Stem cell mechanism of action in neuroplasticity after stroke

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Stroke is the second leading cause of death in the world that may cause a permanent disability. In recent years, stem cell therapy for ischemic stroke has made great progress. Currently, there have been several studies on stem cell therapy in stroke that provide benefits for neuroplasticity using various cell sources and transplant procedures, but the mechanisms are still controversial. Therefore, the aim of this review was to analyze the various mechanisms of stem cells in enhancing neuroplasticity. Stem cell mechanism that increases neuroplasticity can be through various signaling pathways, namely the endothelin-3/EDNRB, SRY-related HMG-box 10 (SOX10), Wnt/β-Catenin, GF1R+ and C-X-C chemokine receptor type 4 (CXCR4) pathways, and through neurotrophic factor (NT-3), specialized extracellular matrix perineuronal net (ECM PNN), microRNA (miR-133b and Ex-miR-17-92+ cluster), as well as modulation of proteins namely phosphorylated collapsin response mediator protein 2 (CRMP2) and increased regulation of phosphorylated-cAMP response element-binding protein (p-CREB), growth associated protein 43 (GAP-43), and synaptophysin (SYP). In conclusion, various signaling pathways and other factors contribute in neuroplasticity increase due to stem cell therapy in stroke.

Keywords:
endothelin-3, micro RNA, neurotrophic factor, signaling pathways

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Introduction

Stroke is the world's second most common cause of death, which may cause a permanent disability that affect the productivity of the sufferers. In addition to have an economic and social impact on the community, stroke also adds to the burden of health financing, and is one of the highest-cost disease [1]. Therefore, success in restoring functional abilities may decrease the cost of stroke treatment. Standard treatment for ischemic stroke is acute reperfusion therapy by giving a thrombolytic drug, or by performing thrombectomy. These treatments may give satisfactory results when are given within three hours of symptom onset. However, early treatment is not always possible to prevent disabilities. Moreover, for hemorrhagic stroke, attempts to stop the hemorrhage are not always satisfactory, and may cause disabilities as the side effects [2]. Therefore, treatments to reduce the disabilities are highly needed. One of the various treatments is a treatment using stem cells.

The use of stem cells in the treatment of ischemic stroke in recent years has made a great improvement. Stem cell types that have been used in human and animal models include stem cells from adipose tissue and umbilical cord blood-derived mesenchymal stem cells [3], amniotic epithelial cells [4], amniotic membrane-derived mesenchymal stem cells, olfactory stem cells, pluripotent adult cells, bone marrow endothelial progenitor cells, electrically stimulated neuronal progenitor cells, or induced pluripotent stem cells (iPSCs) [5]. Stem cells in ischemic stroke induce neuroprotection [4], restoration of motor function [6,7], neurological improvement [6], decrease in infarct volume [8], increased angiogenesis [8,9], and clinical functional recovery [8].

Recovery of functional ability after stroke is mediated by neuroplasticity, which involves structural changes and functional adaptations in reaction to intrinsic or external stimuli in the brain. Neurogenesis, synaptogenesis, and neuroprotection are all linked to neuroplasticity processes. During inflammation, edema, metabolic abnormalities, apoptosis, and nerve fiber loss, which begin shortly after an ischemic event, neuroplasticity in stroke occurs [10]. Currently, some studies on stem cell therapy in stroke have been done, which provided benefits for neuroplasticity [11–20]. Those studies used various cell sources, transplant procedures, and pathway mechanisms to promote neuroplasticity. Neuroplasticity is a complicated phenomenon, which mechanism is still a mystery and relies on the fusion of existing synaptic pathways to form new connections, especially in stroke treatment with stem cells. Therefore, we conducted a review on the use of stem cells in stroke, and discussed the mechanisms by which stem cells enhance neuroplasticity after stroke, by explaining neuroplasticity regulation by various pathways.

