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Secretome of Mesenchymal Stem Cells and Its Potential Protective Effects on Brain Pathologies

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Abstract

Previous studies have indicated that mesenchymal stem cells (MSCs) have a fundamental role in the repair and regeneration of damaged tissues. There is strong evidence showing that much of the beneficial effects of these cells are due to the secretion of bioactive molecules—besides microRNAs, hormones, and neurotrophins—with anti-inflammatory, immunoregulatory, angiogenic, and trophic effects. These factors have been reported by many studies to possess protective effects on the nervous tissue. Although the beneficial effects of the secretory factors of MSCs have been suggested for various neurological diseases, their actions on astrocytic cells are not well understood. Hence, it is important to recognize the specific effects of MSCs derived from adipose tissue, in addition to the differences presented by the secretome, depending on the source and methods of analysis. In this paper, the different sources of MSCs and their main characteristics are described, as well as the most significant advances in regeneration and protection provided by the secretome of MSCs. Also, we discuss the possible neuroprotective mechanisms of action of the MSC-derived biomolecules, with special emphasis on the effect of MSCs derived from adipose tissue and their impact on glial cells and brain pathologies.

Keywords Mesenchymal stem cells · Paracrine factors · Pathologies · Therapeutics · Secretome · Brain

Abbreviations

ASCs	Adult stem cells
AFSCs	Amniotic fluid stem cells
CNS	Central nervous system
CM-A-MSC	Conditioned medium of mesenchymal cells derived from adipose tissue
BM-MSC	Conditioned medium of mesenchymal stem cells derived from bone marrow
CM-MSCs	Conditioned medium of mesenchymal stem cells
DPSCs	Dental plug stem cells
ESCs	Embryonic stem cells
EpSCs	Epithelial stem cells
FSC	Fetal stem cells
HSC	Hematopoietic stem cells
HSC	Hepatic stem cells
HI	Hypoxic-ischemic
IPSC	Induced pluripotent stem cells
A-MSC	Mesenchymal cells derived from adipose tissue
MSCs	Mesenchymal stem cells
BM-MSC	Mesenchymal stem cells derived from bone marrow
hUCB-MSC	Mesenchymal stem cells of the human umbilical cord

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L-MSC	Mesenchymal stromal cells derived from the limbus
NSC	Neural stem cells
PSC	Pluripotent stem cells
ROS	Oxygen species
RNS	Reactive nitrogen species
SCI	Spinal cord injury
TBI	Traumatic brain injury
UCB	Umbilical cord blood
UCPVC	Umbilical cord stem cells
UCPVC	Umbilical cord stem cells
WJSC	Wharton gelatin stem cells

Introduction

Recent studies have demonstrated the ability of mesenchymal stem cells (MSCs) to differentiate into a variety of functional tissues, which are often affected by different diseases and injuries [1–3]. For this reason, MSCs have been considered as one of the most promising agents in regenerative therapy for tissue replacement and repair. Efforts have been focused on studying and applying regenerative therapy with MSC for some diseases and respiratory pathologies, hematopoietic diseases, and diabetes [4, 5] as well as other pathologies that drastically affect the central nervous system (CNS), such as traumatic injury, stroke, and spinal cord injury, among others [6, 7].

This growing interest is based on the acquisition of knowledge to understand the signals that govern the therapeutic and regenerative function of stem cells, especially in tissues without regenerative capacity [8]. The brain tissue is one of the tissues that have this characteristic because it cannot regenerate its nerve cells; in this case, many studies have utilized MSCs to protect and improve neuronal function [9], but ignoring other cells with important functions such as astrocytes. Additionally, the mechanism by which MSCs exert their effect is still not clear. It is even believed that the protective and beneficial effects of MSCs are due to the presence of biomolecules and soluble factors that these cells produce and release into their extracellular environment [10–12].

Previous studies of the secretome of MSCs have revealed a diversity of biomolecules with broad and essential functions that may have important therapeutic implications in the clinic. These biomolecules are anti-apoptotic factors, growth factors such as vascular endothelial growth factor (VEGF), β fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF-1), sphingosine-1-phosphate (S1P) [13], anti-inflammatory factors such as tumor necrosis factor β1 (TGF-β1), interleukin (IL-10) [14], and antimicrobial chemokines (the chemokine (C-X-C motif) ligand (CXCL)10, CXCL8, CXCL1, CXCL6, chemokine (C-C motif) ligand (CCL)20, and CCL5) [15], all being involved in tissue repair and wound closure [16, 17]. Furthermore, it has been found that VEGF, FGF, CCL2 (MCP1), the hepatocyte growth factor (HGF), and IGF-1 have protective functions in different tissues. Specifically, the PDGF-BB, bFGF, endothelial growth factor (EGF) [17, 18], brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) [19–21], IGF-1 [21], and glial-derived nerve growth factor (GDNF) [19] have been reported to exert protective effect specifically on the brain tissue [22, 23]. In this review, we present a general and integrated overview of current knowledge about MSCs, the mechanism of action of their bioactive molecules and/or factors, and the recent progress in the investigation of their potential regenerative and protective action on glial cells such as astrocytes, as well as on different pathologies of the CNS.

