Tissue Eng Regen Med (2021) 18(4):587–611
https://doi.org/10.1007/s13770-021-00334-3

Online ISSN 2212-5469 TERM

REVIEW ARTICLE

Mesenchymal Stem Cell Transplantation for Ischemic Diseases: Mechanisms and Challenges

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Received: 19 December 2020 / Revised: 7 February 2021 / Accepted: 16 February 2021 / Published online: 21 April 2021
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Abstract Ischemic diseases are conditions associated with the restriction or blockage of blood supply to specific tissues. These conditions can cause moderate to severe complications in patients, and can lead to permanent disabilities. Since they are blood vessel-related diseases, ischemic diseases are usually treated with endothelial cells or endothelial progenitor cells that can regenerate new blood vessels. However, in recent years, mesenchymal stem cells (MSCs) have shown potent bioeffects on angiogenesis, thus playing a role in blood regeneration. Indeed, MSCs can trigger angiogenesis at ischemic sites by several mechanisms related to their trans-differentiation potential. These mechanisms include inhibition of apoptosis, stimulation of angiogenesis via angiogenic growth factors, and regulation of immune responses, as well as regulation of scarring to suppress blood vessel regeneration when needed. However, preclinical and clinical trials of MSC transplantation in ischemic diseases have shown some limitations in terms of treatment efficacy. Such studies have emphasized the current challenges of MSC-based therapies. Treatment efficacy could be enhanced if the limitations were better understood and potentially resolved. This review will summarize some of the strategies by which MSCs have been utilized for ischemic disease treatment, and will highlight some challenges of those applications as well as suggesting some strategies to improve treatment efficacy.

Keywords Ischemic diseases · Mesenchymal stem cells · Blood regeneration and angiogenesis

1 Introduction

Ischemic conditions are associated with the restriction or blockage of blood supply to specific tissues, resulting in health complications, many of which can lead to permanent disabilities. Depending on the tissues where ischemic events take place, the lack of oxygen supply is the main factor which defines the damaging consequences. The lack of nutrition delivery and the inability to remove metabolic wastes that accumulate inside the cells can further trigger cell death and tissue necrosis. Ischemia occurs in metabolically dynamic tissues, such as the heart and brain, and may cause severe irreversible damage and even mortality [1]. Other organs, such as the liver and kidney, can also be affected by ischemia due to injury. The most commonly seen types of ischemia in the elderly appears in the limbs, particularly lower limbs, due to peripheral artery diseases (PAD) associated with aging, smoking and diabetes [2]. The current available treatments for ischemia predominantly include the administration of medicines or physical procedures to replenish blood supply to the affected tissues and to prevent further tissue damage. However, treatments for ischemia are invasive and ineffective, especially when severe ischemia has occurred or admission to the hospital is delayed.

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In recent years, cell therapy using mesenchymal stem cells (MSCs) has been proposed as a safe and effective treatment that can be used to improve ischemic conditions. Several clinical trials that have used MSCs for transplantation as a treatment modality have shown improvement in patients with different types of ischemia [3–5]. In ischemic conditions, MSCs are known to function as an immunomodulator, helping to prevent inflammation and tissue damage. MSCs can also generate growth factors that support the regeneration of the vascular network to reconnect the blood supply to the affected tissues, which is the main purpose for utilizing MSC-based therapy. Indeed, the use of MSC transplantation in the treatment of ischemic heart condition has been shown to reduce heart infarction size, improve heart function with increased left ventricle ejection fraction (LVEF), and increase overall recovery and clinical outcomes [4]. In critical limb ischemia (CLI), intramuscular injection of MSCs was found to increase ankle-branchial index (ABI) and ankle pressure at 6-month follow-up, which indicated the improvement of blood flow into the lower limbs [6]. For ischemic brain or stroke conditions, long-term follow-up after MSC treatment showed no negative effects with intravenous application of bone marrow (BM)-derived MSCs (BM-MSCs), and to some extent, showed improved functional outcomes and survival rates [7, 8]. These data demonstrate that MSCs can be safely used to treat ischemic conditions in clinical trials, thereby proposing MSC transplantation as an effective cell therapy in future trials.

However, large-scale trials of MSC-based therapies have been limited in number, hence the controversy over how effective it really is as a therapy. There are data showing that while MSC treatment appeared to be safe, no significant outcome improvements were observed [9]. There has been suggestion that other factors, such as cell dosages, cell sources and individual health profiles, might need to be considered in future trials. The efficacy of MSC-based therapy for ischemic conditions has also been a subject of discussion in the literature. Some have proposed that the variation in efficacy could be due to various factors which can negatively impact the implanted MSCs as well as the host response in vivo [10–12]. These factors might include low survival rate of MSCs in vivo, inefficient cell migration to target tissues, and/or rejection by the host immune system [10–12]. Furthermore, the lack of information on the local ischemic environment associated with different individuals makes it challenging to identify the interaction between MSCs and host tissues, leading to suboptimal selection of MSCs or mis-preparation of suitable MSCs sources for application. Therefore, MSCs with optimal functionality may not be in the final product, leading to ineffective treatment efficacy. Indeed, understanding the interaction between donor cells and host tissues is vitally important for selection of optimal MSC characteristics which presumably would benefit the majority of treated individuals. In this review, we will discuss the changes that occur in the tissue environment following an ischemic event, and the potential of MSCs in modifying these changes in order to improve the ischemic conditions. We will also review the challenges that MSCs face in the in vivo environment, and the factors that impact MSC implant, survival and function in vivo. Understanding the above will help to gain a deeper understanding on the ischemic environment in order to target where the key problems lie, as well as to resolve the problems in a concerted effort.

2 Current status of ischemic disease treatment by MSC transplantation

2.1 The treatment efficacy of some ischemic diseases by MSC transplantation

2.1.1 Myocardial infarction

Myocardial infarction (MI), commonly known as a heart attack, is a disease condition in which disruption of blood flow to a part of the heart causes damage to the heart muscles. About 30% of people have atypical symptoms of this disease, and 5% of people over 75 years have had MI with little or no history of symptoms. Therapeutic efforts to treat MI have included stem cell therapy. In 2018, Kim et al. reported using autologous BM-MSCs to treat MI [13]. In this clinical trial, the authors treated 14 patients with BM-MSCs and compared them to 12 patients in the control group. All treatments were performed after 1 month of percutaneous coronary intervention. After 12 months of follow-up, the authors suggested that intracoronary administration of autologous MSCs significantly improved the LVEF in patients with anterior acute MI [13].

In a phase I, controlled, open-label and dose-escalating study, Penn et al. (2012) treated 19 patients with MI [14] by injection of allogeneic BM-MSCs into the patient's myocardium. The results showed that at the high dose of MSCs (50 million MSCs), MSC transplantation could significantly improve the myocardial damage, ejection fraction and stroke volume 4 months later [14].

In a randomized placebo-controlled trial using autologous MSCs from BM, Mathiasen et al. investigated the treatment efficacy after 6 months of treatment [15] and 4 years of follow-up [16]. In this study, 60 patients were enrolled, with 40 in the treatment group and 20 in the placebo group. After 6 months, there were 51 patients (33 patients in treatment group and 18 in placebo group) who completed the follow-up. Compared to patients in the

placebo group, there were significant improvements ($p < 0.0001$) in LVEF, left ventricular end systolic volume (LVESV), stroke volume and myocardial mass in patients of the treatment group. Furthermore, no serious adverse events were recorded [15]. Additionally, these results were maintained until 12 months after transplantation. After four years of follow-up, there were significantly fewer hospitalizations for angina in the MSC treatment group as compared to the placebo group, and again, no serious adverse events were identified [16].

Recently, there was a clinical trial which compared the treatment efficacy of chronic ischemic cardiomyopathy using allogeneic MSCs from umbilical cord (UC) (i.e. UC-MSCs), autologous mononuclear cells (MNCs) from BM (i.e. BM-MNCs), or placebo (control) [17]. In this study, 44 patients were placed into three groups (control: 16 patients, UC-MSC treatment: 26, and BM-MNC treatment: 12). After 12 months of follow-up, the results showed that significant improvements in LVEF, stroke volume, necrotic myocardium, and 6-min walking test were observed in patients treated with UC-MSCs versus those treated with BM-MNCs or placebo. While BM-MSC transplantation did improve LVEF and stroke volume, it did not decrease myocardium necrosis [17]. Additionally, in a previous study comparing the effects of BM-MSCs and BM-MNCs for the treatment of dilated cardiomyopathy, Xiao et al. [18] showed that after 1-year of follow-up, LVEF, New York Heart Association (NYHA) Functional Classification, and myocardial perfusion were significantly improved in the group receiving BM-MSCs but not BM-MNCs.

Contrary to the above reports, Qayyum et al. [19] suggested that there were no significant changes in patients with chronic ischemic heart diseases treated with autologous MSCs from adipose tissues, compared to control. In this phase II, randomized, double-blinded and placebo-controlled trial, the authors investigated the effects of intramyocardial injection of autologous MSCs and compared them to control. With respect to all criteria related to the improvement of disease, there were no significant differences found between the treatment and control groups. The authors also confirmed that there were no changes in functional parameters or amount of scar tissue in patients treated with stem cells as compared to those in the placebo group [19]. These results were different from other studies using MSCs to treat MI. However, it could be that Qayyum et al. used a different kind of MSCs compared to other studies. Thus, perhaps the source of MSCs can strongly affect treatment efficacy. Moreover, the dose of cells can also directly affect the treatment outcome, as has been well-established in the study by Florea et al. [20]. In their study, they investigated the effects of 2 doses of allogenic BM-MSCs (20 million and 100 million cells) on ischemic cardiomyopathy. The results revealed that only the 100 million dose of MSCs could increase ejection fraction [20].