Various studies on stem cell therapy in stroke

Various studies on stem cell therapy in stroke were listed in Table 1 [11–20], and involved path ways such as endothelin-3/EDNRB, SOX10, and Wnt/β-Catenin. Further, another neuroplasticity promotion was by upregulation of neutrophin-3 (NT-3), by brake down-regulation of perineuronal net (PNN), by microRNA-133b (miR-133b), by microRNA 17-92+ containing exosomes (Ex-miR-17-92+) cluster, by insulin-like growth factor 1 receptor (IGF1R) and CXCR4 signaling pathway, by phosphorylation modulation of collapsin response mediator protein 2 (CRMP2), by up-regulation of phosphorylated-cAMP response element-binding protein (p-CREB), synaptophysin (SYP), and growth associated protein 43 (GAP-43).

A study showed that exosomes from multipotent mesenchymal stromal cells enhanced stroke neurofunctional improvement in middle cerebral artery occlusion (MCAO) rats [21]. Further, combined cell therapy and rehabilitation improved

Table 1 Mechanism of stem cell in the treatment of stroke [11–20]

Reference	Experimental model	Cell/exosome source	Transplantation procedure	Mechanism
[11]	Rat NHI	IGF1R+ hDSCs	7 d post NHI, stereotaxically injection	Autocrine regulatory expression of IGF1R
[12]	Rat MCAO	BMSCs	immediately after MCAO, Intra-arterially injection	the phosphorylated modulation of CRMP2
[13]	Rat MCAO	Exosomes from BMSCs	1 d post MCAO, lateral ventricles injection	Pathway for SOX10, Wnt/β-catenin, and endothelin-3/EDNRB
[14]	Mice MCAO	Human neural stem cells (H9 derived)	Acute phase, ipsilateral cortex (intracerebral injection)	NA
[15]	Rat MCAO	hESC-MSC-CM	Once: 1 hour after MCAO Three times: one, twenty-four, and forty-eight hours after MCAO. Lateral ventricle injection	Growth associated protein 43 (GAP-43), synaptophysin (SYP), and p-CREB are all upregulated
[16]	Rat with cerebral cortex ischemia	BMMCs	1 d post-surgery, left jugular vein injection	upregulation of NT-3
[17]	Mice MCAO	hBM-MSCs	3,5 h post MCAO, intravenous injection	downregulation of PNN
[18]	Rat MCAO	Exosomes from MSCs	2 h post MCAO, intra-arterially injection	miR-133b
[19]	Rat MCAO	Exo-miR-17-92^{+} from MSCs	2 h post MCAO, intravenous injection	Ex-miR-17-92+ cluster
[20]	Rat reperfusion model	IGF1R+ hDSCs	7 d post surgery, intracerebral	IGF1R+ and CXCR4 signaling pathway

BMMCs, bone marrow mononuclear cells; BMSCs, bone marrow stromal cells; CRMP2, Collapsin response mediator protein 2; d, day; GAP43, Growth associated protein 43; GSK3β, Glycogen synthase kinase 3 Beta; hBM-MSCs, human bone marrow mesenchymal stromal cells; hDSCs, human dental pulp derived-mesenchymal stem cells; hESC-MSC-CM, human embryonic stem cell-derived mesenchymal stromal cells-conditioned medium; IGF1R, insulin-like growth factor 1 receptor; MCAO, middle cerebral artery occlusion; mTOR, mechanistic target of rapamycin; NA, not available; NHI, neonatal hypoxia-ischemia; PTEN, phosphatase and tensin homolog; SYP, synaptophysin.

behavioral outcomes in stroke rats [22]. A human study showed that autologous mesenchymal stem cells (MSCs) were feasible and safe in patients with chronic major stroke for improvement of foot motor function and functional recovery [6]. An animal study showed that embryonic stem cell-derived MSCs represented a robust therapeutic approach in MCAO model rats to reduce ischemia of the brain, and it was possible that regulation of synaptic plasticity played a role in this result [15]. Neuroplasticity can occur through various pathways, including: endothelin-3/EDNRB, SOX10, and Wnt/β-Catenin pathways [13], upregulation of neutrophin-3 [16], down regulation of PNN [17], through miR-133b [18], Ex-miR-17-19+ cluster [19], GF1R and CXCR4 signaling pathway [20], the phosphorylation modulation of CRMP2 [12], and up regulation of p-CREB, SYP, and GAP-43 [15].

