41.
86. Zhang G, Li Y, Reuss JL, Liu N, Wu C, Li J, et al. Stable intracerebral transplantation of neural stem cells for the treatment of paralysis due to ischemic stroke. Stem Cells Transl Med. 2019;8(10):999-1007.
87. Qiao LY, Huang FJ, Zhao M, Xie JH, 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 Transplant. 2014;23 Suppl 1:S65-72.
88. Oka S, Yamaki T, Sasaki M, Ukai R, Takemura M, Yokoyama T, et al. Intravenous infusion of autoserum-expanded autologous mesenchymal stem cells in patients with chronic brain injury: Protocol for a phase 2 trial. JMIR Res Protoc. 2022;11(7):e37898.
89. de Celis-Ruiz E, Fuentes B, Alonso de Leciñana M, Gutiérrez-Fernández M, Borobia AM, Gutiérrez-Zúñiga R, et al. Final results of allogeneic adipose tissue-derived mesenchymal stem cells in acute ischemic stroke (AMASCIS): A phase II, randomized, double-blind, placebo-controlled, single-center, pilot clinical trial. Cell Transplant. 2022;31:9636897221083863.
90. Deng L, Peng Q, Wang H, Pan J, Zhou Y, Pan K, et al. Intrathecal injection of allogenic bone marrow-derived mesenchymal stromal cells in treatment of patients with severe ischemic stroke: Study protocol for a randomized controlled observer-blinded trial. Transl Stroke Res. 2019;10(2):170-7.
91. Olmedo-Moreno L, Aguilera Y, Baliña-Sánchez C, Martín-Montalvo A, Capilla-González V. Heterogeneity of in vitro expanded mesenchymal stromal cells and strategies to improve their therapeutic actions. Pharmaceutics. 2022;14(5):1112.
92. Wang Z, Chai C, Wang R, Feng Y, Huang L, Zhang Y, et al. Single-cell transcriptome atlas of human mesenchymal stem cells exploring cellular heterogeneity. Clin Transl Med. 2021;11(12):e650.
93. Chen H, Wen X, Liu S, Sun T, Song H, Wang F, et al. Dissecting heterogeneity reveals a unique BAMBIhigh MFGE8high subpopulation of human UC-MSCs. Adv Sci (Weinh). 2023;10(1):2202510.
94. Chen P, Tang S, Li M, Wang D, Chen C, Qiu Y, et al. Single-cell and spatial transcriptomics decodes wharton's jelly-derived mesenchymal stem cells heterogeneity and a subpopulation with wound repair signatures. Adv Sci (Weinh). 2023;10(4):2204786.