The difference in treatment efficacy between autologous versus allogeneic MSC transplantation was also observed [21]. Hare et al. found that although there was no difference in severe adverse effects from autologous versus allogeneic MSC transplantation, the efficacy of treatment was different. In autologous MSC transplantation, the patients showed improvement in their 6-min walk test and MLHFQ scores, when compared to baseline values. However, this was not observed in patients receiving allogeneic MSC transplanation; moreover, their LV end-diastolic volume was reduced. Hare et al. also suggested that at the low dose of MSCs (20 million cells), there was a greater reduction in LV volume and an increased EF compared with the higher doses of MSCs (100 and 200 million cells) [21].

2.1.2 Ischemic stroke

Ischemic stroke is the most common type of stroke which results from the blockage of an artery supplying blood to the brain. This condition causes brain damage due to the reduction of oxygen to the brain. Currently, mechanical thrombolysis and stenting are strategies that are used to treat acute ischemic stroke [22]. There are certain thrombolysis-inducing drugs that are used to open the blockage of thrombosis [23, 24]. MSCs have also been investigated as a therapeutic platform for treatment of ischemic stroke. Unlike the strategies at present, MSC transplantation is considered as a novel strategy that not only triggers angiogenesis but also promotes brain regeneration after damage.

Honmou et al. treated 12 patients with stroke by transplantation of autologous stem cells expanded in autologous serum-supplemented medium [25]. MSC infusion was performed at 36–133 days post-stroke. In all the patients, no central nervous system tumors, abnormal cell growth, neurological deterioration, venous thromboembolism, systemic malignancy, or systemic infection was observed. The median daily rate of the National Institutes of Health Stroke Scale change was 0.36 during the first week post-infusion, compared to 0.04 from the first day of testing before infusion. The mean lesion volume was also reduced by more than 20% post-infusion [25].

Steinberg et al. (2018) treated 18 patients with chronic ischemic stroke using allogenic modified BM-MSCs [26]. There were 16 of 18 patients who completed 24 months of treatment and displayed significant improvements in the European Stroke Scale (ESS) score ($p < 0.05$), National Institutes of Health Stroke Scale (NIHSS) score ($p < 0.01$), Fugl-Meyer (F-M) total score ($p < 0.01$), and F-M Motor Scale score ($p < 0.01$) [26]. Similar to that study, Levy

et al. (2019) evaluated allogenic BM-MSCs in a phase I/II clinical trial of 36 patients (15 patients in phase I and 21 patients in phase II). The phase I clinical study showed that intravenous transfusion of allogenic MSCs was safe, and the phase II showed that all behavioral endpoints were significantly increaesd over the 12 months of follow-up [27].

In recent publications, Jaillard et al. reported on the efficacy of autologous MSC transplantation for subacute ischemic stroke [28]. In this clinical trial, 16 patients received autologous BM-MSCs while15 patients received placebo control. Treatment efficacy was followed up after 6 months and 2 years of treatment. Although there were no treatment effects on the Barthel Index, the NIHSS, or modified-Rankin scores, there were significant ($p < 0.05$) improvements in the motor-NIHSS, motor Fugl-Meyer score, and task-related fMRI activities of MI-4a and MI-4p [28].

2.1.3 Critical limb ischemia (CLI)

Similar to other ischemic diseases, CLI has also been treated using MSC transplantation. MSCs from various sources, including adipose tissue, bone marrow, umbilical cord and placenta, have all been evaluated as treatment for this disease in the clinic.

In a report by Lu et al. (2019), the authors compared the treatment efficacy of BM-MSCs and BM-MNCs in the treatment of CLI and foot ulcers in patients with diabetes [29]. In the study, 41 patients were randomized into 3 groups: a group receiving autologous BM-MSCs, a group receiving autologous BM-MNCs, and a group receiving saline (control). The results showed that treatment with BM-MSCs significantly improved ulcer healing and recurrence rate compared to treatment with BM-MNCs or with saline. Moreover, compared to the BM-MNC group, the BM-MSC group showed a longer period of limb salvage and blood flow improvement, out to 9 months [29]. In a phase II study with 18 patients, Gupta et al. (2017) also investigated the effects of doses of allogenic BM-MSCs on CLI ischemia. The results of their study suggested that intramuscular injection of BM-MSCs, at a dose of 2 million cells per kg, showed superior clinical benefit [30].

Allogenic umbilical cord-derived MSCs have also been used to treat CLI [31, 32]. Huang et al. conducted 2 clinical trials to evaluate the use of UC-MSCs to treat CLI. After 6 months of follow-up, they found that rest pain, pain-free walking distance, and ulcers were significantly improved in the patients [32]. Moreover, these effects were related to the anti-inflammatory mechanisms and immunomodulation [31]. Allogenic MSCs from placenta have also been evaluated in the treatment of 4 diabetic patients with CLI [33]; the results demonstrated that there were no serious adverse events related to MSC injection during the 24 weeks. The clinical ischemic features improved after 24 weeks of treatment with a significant decrease in resting pain and limb coldness, and a significant increase in scores for pain-free walking distance [33].

3 MSC transplantation for ischemic diseases are superior to other kinds of stem cells

Besides MSCs, different types of adult stem cells included $CD133^+$ cells, mononuclear cells from bone marrow, $CD34^+$ cells, and cardiac stem cells, also were clinically used to treat some ischemic diseases.

$CD133^+$ cells are defined as progenitor or stem cells known as a response for the angiogenesis [36] or protect against stroke [37]. However, some clinical studies represented some contradictory results. In 2014, Nasseri et al. used autologous $CD133^+$ cells from bone marrow to treat ischemic myocardium in 60 patients [38]. They showed that autologous $CD133^+$ cell transplantation did not affect the global left ventricular function and clinical symptoms. Indeed, there were no differences in 6-min walking distance, Minnesota Living with Heart Failure score, or Candian Cardiovascular Society class between treatment groups with $CD133^+$ cells and placebo [38]. These results agreed with a pilot clinical study with 5 patients of ischemic myocardium that Forcillo et al. performed in 2013 [39]. However, it was different from Stamm et al. that intramycocardial delivery of $CD133^+$ cells from bone marrow is safe and provides beneficial effects on the ischemic heart disease patients [40]. Stamm et al. showed that both LV ejection fraction and the perfusion of infarcted myocardium had been improved more in patients treated with $CD133^+$ cells but not in control [40]. In phase II/III, randomized, double-blind, placebo-controlled trial recently published, Naseri et al. (2018) compared the treatment efficacy of patients with myocardial infarction with placebo and followed up for 18 months [41]. They also suggested that bone marrow-derived $CD133^+$ cell transplantation increased left ventricular ejection fraction, improved decreased systolic wall thickening, significantly decreased non-viable segments compared to placebo after 6 and 18 months of transplantation [41]. These contradictory results suggested that some more extensive studies should be performed with some meta-analysis to provide evidence about $CD133^+$ cell transplantation's treatment efficacy for ischemic myocardium.

In the non-expanded and purified form of stem cells from bone marrow, mononuclear cells (MNCs) also were used to treat some ischemic diseases. Some clinical studies also compared the treatment efficacy of MSCs versus MNCs [29, 34]. Heldman et al. (2014) reported a

comparative study of ischemic cardiomyopathy treatment using autologous MSCs and autologous MNCs from bone marrow [34]. There were 19 patients treated with MSCs, 19 patients treated with MNCs, and 10 patients in placebo in this study. The results showed both patients transplanted with MSCs or MNCs improved the Minnesota Living With Heart Failure score compared to placebo. However, the 6-min walk distance only increased in patients transplanted with MSCs compared to placebo ($p = 0.03$); infarct size and regional myocardial function also only improved in patients with MSCs ($p = 0.004$), but not in MNCs, compared to placebo ($p = 0.03$) [34]. In another study, Lu et al. investigated long-term outcomes of transplantation of bone marrow-derived MSCs and compared to treatment efficacy of bone marrow-derived MNCs to treat critical limb ischemia and foot ulcer with diabetes [29]. Lu et al. suggested only in patients with MSC transplantation improved the hazard ratio for amputation, ulcer healing, and recurrence rate but not in patients with MNC transplantation. These primary investigations showed that MSC transplantation could be better than MNC transplantation in some ischemic diseases. However, more further studies should be performed to confirm these observations.

In another approach for heart regeneration after myocardial infarction, the cardiac stem cells were used to treat some patients with myocardial infarction [42]. The autologous cardiac stem cells were isolated and expanded by the cardiosphere culture. Although there was a reduction in scar mass, increased viable heart mass in the patients treated with cardiac stem cells, end-diastolic volume, end-systolic volume, and LVEF did not improve compared to the control [42]. However, different from this study, Chugh et al. confirmed transplantation of autologous cardiac stem cells provided positive results in ischemic cardiomyopathy patients. Patients transplanted with cardiac stem cells showed a marked increase in LVEF and regional EF; reduction of infarct size, and increased in viable tissues compared to control [43].

Like $CD133^+$ stem cells, $CD34^+$ stem cells contain a population of endothelial progenitor cells [36, 44] that can participate in the angiogenesis to regenerate the blood vessels. Some studies showed that CD34 could also express on the very small embryonic-like stem cells [45, 46], which display the huge potential to differentiate into various cells. $CD34^+$ stem cells were used to treat some patients with angina [47]. In a recent publication, Velagapudi et al. performed a meta-analysis of randomized controlled trials with 269 patients treated ischemic heart disease by $CD34^+$ stem cell transplantation. The analysis showed that the administration of autologous $CD34^+$ cells decreased the risk of all-cause mortality, reduced angina frequency, and improved the exercise time compared to the control [48].

Although $CD133^+$ cells, $CD34^+$ cells, mononuclear cells, and cardiac stem cell transplantation can benefit ischemic diseases. These stem cells usually highly expressed human leukocyte antigen [49, 50]; they should be used as autologous sources. That is the main reason that usage of these stem cells is limited. Contrastly, MSCs did not express HLA class II and displayed the high potency of immune modulation [51, 52]. Therefore, MSCs are preferred to use to treat ischemic diseases more than other kinds of stem cells.