Neuroplasticity regulation byEndothelin-3/ EDNRB, SOX10, and Wnt/β-Catenin pathways

Wei *et al.* [13] conducted an *in vivo* study; they gave exosomes enriched in Zeb2/Axin2, which were generated from bone marrow stromal cells (BMSCs) that had been transfected with Zeb2/Axin2 over expression plasmids, to MCAO rat models, with the aim of investigating the effects of Zeb2/Axin-enriched exosomes on functional recovery after a stroke, neurogenesis, and neuroplasticity. After MCAO all rat were placed into three groups at random: the group of MCAO (control), MCAO + BMSC-exosomes (exosome control, exo-ctrl), and MCAO + Zeb2/Axin2-enriched exosomes (exo-Zeb2/Axin2). For all group, phosphate-buffered saline (PBS) or exosomes were administered to the MCAO rat model from the lateral ventricle. The control, exo-ctrl, and exo-Zeb2/Axin2group were injected with PBS, BMSC – exosomes, and exosomes enriched in Zeb2/Axin2 consecutively. The spatial memory and brain function of the MCAO rats were improved dramatically after treatment with Zeb2/Axin2-enriched BMSC exosomes. There was upregulation in nerve growth factor expression and an increased number of neurons in the hippocampus, subventricular zone (SVZ), and cortical regions. Synaptic remodeling in the ischemic boundary zone was promoted by exosomes that were enriched in Zeb2/Axin2 by increasing the amount of synapses, while reversing neurofilament phosphorylation (SMI-31) and synaptophysin (SYN) loss in axons, which was induced by ischemia, and therefore reducing axon damage and increasing proliferation of synapse [13].

Wei *et al.* [13] investigated Exo-Zeb2/Axin2 effect on dendrite or neurite growth in an *in vitro* study using culture method with oxygen and glucose deprivation (OGD) on primary cultured neurons (PCN). In order to simulate ischemia conditions *in vitro,* the OGD model was used, and exo-Zeb2/Axin2 or Exo-ctrl were used to treat PCN. There were four groups, control group (PCN without OGD), group PCN with OGD (OGD group), PCN with OGD+exo-ctrl treatment (OGD+exo-ctrl group), and PCN with OGD+exo-Zeb2/Axin2 treatment (OGD+exo-Zeb2/Axin2 group). After treatment for 24 h, a monoclonal anti-MAP2 antibody immunofluorescence method was used to stain PCN cultures to visualize microtubule-associated protein 2 (MAP2). Laser scanning confocal microscopy was used to capture images, and Image-Pro plus 6.0 was used to count PCNs and analyze neurite outgrowth. Western blot was used to analyze protein expression of endothelin-3/EDNRB, SOX10, and Wnt/β-Catenin. The results showed that the OGD + exo-Zeb2/Axin2 treatment showed increase in glial neuron branching and elongation in comparison to the OGD + exo-ctrl group. When comparing OGD to control group, the OGD had lower levels of Wnt, β-catenin, and SOX10, while endothelin-3 and EDNRB expression was increased. Both exosome treatments showed increase in Wnt, β-catenin, and SOX10 compared to OGD group. Further, OGD+Exo-Zeb2/Axin2 group showed an increase in Wnt, β-catenin, and SOX10, while endothelin-3 and EDNRB showed a decrease when compared to OGD and OGD+Exo-ctrl groups. These findings imply that treatments with exosomes either with or without Zeb2/Axin2 caused up-regulation of Wnt/β-catenin, and SOX10, but down-regulation of endothelin-3 and EDNRB pathways. Finally, BMSC-derived exosomes, which were enriched in Zeb2/Axin2, increased neurite remodeling and synaptic plasticity in the ischemic brain [13].

Neuroplasticity promotion by upregulation of Neutrophin-3

de Fátima Dos Santos Sampaio *et al.* [16] conducted an *in vivo* study in rat model of cerebral cortex ischemia with intravenous administration of bone marrow mononuclear cell (BMMC), and aimed to examine the effects of BMMC treatment on cortico-striatal and cortico-cortical pathway remodeling, as well as neuroprotection and motor function. The rat model was allocated into three groups, naive (healthy and was not subjected to surgery), ischemic control (isc control) and ischemic BMMC (isc BMMC) groups. Animals in the isc control and isc BMMC groups were both subjected to unilateral focal cortical ischemia followed by administration of vehicle and vehicle + BMMC treatment respectively. Analyses of gene expressions were conducted by RT-PCR for pro-apoptotic factors, Bax and Caspase-3 (Casp-3), and anti-apoptotic Bcl-2. In addition, expressions of GAP-43, which played a role in neuronal plasticity, brain derived neurotrophic factor (BDNF), and Neurotropin-3 (NT-3) were measured. The results showed that Bax, as apoptosis-related gene, exhibited a significant reduction in isc BMMC group. Expression of the Bcl-2 gene in the isc control group showed up-regulation of Bcl-2 compared to isc BMMC and healthy groups. There was no significant difference in Bcl-2 expression in the healthy and the isc BMMC groups. However, the isc BMMC group showed dowregulation in Casp-3 expression. Further, the GAP-43 gene expression level did not differ substantially between the groups, although there was a slight reduction in its expression level in the isc control and isc BMMC groups. Indeed, BDNF demonstrated the same in all groups. Further, the isc control had the highest upregulation of NT-3 expression level, compared to healthy rats. While, isc BMMC rats showed an increase NT-3 expression level compared to the healthy one, but lower than the isc control group [16].

So, the results of this study indicated up-regulation of the NT-3 gene compared with healthy group, which appears to be associated with neuronal survival. Ischemic animals that were not treated with BMMC showed increased Bcl-2 expression that inhibited the process of apoptosis. Theoretically, to reduce the lesions' apoptotic effect, in comparison to vehicle-treated isc control rats, BMMC-treated animals should show overexpression of Bcl-2.However, at mRNA expression level the expression was comparable to those found in animals that are healthy, accompanied by a decreased trend in Casp-3 modulation. In morphology analysis, animals with intravenous BMMC transplantation, after 60 days of ischemia, showed increased axonal fibers in the ipsilateral cerebral cortex and in the contralateral neostriatum compared with isc control group; moreover, it also improved neural connectivity and motor skills. This is the evidence of NT-3 gene that within the seven-day therapeutic window after ischemia is involved, and might be significant for long-term neural plasticity when combined with BMMC treatment. Thus, the findings of this study imply that during the acute phase of ischemia, in response to injury, NT-3 is up-regulated, which causes Bcl-2 activation to balance Bax expression

and reduce apoptotic effect, which may promote neuroplasticity after stroke. In comparison to the isc control groups, treatment with BMMCs produced sprouting of axon, critical neuronal circuitry reconnection, and improved performance of motor function, in long term [16].

Neuroplasticity brake downregulation of PNN

Sammali *et al.* [17] conducted a study in mice model of transient occlusion of middle cerebral artery (TOMCA) and administered stem cell therapy using human bone marrow mesenchymal stromal cell (hBM-MSC). The aims of this study were to examine long-term histopathological and functional impact of good manufacturing practice (GMP) compliant hBM-MSC administration on TOMCA mice model, and to understand the effect of hBM-MSC on parvalbumin (PV)-positive and PNN-positive neuronal expression as markers of neuronal plasticity in TOMCA mice model. Animals were divided into 3 groups, sham, TOMCA- phosphate buffered saline (PBS), and TOMCA- hBM-MSC. TOMCA-PBS and TOMCA- hBM-MSC mice were injected with PBS and hBM-MSC, subsequently *via* intravenous (IV), 3.5 h after stroke. Functional examination and fluorescence microscopy was performed to count the number of PV-positive and PNN neurons in brain. Vascular density and neurogenesis were measured by immunohistochemistry [17].