3.1 Treatment efficacy of MSC transplantation in ischemic diseases varies between kinds of MSCs

To date, various kinds of MSCs are used to treat ischemic diseases clinically. Some recent clinical studies introduced in Table 1 showed that both autologous and allogeneic MSCs were used in clinical treatment for ischemic diseases. Hare et al. compared the treatment efficacy of allogenic versus autologous bone marrow-derived mesenchymal stem cells for ischemic cardiomyopathy [21]. In this randomized study, there were 30 patients treated and follow-up to 13 months. Because the sample size is small, some results were non-significant differences between allogeneic and autologous bone marrow-derived MSC transplanted patients. The 1-year incidence of SAEs was 33.3% (n = 5) in allogeneic group while accounted for 53.3% (n = 8) in the autologous group ($p = 0.46$). Only patients with autologous MSCs improved the 6-min walk test and MLHFQ score, but not in allogenic MSC transplanted patients. The allogeneic MSC transplantation reduced the LV end-diastolic volumes. Both autologous and allogeneic MSC transplantation significant reduced mean EED by − 33.21% ($p < 0.001$) [21].

Some kinds of MSCs were used in clinical treatment for ischemic diseases included bone marrow-derived MSCs, adipose-derived MSCs, umbilical cord-derived MSCs, and placenta-derived MSCs. However, there is no comparative study about the treatment efficacy of these sources of MSCs reported. Almost all reports suggested that MSC transplantation improved clinical symptoms of ischemic diseases [13, 14, 16, 17, 20, 21, 25–30, 32, 33, 35]. A study using the autologous MSCs from adipose tissue in chronic ischemic heart disease treatment showed a non-significantly improvement between treatment groups and the control [19]. However, autologous MSCs from adipose tissue could significant improve critical limb ischemia [35]. These observations suggested that the treatment efficacy can depend on different factors such as sources of MSCs, quality and dose of MSCs, kind of ischemic disease, and stage of ischemic disease…

Table 1 Clinical applications of mesenchymal stem cells for ischemic diseases

Diseases	Phase	No. of patients	Sources of MSCs	Types of transplantation	Results	References
Myocardial infarction	Phase I, open label	19	Allogenic MSCs from bone marrow	Myocardium	Safe and well tolerated At dose of > = 50 million of cells, the myocardial damage is significant improved, improved in ejection fraction and stroke volume 4 months later	[14]
Stroke		12	Autologous MSCs from bone marrow	IV	Safe Lesion volume reduced by > 20% at 1 week after infusion The median daily rate of National Institutes of Health Stroke Scale change was 0.36 during the first week post-infusion	[25]
Ischemic heart failure	Randomized, double-blind, placebo-controlled trial	60	Autologous MSCs from bone marrow	Intramyocardial injections	Left ventricular end-systotic volume significant reduced in treatment group, but not in placebo Left ventricular ejection fraction, stroke volume and myocardial mass are also improved Amount of scar tissue is reduced, quality of life is increased in the treatment group, but not in placebo group	[15, 16]
Chronic ischemic cardiomyopathy	Controlled, randomized trial	44	Autologous mononuclear cells from bone marrow; allogenic umbilical cord	Intramyocardial injections	UC-MSCs are better than BM-MNCs in treatment efficacy LVEF, stroke volume improved in UC-MSC group Necrotic myocardium significant reduced in UC-MSC group 6-min walking test signficantly increased in the UC-MSC group	[17]
Subacute ischemic stroke	Controlled, randomized trial	31	Autologous bone marrow-derived MSCs	IV	No treatment effects on the Barthel Index, NIHSS, modified Rankin score Significant improvement in motor-NIHSS, motor-Fugl-Meyer score, task-related fMRI activity in MI-4a and MI-4p Safe and feasible Motor	[28]
Myocardial infarction	Controlled trial	26	Autologous bone marrow-derived MSCs	Intracoronary delivery	Autologous BM-MSC was tolerable and safe with significant improvement in LVEF at 4 months and 12 months after transplantation	[13]
Chronic ischemic heart disease	Phase II, randomized, double blinded, placebo controlled trial	60	Autologous MSCs from adipose tissue	Intramyocardial injections	Non-significant differences between stress, rest values between control and treatment groups LVEF, myocardial mass, stroke volume, left ventricle end-diastolic volume, end-systolic volume changed non-signficantly between control and treatment groups Scar tissue was unchanged between 2 groups of control and treatment	[19]

Table 1 continued

Diseases	Phase	No. of patients	Sources of MSCs	Types of transplantation	Results	References
Ischemic cardiomyopathy	Blinded trial	30	Allogenic MSCs from bone marrow	Transendocardial injection	All patients with dose of 20 and 100 million of cells reduced scar size, but only patients with 100 million cells increased ejection fraction	[20]
Ischemic cardiomyopathy	Phase ½ randomized trial	30	Allogenic and autologous MSCs from bone marrow	Transendocardial injection	Both allogenic and autologous MSCs were both associated with low rates of SAEs, including immunologic reactions MSC injection affected patient functional capacity, quality of life, and ventricular remodeling	[21]
Ischemic cardiomyopathy	Phase 1 and 2 randomized, blinded, placebo-controlled study	65	Autologous MSCs and MNCs from bone marrow	Transendocardial injection	The 1-year incidence of SAEs are similar in groups of MSCs, MNCs and placebo Both MSCs and MNCs transplantation improved the Minnesota Living With Heart Failture score; however, the 6-min walk distance, infarct size, regional myocardial funtion only improved in the group of MSC transplantation, but not in MNCs or placebo	[34]
Ischemic stroke	Phase 1/2a, open label, single-arm	18	Allogenic-modified bone marrow derived MSCs	Administered to the target sites	There are 1 treatment emergent adverse event No patients withdrew due to adverse events 88.9% patients got headache 16/18 patients completed 24 months of follow-up with significant improvements from the baseline for European Stroke Scale (ESS) score, $p < 0.05$); National Institutes of Health Stroke Scale (NIHSS) score, $p < 0.01$, Fugl-Meyer (F-M) total score, 19.4 $p < 0.01$); and F-M motor scale score, $p < 0.01$ No significant changes in the modified Rankin Scale score	[26]
Chronic stroke	Phase I/II	15 in Phase I and 21 in phase II	Allogenic bone marrow-derived MSCs	IV	All behavioral end points showed significant gains over 12 months of follow-up	[27]
Critical limb ischemia and foot ulcer	Pilot study with control	41	Autologous BM-MSCs and BM-MNCs	Intramuscularly injection	Patients with BM-MSCs, but not in patients with BM-MNCs, were significant different in ulcer healing and recurrence rate compared to control at 9 months of follow-up Treatment of BMMSCs, leaded to longer time of limb blood flow and ulcerative healing, reduced ulcer recurrence and amputation in 9 months	[29]
Critical limb ischemia	Phase II, prospective, nonrandomized, open-label	18	Allogenic BM-MSCs	Intramuscularly injection	Admimistration of BM-MSCs at a dose of 2 million cell per kg showed the clinical benefit	[30]

Table 1 continued

Diseases	Phase	No. of patients	Sources of MSCs	Types of transplantation	Results	References
Critical limb ischemia	Pilot study	26	Allogenic UC-MSCs	Intramuscularly injection	No patients underwent lower limb amputation, not adverse effects related to transplantation Rest pain, pain free walking distance and uclers significantly improved at the end of the 6-months follow-up	[32]
Critical limb ischemia	Pilot study	4	Allogenic MSCs from placenta	Intramuscularly injection	The clinical ischemic features of patients were improved 24 weeks after treatment with MSCs The scores of resting pain and limb coldness significantly decreased Pain free walking distance significant increased from the baseline to 24 wks	[33]
Critical limb ischemia	Pilot study	15	Autologous MSCs from adipose tissue	Intramuscularly injection	Multiple injection of AD-MSCs cause no complications during 6 months follow-up Clinical approvemet was recorded in 66.7% pateitns At 6 months, significant improvement in pain rating scales, and claudication walking distance were recorded	[35]

4 The biology of ischemia

Ischemia creates a sudden decrease and loss of blood supply to the affected area, which results in several consequences to the microenvironment, directly affecting the survival of the cells in the surrounding tissues. Depending on the degree and time of restriction, the consequences brought by ischemia can be reversible or irreversible. At the cell level, the ischemic condition results in changes in oxygen supply and nutrition deprivation, forcing the cells to turn on different mechanisms to deal with the environment (Fig. 1).

4.1 Nutrition deprivation

The reduction in oxygen supply can affect the ischemic condition at various degrees, depending on the need for oxidative metabolism in the specific tissues. The decrease in oxygen supply firstly lowers metabolism that requires oxygen, such as oxidative phosphorylation and beta-oxidation, resulting in a decrease in ATP synthesis. For instance, energy metabolism-related genes were among those downregulated in ischemic limb mouse models [53]. In rat ischemic heart, the low blood flow has been shown to reduce 84% of oxygen consumption, leading to 51% reduction of ATP and peak lactate production at 20 min of the ischemic event [54]. The increase in lactate production was found to be a result of ischemia due to the reduction in oxidative phosphorylation and increase in glycolysis and glycogen breakdown [55].

The attempt for ischemia-affected cells to increase glycolysis during the ischemic condition (to replenish energy requirement) is supported by multiple mechanisms. It has been suggested that ischemic conditions may increase anaerobic glycolysis as a result of reduced oxygen to maintain the levels of ATP and to help improve the survival of hepatocytes [56]. These data suggest that switching from aerobic metabolism to anaerobic metabolism is one of the strategies of cells at the ischemic site to maintain the energy pool that is needed for them to function. However, at the same time, when the blood supply is reduced, the decrease in glucose supply at the affected area also causes the cells to have short glycolysis. In a study investigating blood glucose in the subcutaneous tissue and skeletal muscles of patients with CLI, it was shown that a reduction in blood perfusion resulted in the decrease of glucose concentration in both the subcutaneous and intramuscular catheters. However, there was a reduction of lactate concentration only in the intramuscular measurement [57]. A similar glucose reduction was also found in ischemic models in rats [58], suggesting that reduced glucose concentration is a sensitive indicator of ischemic condition [59].