The results showed that in TOMCA mice model, therapy with hBM-MSC decreased sensory impairments, caused better cognitive and exploratory capabilities, and increased neuron survival in the perilesional region. Moreover, hBM-MSCs enhanced vascular density and neurogenesis in SVZ, and decreased PV-positive neurons surrounded by PNN. PNN is a distinct extracellular matrix (ECM) that surrounds the cell body, early axonal segments, and apical dendrites of certain neurons and is made up of chondroitin sulfate proteoglycans (CSPs). PNN preferentially surrounds PV expressing GABAergic inter-neurons, which are fast-spiking interneurons that play a key role in brain circuit activity control. When the PNN is reduced, reduced PNN facilitates restorative plasticity through circuit remodeling in the perilesional cortex; and hBM-MSCs was shown to reduce PNN. In this study, protection was linked to a decrease in the fraction of PV-positive inter-neurons surrounded by PNN. PNNs are referred to be a 'plasticity brake' since their absence encourages tissue remodeling in adults. As a result, the finding of a lower percentage of PNN-encircled inter-neurons in TOMCA – hBM-MSC group implies that hBM-MSC treatment can boost tissue remodeling and functional recovery. Therefore, this study showed that IV GMP-compliant hBM-MSC improved functional recovery in stroke mice by promoting neural protection and neuroplasticity [17].

Neuroplasticity promotion by miR-133b

Xin *et al.* [18] aimed to investigate whether miR-133b over expressed exosomes when compared to natural MSC exosomes (control) demonstrated a significant therapeutic impact. The *in vitro* research aimed to culture MSCs and isolate their exosomes, and the exosomes were used for treatment of primary astrocytes. MSCs were isolated from the bone marrow of Wistar rats, cultured, and then engineered using a lentiviral vector. From this process, two types of MSCs were obtained, namely microRNA-133b$^+$over-expressingMSCs (miR-133b$^+$MSCs) and natural MSCs (control MSCs), which were used to produce exosomes, namely miR-133b$^+$MSCexosomes (miR-133b$^+$-Ex) and control MSC exomes (Con Ex) respectively. To mimic ischemic conditions in an *in vitro*, an oxygen and glucose deficient (OGD) culture system was used, while in vivo research was conducted on MCAO rat model. After MCAO induction, twenty-four hours later, rats were randomly assigned to intra-arterial (IA) Con-Ex or miR-133b$^+$-Ex group and 0.5 ml PBS (control group). In addition, immunohistochemistry and Western blot were used to evaluate functional recovery [18].

The findings revealed that in vivo treatment with miR-133b$^+$-Ex increased neurologic outcomes, i.e. synaptic plasticity and neurite remodeling in the ischemia boundary zone (IBZ), and increased exosomes in IBZ, compared to Con Ex. In vitro, miR-133b$^+$-Ex group showed increased exosome release from OGD primary astrocyte culture, and down-regulation of protein levels of Rab9 effector protein with kelch motifs (RABEPK). Downregulation of RABEPK expression after miR-133b$^+$-Ex treatment might alter astrocyte exosome release. Thus, it can be concluded that miR-133b$^+$-Ex treatment, which was delivered through IA, improves neural plasticity and functional recovery following a stroke. It is supposed that high levels of mature miR-133b containing exosomes promote the release of exosomes from astrocytes through down regulation of RABEPK [18].