While glucose is a clear indicator, other metabolic pathways are also expected to be affected by the decrease in blood supply during ischemia. In a recent study that investigated the transcriptome in the skeletal muscle of CLI

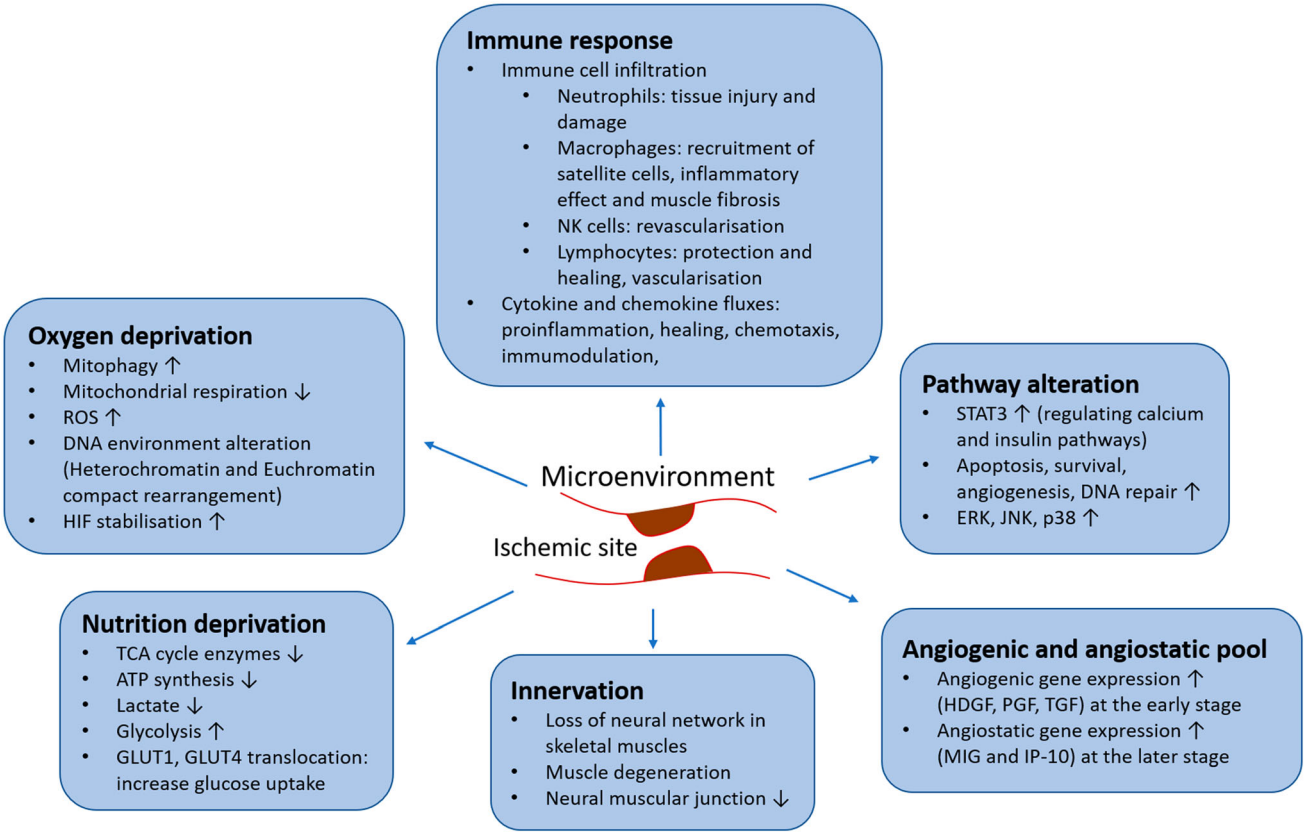

Fig. 1 Changes in the microenvironment surrounding ischemic site. These changes create the deprivation in oxygen and nutrition, leading to cell pathway alteration. Immune and nerve system in the local area are also affected and activated, resulting various degrees of tissue damage. The changes in angiogenic and angiostatic pool is modified to help the local tissue to adapt to the new condition

patients, it was found that the expression of succinate dehydrogenase (the enzyme involved in mitochondrial respiration and TCA cycle) was reduced [60]. In prolonged ischemia, the production of lactate and pyruvate decreases gradually until ischemia is completed [54, 55]. At the cell level, in order to enhance the rate of glycolysis, GLUT1 and GLUT4 expression was translocated to different compartments of myocytes and to the cardiac capillary to enhance the uptake of glucose [61, 62]. Particularly, it has been shown in rats with ischemic hearts that GLUT1 was translocated from the cardiac tissue into the capillary endothelium to enhance glucose absorption to cardiac cells [62]. In a similar model, GLUT4 was found to be translocated to the plasma membrane of cardiac myocytes during heart ischemia [61], or to the sarcolemma and T tubules of myocardiocytes to maximize the transport of glucose into the cells [62]. These data suggest that the reduction in blood supply to the ischemic tissues affect the cells to perform different mechanisms to adapt to the short supply of glucose and to maintain the energy levels for normal functioning.

Although there is knowledge about glycolysis and oxidative phosphorylation in ischemic conditions, there is still limited information about amino acid metabolism that has either been published or studied. In areas that need high levels of amino acids for physical activity, such as skeletal muscles, the requirement of amino acids is crucial to maintain tissue function and recovery. For example, resistance exercises recapitulate a small degree of reduced blood flow that occurs in ischemic muscles, and it was found that amino acid intake played an important role in protein recovery of the leg muscle [63]. Thus, a supply of amino acids at the ischemic site appears to be essential for the prevention of ischemic damage. Indeed, it has been shown in animal ischemic heart models that treatment with glutamine induces a protective effect to the cardiac muscle following ischemia and reperfusion, due to the production of O-linked N-acetyl-glucosamine (O-GlcNAc), the key player in the cardiac protection mechanism [64–66]. The involvement of O-GlcNAc in the activation of hexosamine biosynthesis pathway is the main driving factor in cardio-protection and is most likely to be related to ATP levels and other endogenous stress-activated pathways rather than protein synthesis [64, 66]. This suggests that the recovery mechanism for ischemic condition relies greatly on the ability to maintain energy and suppress stress induction

that potentially leads to apoptosis. Glutamine and glutamate also play an important role in controlling action potential duration (APD), an indicator of electrical measure of cardiac function, to exert cardiac protection [64]. Thus, it is important to understand needs of tissues for amino acid metabolism during ischemia. It may also be important to know which amino acids are critically required or changed at the onset of the ischemic event and during prolonged periods of ischemia.

The above evidence suggest that a reduction in nutrition supply greatly affects the cells at the ischemic tissues. Hence, nutrition supply is an important factor for consideration in treatment, especially when using cell-based therapy. Ensuring that nutrition is affordable for donor cells to function and enhancing the ability of donor cells to adapt to limited nutrition supply in the ischemic environment are key factors to achieving success in cell-based therapy for ischemia.

4.2 Oxygen deprivation

The reduction of oxygen supply directly affects the respiration of cells. Mitochondria are known as the site of metabolism and respiration. Thus, the reduction in oxygen supply due to ischemic events poses potential damage to the function of the mitochondria. In fact, mitophagy was found to increase at day 14 after a hindlimb ischemic event in mice, indicating evidence of mitochondrial damage [67]. In a study that analyzed the gene expression profile of gastrocnemius muscle, both respiration capacity and expression of the genes associated with cellular respiration and mitochondrial inner membrane significantly decreased in patients with CLI [60]. Particularly, genes that are involved in the formation of the electron transport chain, such as cytochrome c oxidase (COX) proteins, ATP synthase, and ubiquinol-cytochrome C reductase (UQCR), were found to be reduced in the skeletal muscle of CLI patients compared to healthy adults [60]. Following the reduction in mitochondrial function and respiration, reactive oxygen species (ROS) were shown to be increased as a result of electron transport chain failure during the development of ischemia [68]. ROS were detected in ischemic hearts in rat models and the increase in ROS production was found to be correlated with an increase in necrosis and cell death, which could be reduced by the use of antioxidants, such as manganese superoxide dismutase (MnSOD) [68, 69].

However, the precise mechanisms and the functions of ROS during the ischemic period remain unresolved questions. The reperfusion of blood flow and replenishment of oxygen supply to the ischemic hearts were shown to result in a burst of ROS production [70, 71], which could further damage the cells at the ischemic site. Cells of the ischemic area are prone to ROS production, but it is not known whether the use of antioxidants can successfully inhibit the necrotic cascade. In this case, saving the mitochondria from being dysfunctional is the key factor to efficiently restore cell respiration and prevent the consequences of ischemia. In fact, the reduction in oxygen and nutrition supply can affect cells at the genomic level. Deprivation of oxygen and nutrition could cause alterations in the DNA environment of cardiomyocytes during a period of hypoxic culture, resulting in the restructuring of chromatins in which heterochromatin and euchromatin are compacted and rearranged in the cell nuclei [72]. Even in vitro, culture of cardiac cells in hypoxic conditions was found to lead to a cascade of reactions, including restricted access to histones and redistribution of polyamides to the nucleus, resulting in the inhibition of transcription [72]. In fact, the compaction of chromatin during hypoxia is correlated with a wide suppression of gene expression [73], suggesting that the lack of oxygen supply in the cell environment can cause remarkable changes in cell growth and behavior.

The lack of oxygen supply creates a hypoxic condition that triggers the stabilization of hypoxic induced factors (HIF), which play an important role in the regulation of metabolism and redox homeostasis [74]. HIF has been shown to have positive effects on rescuing cardiac muscle in ischemia via ischemic preconditioning of the heart, a condition with repetitive ischemic-reperfusion events that trigger cardiac protection [75]. Endothelial cell expression of HIF was found to reduce the inflammation response of renal cells, and only HIF-2α acted on vascular cell adhesion molecule-1 (Vcam1) to inhibit the infiltration of neutrophils during the recovery phase, which was necessary to protect the kidney from ischemic injury [76]. The enhanced HIF expression by adenovirus into ischemic limbs of diabetic mice showed that HIF-1α plays an important role in the recovery of the vessels and increases the recruitment of angiogenic cells, suggesting that HIF-1α is an important factor in response to the ischemic condition in diabetes [77]. Understanding the changes in oxygen supply at the ischemic tissues and how cells adapt to the hypoxic environment can be useful to predict the outcomes of the ischemic condition and help to devise strategies to support the cells in overcoming the challenges of low oxygen supply.

4.3 Pathway alterations

The reduction in oxygen and nutrition supply following ischemia can cause cellular pathway alterations to adapt to the new conditions and to rescue the ischemic injury. Depending on the tissues where the injury has occurred, different cellular pathways may be predominant in the response to the ischemic event. In repetitive ischemic

reperfusion (I/R) periods of pig hearts, it was shown that repetitive I/R plays an important role in cardioprotection of the hearts. During examinations at the onset of ischemia, Pavo et al. showed that I/R affected a wide range of cellular pathways, including calcium and insulin signaling and adipocytokine pathways, as early as 5 h after the repetitive I/R events, which were regulated by the overexpression of STAT3 of the PI3K/Akt and the JAK/STAT pathways [78]. STAT3 signaling has also been shown to be important in the regulation of satellite cell recruitment in muscle injury and promoting angiogenesis in experimental peripheral arterial disease (PAD), a pathogenic cause leading to hind limb ischemia [79, 80]. Besides, pathways related to apoptosis, survival, angiogenesis, DNA repair, oxidative stress and metabolism were also observed in both distal and remote areas of the ischemic site after cycles of repetitive I/R [78]. These pathways were suggested to play an important role in protecting cardiac muscles from further injury; some of these pathways induce cardioprotection by applying repetitive I/R. The ERK, JNK, and p38 pathways were found to be elevated in I/R in rat spinal cord after hind limb ischemia, in which the activation of ERK and JNK by phosphorylation was correlated with the production of ROS [81].

Even though these results may require further validation (since the authors could not measure ROS directly), they have raised the possibility that ROS could be important signals for the activation of tissue survival and angiogenesis pathways via other remote tissues [82]. In response to ischemia, the rat brain has been shown to have an enhanced Notch1 signaling pathway, the pathway that is involved in the recruitment of precursor neural cells, as well as in the selection and stabilization of tip and stalk cells in angiogenesis [83–86]. Therefore, Notch signaling has been identified to be critically associated with the pathogenesis of brain ischemia and can be targeted to boost the recovery of ischemic and stroke conditions. Different chemicals have been examined to enhance Notch signaling as a potential therapeutic target in brain ischemia [87, 88]. The data suggest that cell signaling alterations play an important role in preventing tissue injury and promoting the protection of the ischemic areas. Understanding the function of different pathways can efficiently enhance the recovery of ischemic conditions which can be targeted for therapeutic purposes.

4.4 Immune response

The involvement of immunity is a prominent response of the body when ischemia is triggered. Multiple immune responses are known to occur at the ischemic site; some of these responses include cytokine and chemokine production, followed by immune cell infiltration and activation. Depending on the type of reaction, an immune response can either cause damage to the tissues or induce protection to prevent tissue injury. Both innate and adaptive immune responses have been shown to play pivotal roles in the progression and recovery of ischemic tissues [89, 90]. Immune cell infiltration to the affected area occurs within hours after heart ischemia occurs, and is also associated with the influx of cytokines and chemokines [91]. In ischemic heart, it was found that during the onset of MI, immune cells (such as neutrophils, M2-macrophages, natural killer (NK) cells and T-cells) were significantly recruited to the affected area [91, 92]. Among these cells, neutrophils were those that presented at the early stage, while macrophages were the most abundant cells in the affected area after 3 days of MI [91, 92]. Macrophages were also involved in the recruitment of satellite cells that are important for the regeneration of damaged muscles [93].

Along with immune cell infiltration, the ischemic heart also experiences fluxes of cytokines and chemokines from both the local tissues and the serum, which dynamically change with time during the prolonged period [91]. It was found that the expression of cytokines and chemokines was accelerated as early as 6 h after femoral artery ligation in mice and peaked after the first day ischemia took place [53]. The presence of cytokines (such as TNF-α, IL-6, IL-4 and IL-10) and chemokines (such as CXCR7, MIP-2 (macrophage inflammatory protein 2 or also named CXCL2), and CCR2) are known to exert both pro-inflammatory and protective function during the ischemic period [91, 92]. Circulating chemokines, such as MIP-2, were also found to be involved in the injury of lung, a distant tissue, in mice with hindlimb ischemia [94]. On the other hand, the addition of regulatory T (T_{reg}) cells was shown to induce protection against tissue injury in mice with ischemic kidneys [95]. In this case, the expression of PD-1 ligand on T_{reg} cells was responsible for their ability to protect the kidneys from damage; furthermore, the blockage of PD-1 ligand prior to kidney ischemia resulted in increased kidney injury [95]. The role of different chemokines and cytokines requires further investigation to unravel if they are required for cell–cell interaction in the ischemic area. Several studies have indicated the role of chemotactic interaction of immune cells in the recruitment of angiogenic cells, which improved the recovery of ischemic tissues. For example, the chemotactic axis of CCR7 with CCL19 and CCL21 could promote the accumulation of $CD4^+$ T cells, which are essential for arterial remodeling in the mouse limb ischemia model [96]. These evidence suggest that the immune response is not only a local effect on the ischemic site but is also a systemic effect.

The damaging and protecting roles of immune cell infiltration during ischemia can be interchangeable, depending on the time when the cells are recruited to the ischemic sites. For example, the accumulation of neutrophils has been known to be damaging to the ischemic heart, potentially resulting in I/R injury. Moreover, other immune cells, such as macrophages and lymphocytes, could mediate protective and healing effects [97–99]. In the ischemic limb, lymphocytes and NK cells exerted their healing effects via the revascularization process [100, 101], while macrophages exerted their inflammatory effects, which often resulted in muscle fibrosis after ischemia [102].

It is noted that the expression profiles of immune cells are critical in the determination of whether an injury or a protective effect might occur in the ischemic tissues. For instance, the expression of PHD3 (prolyl-4-hydroxylase domain enzyme 3) on macrophages was shown to be correlated with increased fibrosis in skeletal muscles of the ischemic hindlimb, and that the depletion of PHD3 significantly rescued the damaging effect [102]. However, the transmembrane protein, toll-like receptor 4 (TLR-4), has been shown to be important for the accumulation of neutrophils in the extracellular muscle space during the ischemic condition, which is correlated with tissue injury [103]. Several studies have described the remodeling role of immune cells during the ischemic event. Particularly, neutrophils are responsible for both tissue injury during ischemia and tissue remodeling after ischemia. The infiltration of neutrophils can form plagues, with neutrophil accumulation on the arterial walls; moreover, the neutrophils help in vessel rupture in the later stages by interacting with circulating immune cells and vascular cells [104].

The accumulation of immune cells in the ischemic area also plays an important role in the cell-to-cell interaction, an important factor for the remodeling and the recovery process [89, 90]. Of note, the presence of immune cells with the secretion of cytokines and chemokines into the ischemic area are critical in the chemotactic process, which is essential for the recruitment of myoblast precursor cells and vascular precursor cells to the ischemic site [53, 97]. In mouse models, the role of $CD8^+$ T cells in the ischemic area has been investigated, which showed that $CD8^+$ T cells play an important role in the recruitment of $CD4^+$ T cells, regulation of immune response, and support of blood flow recovery to the ischemic site via the expression of IL-16 [100]. Even though the precise mechanism for this effect has not yet been elucidated, the results of the study in mice have suggested the importance of the immune response in the healing of ischemia. Similarly, the presence of NK cells and $CD4^+$ T cells have been shown to be correlated with improved arteriogenesis in mice with ischemic limbs [101, 105]. The depletion of $CD8^+$ T cells, $CD4^+$ T cells and NK cells can result in severe impairment of the limbs and can significantly impair arterial and collateral genesis [100, 101, 105]. The activation of $CD4^+$ T cells, especially T_{reg} cells, was shown to be critical for wound healing in mice with MI, which was suggested to be related to the ability to induce collagen secretion from other cells in the affected area [106]. Even though the precise mechanisms of these phenomena have not been well-understood, the activation of T cells during ischemic events was found to play an important role in promoting neovascularization and angiogenesis in ischemic limbs via the co-stimulation of other cell types, such as monocytes and vascular smooth muscle cells (VSMCs) [107, 108]. In particular, the stimulation of $CD14^+$ monocytes by T cells was found to lead to enhanced expression of pro-angiogenic and pro-arteriogenic factors, such as vascular endothelial growth factor (VEGF), interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), and platelet-derived growth factor (PDGF)-BB [108]. This effect may be due to the ability of monocytes to produce monocyte chemoattractant protein (MCP)-1 which, in turn, recruits macrophages that induce the expression of TNF-α and VEGF [109].

Thus, as an important factor during the ischemic event, the immune response can contribute broadly to both injury and recovery of the condition. The acceleration of immune responses is dependent not only on the onset and severity of the condition but also on the medical history and genetic profiles of different individuals. While other factors are important, genetic profiles have appeared to be critical for the prognosis of any ischemic-related complications and for development of efficient treatments for different individuals. Specifically, it will be important to understand how different people with different genetic profiles progress during ischemia and how they adapt to different types of treatments. Ideally, a therapeutic target that includes immune modulation and adaptation will be promising for a broad range of patients.