Neuroplasticity enhancement by Ex-miR-17-92+ cluster

A study by Xin *et al.* [19] aimed to investigate exosomes, which enriched in miR-17-92+ clusters (miR-17-92+Exo), recovered from MSCs that were transvected with a miR-17-92+cluster containing plasmid. The miR-17-92+Exo were compared to exoxomes from natural MSC (Con-Exo) to see whether miR-17-92+Exo improved neurological recovery in stroke. The stroke model was MCAO mice model. Twenty-four hours after stroke induction, Con-Exo, miR-17-92+Exo, or liposomes were administered intravenously. On day 28 after MCAO the mice were sacrificed. Histochemical, immunohistochemical, and Golgi-Cox staining method was performed to examine myelin, synaptic, axonal and dendritic changes. PTEN and mTOR, Akt, as well as GSK-3βwere detected using Western blot of total protein that was isolated from frozen brain IBZ area [19]. The results showed that when compared to liposomes, both exosome therapies increased considerably the density of axon-myelin bundles, and significantly increased synaptophysin immunoreactivity in IBZ. However, miR-17-92+Exo showed better result compared to Con-Exo. Dendritic plasticity that was analyzed by Golgi silver impregnation showed that both exosome therapies boosted primary and secondary neurite branching and spinal density substantially compared to liposome treatment, and miR-17-92+Exo showed better result compared to Con-Exo. Both exosome treatments showed the presence of newly generated neurons, oligodendrocyte progenitor cells and mature oligodendrocytes that were significantly increased compared to liposome treatment, butmiR-17-92 +Exo showed better result compared to Con-Exo in neurogenesis and oligodendrogenesis. Using Western blot, it was shown that when compared to liposomes, both exosome therapies dramatically reduced PTEN levels and boosted mTOR, and Akt as well as GSK-3β phosphorylation. Moreover, in IBZ neurons, phosphorilated GSK3β (p-GSK-3β) was found. Thus exosome therapies target PTEN and its downstream pathways. Inactivation of GSK-3β in neurons is due to PTEN down regulation, which causes activation of the downstream signaling pathway PI3K/Akt/mTOR. In the case of GSK-3β inactivation, it plays a crucial role in axon regeneration and supports axonal development and recovery in the CNS. It can be concluded that cluster of miR-17-92 boost oligodendrogenesis, neurogenesis, and brain plasticity and dramatically improve the therapeutic efficacy of exosomes that are used to treat stroke. When PTEN expression is down regulated, PTEN downstream proteins such as mTOR and Akt are activated, while GSK-3 activity is inhibited, which are part of the molecular basis for neurological restorative alterations [19].

Neuroplasticity enhancement by IGF1R+ and CXCR4 signaling pathways

Lee HT *et al.* [20] conducted investigations in vitro and in vivo on stem cells in stroke. The in vitro study aimed to investigate the molecular mechanism of incubated human dental pulp stem cells (hDSC) in a culture system, which could maintain enhanced IGF-1R expression in correlation to long-term self-renewal capability. While the in vivo study hypothesized that cross-talk between signaling pathways, which involved IGF-1R and CXCR4, in hDSCs with increased expression of IGF-1R (hDSCs-IGF-1R) might promote neurite repair and neuroplasticity in hDSCs-IGF-1R transplanted stroke models through autocrine/paracrine activation of survival signals. Sources of stem cells (hDSCs-IGF-1R) were cultured in2 percent human cord-blood serum (huUCS) or 10% FCS containing DMEM-LG medium. To calculate the concentrations of IGF-1 and platelet-derived growth factor BB (PDGF-BB) in FCS and huUCS, ELISA method was performed. In the in vivo study, the experimental animals were rat model with an ischemic brain. One week after brain ischemia, animals were injected stereotaxically. Control animals were only given phosphate buffered saline (PBS), while treated animals got hDSCs-IGF-1R. For the blocking experiment, twice weekly for two weeks, CXCR4 was neutralized byCXCR4 inhibitory antibody (CXCR4-Ab) intraperitoneal (IP) injection into hDSCs-IGF-1R-treated animals. In addition, hDSCs-IGF-1R implanted rats were given IGF-1R inhibitor (picropodopyllin [PPP]) 20 mg/kg/dayIP for three days. Molecular studies were carried out by immunohistochemistry, Western blot, RT-PCR, and microPET scanning [20].

The results showed that when hDSCs-IGF-1R cells were grown in huUCS, IGF-1R expression could be maintained. Cytokine arrays indicated that the levels of several growth factors in huUCS culture were higher, including IGF-1 and PDGF-BB, compared to FCS. These cytokines influenced signaling in hDSCs-IGF-1Rand might help with engraftment during the transplant process. Key aspects of signaling such as Giα2, Gβ, and phosphotyrosine (pY) were immunoprecipitated from IGF-1and/or stromal cell-derived factor 1 alpha (SDF-1) treated lysed cells using