4.5 Innervation

Nerve distribution is important for the growth, development and regeneration of active muscles, especially skeletal muscles. The level of impact of an ischemic event in muscle, in turn, can result in the loss of the neural network. It has been shown that the degree of ischemic injury may be associated with neurological pathology and recovery of the ischemic tissue, where neurodegeneration around the ischemic site may correspond with the severity of muscle atrophy [110, 111]. Furthermore, the prolonged damage of the neural network often results in severe muscle degeneration. For example, long-term damage in

replanted limbs in rats was shown to have a significant impact on the recovery of skeletal muscles since the damage reduced the muscle weight and resulted in smaller muscle cells [111]. Accordingly, it was noticed that the reduction in arterial flow did not lead to significant changes in muscle atrophy, and only denervation ischemia caused severe muscle degeneration [111]. In mice, denervation of motor units and neuromuscular junction appeared a few days after an ischemic event and lasted for 56 days after ischemia, leading to muscle degeneration [67]. The remodeling of subsynaptic nuclei at the neural muscular junction and mitochondria along the muscle fibers was found to play an important role in the repair of denervation and, therefore, was critical for muscle regeneration [67]. When it comes to ischemia with nerve damage, it has been shown that mice with ischemia accompanied with pain showed lower muscle weight and decreased physical activity [110], suggesting that the preservation of the neural network plays an important role in the ischemic condition. The latter needs to be considered when searching for appropriate treatments. Understanding the neural conditions in each ischemic case may be important for the control of ischemic damage. Moreover, treatment of an ischemic disease may need to be considered in combination with treatment of denervation to potentially gain the best outcome.

4.6 Angiogenic and angiostatic pool

Reduced blood supply causes the expression of various transcriptional factors to be influenced by changes in the living environment in ischemia. The production of several growth factors, including angiogenic and angiostatic factors, results in new vascular formation at the ischemic injured site to replenish blood flow. The induction of these factors after an ischemic event plays an important role in the balance between the formation and maturation of new blood vessels, since neovascularization without a proper maturation process can result in a more severe condition when a rupture occurs in the newly formed vessels. The expression of angiogenic genes, such as hepatoma-derived growth factor (HDGF), placental growth factor (PGF), and transforming growth factor (TGF), were among those that were highly expressed in the ischemic limb site after the ligation of femoral artery [53]. However, the expression of genes that are known as angiogenic factors, such as angiopoietin 1 (ANG1), vascular endothelial growth factor B and C (VEGF-B and C), and fibroblast growth factor (FGF), was not detected or increased, while angiostatic-related genes, such as monokine induced by IFN-ϒ (MIG) and IFN-ϒ-inducible protein-10, were significantly upregulated at the later stage of ischemia [53]. This evidence raises questions about how angiogenic homeostasis is maintained during the ischemic condition to support the recovery of the subjects. In fact, it is known that at the early stage of ischemia, the production of angiogenic factors is regulated so that the process of vessel cell recruitment and maturation are maintained to ensure the stable formation of mature vessels without the overgrowth of neo-vessels [112]. Therefore, the constitution and concomitant levels of angiogenic and angiostatic proteins in the ischemic environment are critical for the recovery outcome. Ensuring the replenishment of angiogenic and angiostatic factors in ischemic tissues is, therefore, an important criterion to achieve success in cell therapy.

5 Mechanisms of MSC transplantation for ischemia

MSCs hold great potential for therapeutic clinical applications since they possess specific unique characteristics that cannot be found in other cell types. Importantly, MSCs can be expanded unlimitedly and stably in vitro, allowing them to be used in large quantity with low variation, thus meeting the consistency criterion required for large-scale applications [113]. Other characteristics that potentiate MSCs for therapeutic use include their ability to home to target tissues, to differentiate, to generate the microenvironment, and to immunomodulate. For conditions such as ischemia, these characteristics are especially useful to cope with changes in the microenvironment and to support the healing of tissues (Fig. 2).

5.1 Homing capacity

MSCs are known for their ability to assist in the healing and recovery of damaged tissues by creating a microenvironment for regeneration. This is associated with the ability of MSCs to migrate from their site of origin to the site of injury. The ability of MSCs to travel and home to different tissue sites has been widely studied and discussed due to their expression of several molecules that assist with the process of cell movement [114]. MSCs can either be applied locally to the site of lesion or systemically in the circulation. Indeed, the transendothelial migration capacity of MSCs is an important characteristic which makes them advantageous in transplantation medicine [115]. Systemic transfer of MSCs involves steps that require the cells to attach to the endothelium and roll along the blood vessels to the injured tissues before the cells cross the endothelial membrane and successfully migrate across the tissue to reach the injured site [115, 116].

If administered systemically, the homing mechanism of MSCs to damaged tissues includes the attachment of MSCs to the endothelial cell (EC) walls via the interaction of

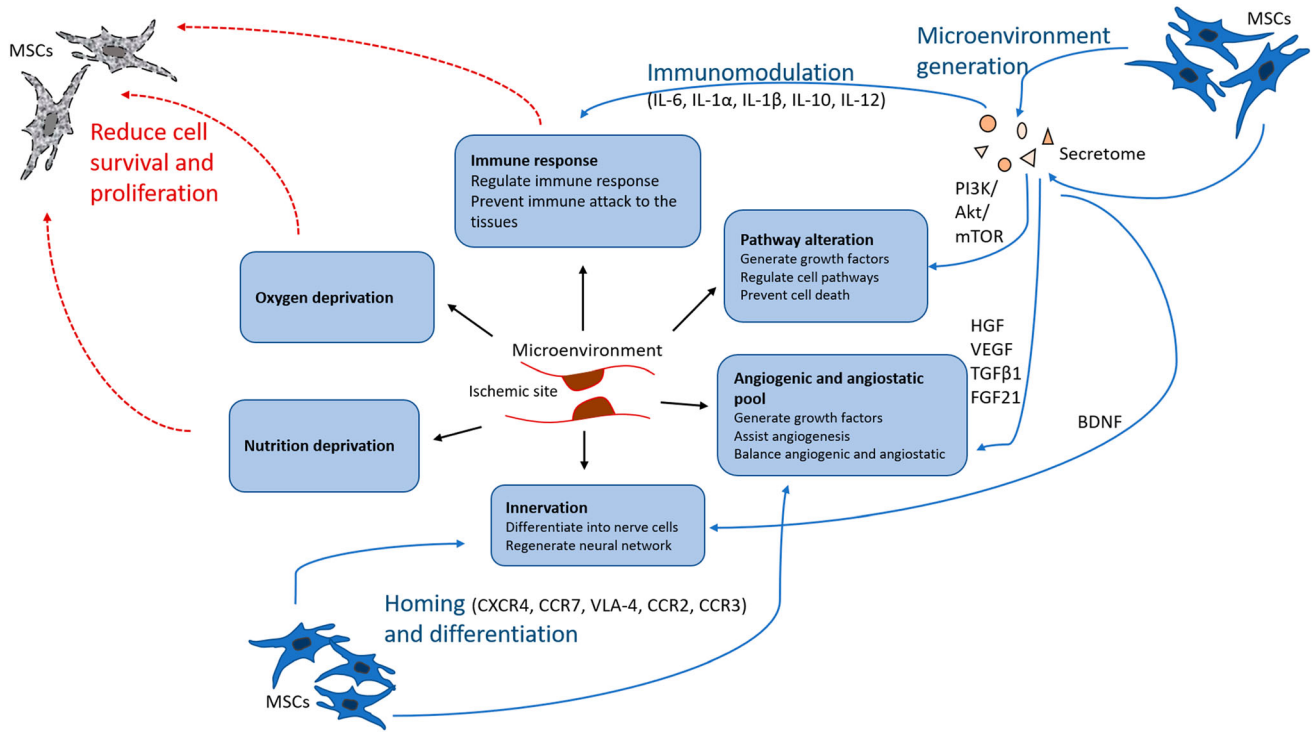

Fig. 2 Schematic of how the changes in ischemic microenvironment can affect MSCs (red arrow), and in turned, be modified by the presence of MSC administration (blue arrow). Changes in the environment such as oxygen and nutrition deprivation and host immune response can negatively affect the survival and proliferation of MSCs. On the other hand, the administration of MSCs during ischemic condition can help to reverse the damages caused by ischemic condition. Improvement of the condition is induced by the effect generated from MSCs characteristics of immunomodulation, microenvironment generation, homing and differentiation. MSCs generate secretome containing growth factors, cytokines and chemokines to modulate the host immune response, resolving cell death signal and balancing angiogenic and angiostatic pool in the ischemic site. MSCs can also home and guide the regeneration of nerve in the ischemic tissue to support tissue recovery

CD44 on MSCs and selectins on the EC surface, which enable the MSCs to roll along the vessel wall [114]. Once in the vessel, MSCs are activated via the chemotactic effects of CXCR4, CXCR7 and other chemokines on the MSC surface with stromal derived factor-1 (SDF-1) on ECs, which also help MSCs to move towards the injury site [117]. Indeed, the expression of CXCR4 on MSCs has been shown to have controversial effects on the homing capacity of the cells. Some studies have suggested that the high expression of CXCR4 is associated with bone marrow homing but not transendothelial migration, while many studies have shown the role of CXCR4/SDF-1 chemotaxis in MSC migration to various tissues [101, 118–121]. Once reaching the destination, MSCs are arrested by the interaction of the very late antigen 4 (VLA-4), which is formed by the combination of integrin α4 and β1, with vascular cell adhesion molecule 1 (VCAM-1) [115, 116]. At the site of the arrest, transmigration or diapedesis occurs, allowing MSCs to cross the endothelial membrane into the interstitial space of the target tissue. To cross the endothelial membrane, MSCs are supported by the activity of matrix metalloproteinases (MMPs), which help to lyse the basement membrane of the endothelium, assisting MSCs to enter the interstitium [122]. Inside the interstitial space, MSCs and their secreted cytokines (such as CCR2, CCR3 and CXCR4) respond to several factors, including SDF-1, platelet derived growth factor (PDGF), IL-8 and insulin-like growth factor (IGF), to migrate to the target sites [123–125].

5.2 Differentiation capacity

MSCs are well-known for their differentiation potential. Several studies have been conducted to evaluate the differentiation of MSCs into certain cell types in vitro; many of them are of special interest, such as adipocytes, osteoblast and chondrocytes, while others are non-MSCs, such as hepatocytes, neurons and pancreatic islet cells [126–129]. Other cell types including smooth muscle cells, cardiomyocytes and keratocytes have also been successfully derived from different sources of MSCs [130, 131]. Depending on the origin of MSCs, the differentiation process can be optimized by selecting the MSC populations from certain sources; for example, BM-derived MSCs are

the best source of cells for neural differentiation while MSCs from BM or adipose tissue may be more suitable for vascular muscle cell and adipocyte induction [129, 132].

MSCs with a certain expression profile may also be more favorable to commit to particular cell types. Thus, the selection of different MSC subpopulations in order to perform specific differentiation has also been a solution for optimization. For instance, MSCs with high expression of CD146 have been shown to be associated with vascular muscle cell differentiation. Therefore, the selection of the MSCs may be based on their advantage in angiogenesis [131]. In connection with homing, the above evidence raises a possibility that the selection of MSC subpopulations with high expression of both CXCR4 and CD146 may enhance the efficacy of MSCs in treating ischemic conditions. This is because MSCs with high expression of CXCR4 were shown to be efficient in homing to the injured sites, such as ischemic heart and brain [119, 133–135]. Other MSC subpopulations were also shown to be associated with different cell type commitments; for instance, $CD200^+$ MSCs showed high osteogenic potential while $CD140^+$ MSCs showed high adipogenic potential [136]. However, it must be noted that controlling the differentiation of MSCs in vivo is a challenging task that requires a broad knowledge of MSC behavior and function. Thus, further investigations of MSC differentiation in vitro and in vivo are warranted to understand the factors that can affect MSC differentiation in vivo.

5.3 Microenvironment generation

The ability to create a microenvironment for healing and homeostasis is another major characteristic of MSCs that is useful in the recovery of damaged tissues. Indeed, MSCs are the foundation for regenerative medicine. The purpose of using stem cells in regeneration not only relies on the ability of the transplanted cells to support the formation and healing of the injured tissues but also to prevent the damages that may cause irreversible tissue consequences, such as malfunction or dysfunction. MSCs with their varied secretion of protein molecules have been widely studied. Isolation of extracellular vesicles from MSCs has revealed several growth factors- such as hepatocyte growth factor (HGF), VEGF, transforming growth factor-β1 (TGFβ1), fibroblast growth factor 21 (FGF-21), and others- which enhance the ability of MSCs in endogenous repair and healing of tissues, including the promotion of vascularization to maintain tissue survival [137–140]. MSCs also secrete neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), to enhance neurogenesis in the case of the ischemic brain [141].

It was found that in the rat model of stroke, animals that were treated with activated MSCs had a significant increase in the number of cells of the oligodendrocytic lineage which express platelet-derived growth factor receptor α (PDGFRα), known to support the lipogenesis process [142]. The authors also identified several molecules in the secretome of activated MSCs that may have an important role in the healing and recovery of the damaged brain [142]. Furthermore, the presence of MSCs also helps to regulate autophagy to support nerve cell survival in cerebral ischemia via the activation of Akt/mTOR signaling pathway [143, 144]. This evidence suggests that MSCs have the potential to induce a healing environment in the injured tissues, which can be monitored by various mechanisms. In fact, several studies have put effort into enhancing the ability of MSCs to secrete exosomes that can be used for healing, but especially to promote angiogenesis as a part of the recovery process [138, 145, 146]. Stimulation and triggering factors can be applied when culturing MSCs to push the expression of angiogenic factors from MSCs and to push the secretion of these factors into the environment so that secretomes can be applied to the injured areas. Different types of triggers are exploited- including mechanical boundary culture conditions, hypoxic culture, and gene expression transfection- which promote angiogenesis and prevent apoptosis at the damaged tissue [145, 147, 148]. For example, exosomes from HIF-over-expressing MSCs showed an increased content of miRNA and proteins related to cell cycle, glycolysis and angiogenesis [147]. High levels of angiogenic factors, such as MMP-2, VEGF and TGF-β1, were found in the conditioned media of mechanically-stimulated MSCs [145]. It was found that even though the secretion of VEGF-A was not present in the conditioned medium from umbilical cord-derived MSCs (UC-MSCs), the effect on angiogenesis was maintained, suggesting that other factors were involved in angiogenic capacity [149]. Some signaling pathways have been identified as being responsible for the regulation of vascularization, such as the Notch signaling pathway and its downstream molecules Jagged-1 [147, 150]. Even though the identification of the triggering factors and activators in MSCs on angiogenesis may require further studies, the potential of using MSCs and their secretomes as a biomaterial in transplantation to treat ischemic diseases is, undoubtedly, promising.

5.4 Immunomodulation

The secretome from MSCs can modulate the immune response at the site of injury, preventing cell apoptosis and tissue necrosis mediated by the immune system. In vitro, when BM-MSCs and adipose-derived stem cells (ADSCs) were co-cultured with endothelial cells, high levels of IL-6 were detected in the media, which correlated with the reduction in neutrophil adhesion, suggesting that MSCs

performed immunomodulation via the release of IL-6 into the environment [151]. Other secreted cytokines may include IL-1α, IL-1β, IL-10, and IL-12, which play important roles in the regulation of inflammation and immune modulation [152, 153].

While MSCs, in general, have been shown to perform immunomodulation at different levels, the presence of some subpopulations with specific origins has been shown to be more pronounced in immunomodulation function. For examples, BM-MSCs with high expression of CD146 were found to be more sensitive to immune stimulation, in which the exposure to inflammatory priming caused the cells to increase the expression of proteins related to immunomodulation, such as IL-10, IL-3, IL-4, and toll-like receptors, than cells with low or no expression of CD146 [153]. MSCs with low expression of CD105, from both human and mouse origins, have also been reported to have stronger immunomodulatory function compared to MSCs with high CD105 [154, 155]. Similarly, CD106 + MSCs were found to express more immunomodulatory factors than negative counterparts, which resulted in better T_{reg} induction and inflammatory suppression [152]. Other types of MSC immunomodulation include the suppression of stimulated immune cell proliferation, T cell differentiation and macrophage activation, the induced polarization of macrophages into anti-inflammatory M2 phenotype, and the reduction in pro-inflammatory factors [153, 156, 157]. The ability of MSCs to inhibit INF-ϒ and induce IL-4 secretion has also been shown to shift the induction of T_H1 cells to T_H2 cells, which effectively reduces proinflammation and promotes anti-inflammation responses in graft-versus-host disease (GvHD) [158]. Immune activation of IFN-ϒ on MSCs can prolong the survival of MSCs by suppressing host T cell activation. Thus, the induced expression of IFN-ϒ on MSCs has been shown to be effective when co-transplanted with activated peripheral blood mononuclear cells [159]. These evidence suggest that MSCs can exert potent immunomodulation to assist in the control of tissue damage caused by immune responses while at the same time exerting potent healing effects. In conclusion, the use of MSCs can either facilitate recovery or suppression of immune responses; thus, the selection of the MSC subpopulation with high potency in tissue healing and protection can be optimized to enhance the use of MSCs in therapy.

6 Challenges of using MSCs in vivo and strategies to improve therapeutic efficacy of MSCs

The potential of MSCs in cell therapy has been proposed and investigated both in vitro and in vivo. However, several challenges directly affect the efficacy of MSCs in therapeutic applications, especially in the in vivo environment. These challenges include the low survival rate of MSCs in the in vivo environment, low growth rate, low ability to proliferate, and rejection from the host immune system. Using MSCs in cell therapy requires selected MSCs to be grown and expanded in vitro, after which the cells are transferred to the in vivo environment, either systemically or locally to the affected sites as a cell treatment. The changes in the environment provide the biggest challenge to the adaptation of MSCs and can effectively reduce the ability of MSCs to perform their function. Understanding the key challenges that MSCs may face in vivo will help to find solutions to improve the cell characteristics that support and sustain them in vivo, as well as solutions to increase the efficiency of cell therapy. Furthermore, it is critical to have good knowledge about the possible causes and mechanisms of these challenges so that they can be addressed and solved appropriately using different strategies (Table 2).

6.1 Survival and migration

The changes from in vitro to in vivo environment, especially in ischemic conditions, cause MSCs to face several microenvironmental restrictions, including nutrition deficiency, hypoxia, and oxidative stress (Fig. 2). For example, the injection of human MSCs into mouse ischemic heart showed that most of the injected cells were relocated into different organs, and only a small number of cells (∼ 0.44%) were found in the heart at day 4 after injection [178]. In another study, when MSCs were transferred into patients via a coronary artery (intracoronary), only 1.3%-2.6% of cells were detected after 7 days, and only background detection was found when transferred to the patients via an antecubital vein (intravenously) [162]. Similarly, less than 1% MSCs were found in rat infarcted heart after 7 days of in vivo transfer [179]. These evidence suggest that in vivo, MSCs not only face survival problems but also face difficulty in migration, especially when administered systemically [180].