anti-CXCR4, anti-IGF-1R, or control IgG, then evaluated using Western blot to look for potential connections between IGF-1R and CXCR4 as well as their signaling pathways. When hDSCs-IGF-1R cells were treated with either IGF-1or SDF-1 or both, IGF-1R or CXCR4 immunoprecipitation (IP) that was followed by immunoblotting of IGF-1R or CXCR4 revealed a substantial increase in the active form of Giα2 and Gβ, IGF-1R, CXCR4, and pY expression relative to controls. CXCR4-Ab and/or picropodopyllin (PPP) were applied to hDSCs-IGF-1R to further test whether downstream signaling stimulation of ERK1/2 and Akt via receptor cross-talk between IGF-1R and CXCR4 occurs. Interestingly, PPP inhibits IGF-1 and SDF-1 signaling in hDSCs-IGF-1R, resulting in lower levels of p-ERK1/2 and p-Akt. Similarly, the rise in p-ERK1/2 and p-Akt that was caused by SDF-1 and/or IGF1 in hDSCs-IGF-1R was inhibited by pre-treatment with CXCR4-Ab. Therefore, these findings suggest that CXCR4 is required for IGF-1R signaling pathway activation and vice versa. In particular, the interplay and synergistic activation of the two types of receptors of the two signaling pathways in hDSCs-IGF-1R was evidenced by a cross-talk between IGF-1R/IGF-1 and CXCR4/SDF-1 signaling pathways in both directions. In a rat stroke model, interplay between CXCR4 and IGF1R was shown to induce neuroplasticity in rats, which received hDSCs-IGF-1R, i.e. boosting the neurological system to increase metabolic activity of glucose, angiogenesis, as well as anti-inflammatory properties. Therefore, in the hUCS culture system, the presence of PDGF aids in the maintenance of hDSC IGF1R expression. Thus, it can be concluded that hDSCs-IGF-1R implantation provides a combined input from IGF1R and CXCR4 signaling that improves neuroplasticity [20].

Neuroplasticity promotion by the phosphorylation modulation of CRMP2

He *et al.* [12] conducted a study to investigate the role of CRMP2 in rats with ischemia of the brain that was treated with bone marrow stem cells (BMSCs). The femur, tibiae, and humeral bones were used to isolate BMSCs. Experimental animals were grouped into 3 groups, sham, MCAO, and MCAO-BMSC. In the MCAO-BMSC group, MCAO rats were given BMSC in phosphate buffered saline (PBS) suspension intra-arterially (IA). In the MCAO group, MCAO rats got PBS IA. In the sham group, rats simply had mock surgery and PBS IA. All IA injections were carried out right after the model was created. To measure neurological conditions, the modified neurological severity score (mNSS) was utilized at 3, 7, and 14 days postsurgery (dpo), and at 14 dpo, electrophysiological evaluation was performed. Furthermore, brain tissue was obtained for Western blot, RT-PCR, and immunohistochemistry examination after all rats were killed [12].

The results showed that BMSC treatment improved neurobehavioral skills that had been impaired by ischemic brain injury; in addition, there was an increase in SYP and GAP43 levels. In MCAO-BMSC rats, the levels of phosphorylated CRMP2 (p-CRMP2) and phosphorylation-mediated proteins including Cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 Beta (GSK3β) were significantly reduced. BMSCs might activate endogenous signaling pathways through neurotropin secretion, to reduce GSK3β expression, thereby ultimately downregulating CRMP2 phosphorylation. As a result, p-CRMP2 decreased levels encouraged neurite and axonal development. Furthermore, in the MCAO-BMSC group, GSK3β-mediated factors such as neurotrophins and signaling factors were all considerably elevated. The neurotrophins were ciliary neurothropic factor (CNTF), transforming growth factor beta 1 (TGFβ1), platelet derived growth factor subunit B (PDGFB), brain-derived neurothropic factor (BDNF) and nerve growth factor (NGF), while the signaling factors were protein kinase C (PKC) signal tranducer, phosphatidylinositol-3-kinase (PI3K), mitogen-activated protein kinase (MEK), activator of transcription 3 (Stat3), and sarcoma proto-oncogene (Src). On the basis of these findings, it can be concluded that the phosphorylation modulation of CRMP2 may be linked to the neuroplasticity due to BMSC effects on cerebral ischemia. CRMP2 or dihydropyrimidinase-like protein-2 (DPYSL2) is a protein that is found in the central nervous system that is widely expressed, particularly in developing neurons. CRMP2 influences microtubule dynamics, neurite development, and retraction, all of which are important in axonal elongation [12].