Problems with in vivo survival and migration in MSCs after transfer have led to an emerging interest in research on modifications of in vitro cell culture of MSCs to investigate ways to enhance the retention of MSCs in vivo and increase the migration of MSCs towards the target sites. Several studies have demonstrated different strategies to improve the survival and migration of MSCs. Stimulation that prolongs the survival of MSCs, including the modulation of autophagy and hypoxic pre-culturing, could effectively protect MSCs from apoptosis and hypoxic stress in animal models [143, 160]. It was found that long-term starvation of MSCs in culture, even though it changes the cell morphology and phenotype, did not change the cell

Table 2 In vivo challenges that possibly affect MSCs efficacy in therapeutic application and strategies to improve MSCs adaptation

In vivo challenges for MSCs	Improvement strategies
Survival	Modulation of autophagy [143]
	Hypoxic preconditioning [160]
	Long-term starvation in culture [161]
	Selective transfer of $CD34^+$ MSCs [162]
	Encapsulation of MSCs in microgel and lysophosphatidic acid (LPA) pretreatment [163]
Homing	Selective transfer of CXCR4 MSCs or cell surface engineering with CXCR4 + [164, 165]
	Selective transfer of $CD34^+$ MSCs [162]
	Ultrasound [166]
	Magnetic attraction using of superparamagnetic iron oxide (SPIO) nanoparticles [167]
	Enhancing the expression of CD44 by culturing MSCs on hyaluronic acid (HA) coated plated [168]
	Coculture with endothelial cells [169]
Proliferation	Preconditioning in hyperoxic condition and pan-caspase inhibitor treatment [170]
Host immune response	IFN-ϒ loaded microparticle priming of MSC spheroids [159]
	3D aggregates of MSCs
	Encapsulation of MSCs in microgel [163]
	Selection of CD106 + MSCs population [152]
	Selection of CD105- MSCs population [154, 155]
	Pretreatment of MSCs with IL-17A [171]
	Pretreatment of MSCs with IL-1β [172]
	Pro-inflammatory treatment of MSCs [173]
Trans-differentiation	Combination with nitric oxide releasing hydrogel [174]
	Inhibition of cycloxygenase-2 [175]
	Increase of Akt and NFkB signals [176]
	Transfection with ETV2 [177]

stemness and genetic stability, which helped the cells reach quiescence and improve survival/adaptation in the in vivo environment [161]. The selection of certain MSC subpopulations has also shown advantages in enhancing cell survival in vivo. Particularly, selective transfer of $CD34^+$ enriched MSCs showed a high cell retention rate in the heart compared to unselected MSCs [162]. Another strategy to improve MSC survival includes the encapsulation of MSCs in microgel before intravenous delivery and the induction of protection via treatment with lysophosphatidic acid (LPA), which showed that the survival time of MSCs increased fivefold over unencapsulated cells and the number of detected bare cells was doubled [163]. The enhancement of MSC migration has been tested with different strategies, including using mechanical and biological mechanisms. For instance, ultrasound of the CLI area was used to direct the homing of MSCs to the target site, and thereby to improve the efficacy of the treatment [166]. Even though more investigations on survival of MSCs in in vivo ischemic conditions are necessary, the improvements in cell survival and migration are, indeed, possible. This emphasizes the importance of enhancing survival rate and migration of MSCs when used as therapeutic applications.

6.2 Proliferation and differentiation

The exposure to hypoxia and nutrition restriction during ischemic conditions not only causes the reduction in cell survival but also influences the propagation of MSCs in the in vivo environment, which is followed by the risk of functional loss [181]. Several efforts have been made to improve the proliferation rate of MSCs in hypoxia, which includes the preconditioning of MSCs in hyperoxic conditions and pan-caspase inhibitor treatment [170]. However, whether these conditions provide similar effects in vivo requires further examination. Alternatively, the transfer of MSCs at the same time with T_{regs} has been shown to be effective at protecting MSCs and supporting the proliferation of MSCs in vivo in an ischemic heart model [182]. The evidence showed that the propagation capacity of MSCs in vivo could also be improved by appropriate preconditioning culture of MSCs. However, given the small number of studies, the efficiency of strategies to improve MSC proliferation are still limited.

This could be due to the fact that the understanding of MSC behavior in vivo is not well-understood and the need to maintain MSC survival and migration in vivo are already major issues, making it more challenging to target the key factors that directly affect MSC proliferation. Further investigation is warranted to explore the mechanism that can be used to efficiently enhance MSC survival, migration and proliferation in therapeutic application.

Although transdifferentiation of MSCs into endothelial cells in both in vitro and in vivo conditions have been reported in some publications [183–187], the transdifferentiation process requires particular conditions, although the requirements are still unclear. In the commentary by Crisan (2013), the author discussed the results of the study by Corotchi and colleagues who evaluated the transdifferentiation of MSCs from Wharton's jelly into endothelial cells [188] and who suggested the transdifferentiation potential of MSCs into endothelial cells, although the transdifferentiation remains controversial [185].

6.3 Host immune response

MSCs have low expression of the major histocompatibility complex (MHC) class I, no MHC class II, and low expression of costimulatory molecules (such as CD40, CD40 ligand, B7-1 and B7-2), which help MSCs to suppress the acceleration of T cells from the host immune system and inhibit NK cells from the host immune system during transplantation [158, 189, 190]. Even though MSCs possess great immunomodulation properties, such as being able to self-downregulate MHC-I after immune stimulation to maintain low immunogenicity [190], the transplantation of allogenic MSCs still face rejection by the host immune system. Notably, T cell activation and proliferation were only found following the intracranial injection of allogenic MSCs but not autologous MSCs [191, 192]. It has been widely discussed whether MSCs are as immune-privileged as thought and that mismatched MSCs are the major cause of host rejection in MSC transplantation [189, 193]. Studies in mice have shown that grafting of allogeneic MSCs mismatched in MHC-I and II were actively rejected by the host immune system, which was shown by the increase in T cells and NK cells in the host mice [194]. Different mechanisms leading to donor cell rejection have been well-described, including direct, indirect, or semi-direct allorecognition of MHC-mismatched MSCs [189, 195].

In direct allorecognition, MHC molecules on donor MSCs are recognized directly by host T cells, which activates T cells and accelerates immune responses to MSCs [189]. Indirect allorecognition of the host immune system works by the recognition of MHC peptides shed from the surface of MSCs by antigen-presenting cells (APCs) or dendritic cells (DCs), which then transfer the signals to activate T cells and trigger the immune system [189]. Semi-direct allorecognition includes the antigen-presenting properties of MHC molecules recognized by APCs or DCs, which will present them directly to T cells to activate the rejection response [195, 196]. Thus, suppressing MHC mismatch recognition appears to be the key to improving MSC survival during allogeneic graft. The immunosuppressive ability of MSCs is also helpful in GvHD [197–199]. Improving the immunomodulation ability of MSCs not only supports the cells to survive in the in vivo microenvironment but also enhances the cell capacity by improving the efficiency of MSCs in regulating the host immune response. Changes in culture condition and treatment have been shown to be effective in improving MSC ability to suppress the immune system [159, 200]. However, the mix of literature demonstrating MSC-mediated immune rejection and privilege might suggest that different MSC lines and individual host profiles may be correlating in performing either a strong or weak host immune response. Therefore, to predict the outcomes of a host immune response during MSC transplantation, a thorough examination is required to identify the correlation between host immune system reactions in different combinations of allogenic MSCs and host profiles.

7 How to overcome the challenges

In recent decades, the increase of aging populations has been met with the emerging incidence of vascular diseases, including ischemia, MI and stroke. The disease pathogenesis coming from blood flow restriction has become a serious health condition that results in decreased patient quality of life and increased burden on the health system. The restriction of blood supply critically changes the tissue function as the demands for nutrition and oxygen to maintain normal cell and tissue activity are not met, leading to complications including cell pathway alterations and immune response alterations, which may either support the tissue to adapt to the new conditions or may negatively accelerate apoptosis and necrosis as a consequence. Understanding the changes that occur within different cells at the ischemic sites will be beneficial in identifying the potential risk that may take place and addressing the promising targets that can be used to best support the treatment. The requirement to quickly regenerate the blood supply is critical to prevent severe and irreversible damage associated with ischemic events. Several therapies have been proposed and put into clinical trials, aiming to either prevent the damage of restricted blood flow or to enforce the recovery after the ischemic events. However, no current treatment has appeared to be effective in treating ischemic conditions, and most of them are temporary. Thus, there is

a great need for new therapies that can safely improve and enhance the recovery after ischemic injury.

MSCs have recently been used in several applications due to their ability to self-renew and expand, differentiate into different cell types, home to target tissues, and exert immune modulation. MSCs have been effectively used as a cell therapy in different types of vascular diseases, including peripheral arterial diseases, ischemic heart, ischemic brain, and stroke [201, 202]. However, inconsistent clinical outcomes and limited data on post-treatment improvements have prevented large-scale trials of MSC transplantation and prevented MSCs to be further investigated as a potential cell therapy. These challenges not only come from the changes between in vitro and in vivo environments but are also derived from the alterations/modifications that occur at the ischemic sites, where nutrition and oxygen supply become short and host immune response is accelerated. Among the factors, nutrition and oxygen deprivation and host immune responses, as well as low adaptation, are the key factors that affect the survival of MSCs. The low homing potential of MSCs comes from the pre-treatment culture of the cells and the low adaptation in the in vivo environment, which causes the MSCs to express low chemotactic factors [115, 116]. Generally, there has not been much evidence in the proliferation capacity of MSCs in vivo after transplantation. Even though MSC proliferation may lead to concerns related to tumor formation, the ability to maintain MSC characteristics with controlled growth and proliferation may be a new aspect for future research of MSC-based therapy. Therefore, understanding of the challenges of MSCs in vivo is important to address where improvement can be made for future research.

In fact, several studies have proposed different modifications that can be applied to improve MSC characteristics to support the in vivo adaptation of MSCs, which have shown effective improvement in different models [12, 166, 203]. The improvement of MSC characteristics should be in coordination with other factors, such as the local environment conditions at the ischemic sites and the patient's health profile. For example, the combination of MSC therapy with other cell types and treatments may be required in patients with different conditions, such as hypertension, obesity, diabetes or cancer, as the associated conditions may lower the efficacy of MSC treatment due to microenvironment changes. Thus, to achieve better outcomes with using MSC transplantation in ischemic conditions, further investigation of MSC-based therapy in various patients with different health problems is required to fully evaluate the efficacy of MSC transplantation for large-scale applications.

8 Conclusion

MSC transplantation provides a promising therapy for ischemic disease treatment. MSCs not only participate in angiogenesis but also promote the regeneration process of injured tissues by various mechanisms. However, the treatment efficacy of ischemic diseases by MSC transplantation has been met with challenges related to the proliferation as well as differentiation of MSCs at injured tissues. Some novel strategies to overcome these challenges have been suggested in recent studies that promote the treatment efficacy in animal models. Therefore, more preclinical trials and clinical trials using these new strategies are essential to evaluate and optimize this therapy for ischemic diseases.

Acknowledgements All authors equally contributed in this work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

Ethical statement There are no animal experiments carried out for this article.

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