Neuroplasticity Enhancement via up-regulation of p-CREB, SYP, and GAP-43

A stroke stem cell research, which was conducted by Asgari Taei *et al.* [15], aimed to look into the impact of human embryonic stem cell-derived MSC conditioned medium (hESC-MSC-CM) on experimental ischemic stroke. Wistar rats were subjected to ischemic stroke by a 90 min MCAO. Animals were randomly assigned to

five groups i.e.: sham group, which received sham surgery without MCAO+ intra-cerebro-ventricular (ICV) cannulation into the left lateral ventricle (ICV-C-LLV), MCAO + normal saline (NS) via ICV-C-LLV (control group), MCAO + DMEM via ICV-C-LLV at 1, 24, and 48 h after MCAO induction (vehicle group), MCAO + single hESC-MSC-CM via ICV-C-LLV 1 h after MCAO induction (CM-1 group), and MCAO + three times hESC-MSC-CM via ICV-C-LLV at 1, 24, and 48 h after MCAO induction (CM-3 group). After MCAO, on days 1, 3, and 7, neurological function was assessed using the mNSS and Bederson test. On days three and seven, the infarct volume and the amount of edema in the brain were measured. Seven days following surgery, the CREB, p-CREB, GAP-43, and SYP levels were measured in cortical tissue in the peri-ischemic area [15].

The findings revealed that on day 7, when compared to control and vehicle group, CM-1 group demonstrated a significant reduction in cortical and total infarct volume, but not infarct volume in the striatum. In comparison to the control group, CM-3 group significantly reduced total, cortical, and striatal infarct volume. CM-1 and CM-3 groups both reduced cerebral edema when compared to the control group. SYP and GAP-43 protein expression levels in the peri-ischemic brain were also examined using IHC labeling on the seventh post-MCAO day. When compared to the sham group, the SYP and GAP-43 positive cells in the control and vehicle groups were lower. When compared to control and vehicle group, the CM-1 group showed no effect on SYP and GAP-43positive cells. However, Compared to control and vehicle group, the number of SYP and GAP-43 positive cells in the cortical tissue of peri-ischemic area were increased in the CM-3 group. In addition, SYP, GAP-43, CREB, and p-CREB protein levels in peri-ischemic cortical tissue were also examined by Western blot analysis. On the seventh day after MCAO, SYP and GAP-43 protein levels, and CREB – p-CREB/CREB ratios were all reduced dramatically in the peri-ischemic area of cortex in control and vehicle group. GAP-43, which is a cytoplasmic membrane phosphoprotein, is found in axonal growth cones and linked to new axon growth, search for routes in branching axon, synaptogenesis, and remodeling of synapse, and is regarded as a marker of axonal germination. SYP is a protein, which is found in synaptic vesicles, and is involved in synaptic plasticity and synaptogenesis. On the other hand, p-CREB reduces motor impairments after a stroke by increasing excitability of neurons in the circuits near the place of stroke. Three injections of hESC-MSC-CM via ICV-C-LLV, on the other hand, returned p-CREB, SYP, and GAP-43 levels to a normal state. As a result, overexpression of p-CREB, SYP, and GAP-43 in the cortex of the peri-infarct area might be involved in hESC-MSC-CM neuroprotection via axonal development and synaptic plasticity [15].

Conclusion

Stem cell mechanism that increases neuroplasticity may be through various signaling pathways, namely the SOX10, endothelin-3/EDNRB and Wnt/β-Catenin pathway, GF1R+ and CXCR4 pathway, neurotrophic factor (NT-3), specialized ECM (PNN), microRNA (miR-133b and Ex-miR-17-92+ cluster) and modulation of proteins namely phosphorylated CRMP2 and increased p-CREB, SYP, and GAP-43.

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Conflicts of interest

There are no conflicts of interest.

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