Diversity of MSC Sources: Greater Opportunity in Cell Therapy

MSCs can be found in a variety of tissues in the body, and they are the source of a natural response to traumatic onset and cell tissue regeneration. It is important to separate the embryonic stem cells (ESCs) from fetal stem cells (FSCs) and adult stem cells (ASCs), since they differ in the degree of cellular fate [24].

ESCs are pluripotent, while FSCs and ASCs are multipotent. The pluripotent state implies that it can give rise to more than one type of differentiated cell line and multipotent cells have a restrictive potency due to the acquired cell's ability to form a type of tissue depending on the germ layer from which they originated [25, 26]. ESCs come from lineages of blast cells and gastric (blastocyst, trophectoderm, and internal cell mass) [27–30]. On the other hand, it was initially thought that all the cell lineages of the ASCs came from the mesoderm embryonic tissue [27], but later on, it was observed that ASC cells differentiated into functional cells that originated from the ectoderm and the endoderm [31]. This suggests that ASCs originally formed from the three embryonic germ layers (mesoderm, ectoderm, and endoderm). ASCs have been found in almost all tissues, regardless the tissue has a great regenerative capacity or not, denoting the important role as progenitors [8].

In adult individuals, the stem cells are located in niches in the perivascular zone of all organs which are microenvironments that provide protection for different stimuli, like differentiation and apoptosis, in addition to allowing them to maintain a balance between self-renewal and differentiation [32–35]. Even if all niches share the same expression of key molecules in different tissues, each niche has a molecular identity associated with the tissue to which it is related. They even share characteristics of pericytes in terms of phenotype, marker expression, and differentiation capacity [32, 36]. This

identity is mainly reflected in the presence of membrane receptors that are specific to each tissue for extracellular matrix proteins and associated growth factors of the surrounding tissues [32, 33, 37]. The common molecules between niches can play diverse roles according to the embryonic origin and the specific functions of the specific tissues [33].

According to Bieback et al. [38], MSCs comprise a diverse population of multipotent cells capable of differentiating into an osteogenic, adipogenic, or chondrogenic lineage that makes them candidates to develop new therapeutic strategies based on the cell types, such as the treatment of mesenchymal tissue injury or support in the transplantation of hematopoietic stem cells (HSCs) [39]. It has been known that MSCs are traditionally obtained from bone marrow, but they have now been isolated from adipose tissues through lipoaspirates in higher volumes with respect to the one obtained from bone marrow and umbilical cord blood (UCB) [40]. Among the advantages of using MSCs are that they can be easily obtained, have rapid proliferation, and the ability to migrate to the site of inflammation [41]. In addition, MSCs can be isolated, cultured, and differentiated into different cell types. Some time ago, the use of MSC was focused on differentiation and transplant in different areas with some type of injury such as bone, tendon, and heart, among others [42–44]. However, this type of transplant treatments began to have adverse effects due to the ability to promote tumor growth and metastasis, problems that continue to generate concerns in the field of regenerative medicine [45]. For example, it has been reported that MSCs administered systemically can be recruited and migrated to tumors [46] and that can contribute to tumor pathogenesis through the support of tumor microenvironments [47]. It has even been recently shown that mesenchymal cells increase metastatic potential at various stages of growth progression of the primary tumor [48].

Characteristics, Therapeutic Benefits, and Controversies in the Use of Embryonic Stem Cells

The discovery of embryonic mesenchymal stem cells as a tool for regenerative tissue engineering was based on the study of teratocarcinomas. Teratocarcinomas are tumors that present differentiated tissues, sometimes totally differentiated (mainly teeth or hair). Once isolated and cultured, these cells were found to have blastocyst and pluripotent properties [49]. In fact, this opens the investigation of ESC as a tool for transgenesis and proliferation treatments. Depending on the source of ESCs , they would have different molecular characteristics . During in vitro fertilization procedures, ESC can be obtained from the blastocyst period at the time of pre-implantation, until the end of the gastrulation period [50]. As the first cells of the embryo break down, they have a greater commitment and their power varieties depend on the location within the embryo.

Besides the controversy due to the ethics involved in working with human embryos [51, 52], the in vitro issue of low embryo generation rate [53], and differentiation to obtain a heterogeneous population [53], ESCs are currently being evaluated for several purposes. These include development of a population of cardiac progenitor cells [54, 55] and sub-retinal transplantation of retinal pigment epithelium (EPI) derived from hESC in patients with macular dystrophy of stargardt and age-related macular degeneration [56, 57]. As reported with other stem cells, there are reports on the generation of tumors in transplants of ESCs, which is an obstacle for the clinical use of these cells [49, 51, 52]. It is considered that this negative outcome is related to both the pluripotency of ESCs and the fact that the blastocyst cytoplasm expresses many proteins promoting cell division and proliferation. Another difficulty to work with ESCs is that the transplanted cells do not function and integrate properly in the organs, thereby inducing immune rejection [51, 58]. These limitations have prevented the use of human embryonic cell lines and implementation of their therapeutic potential [53].

Genetic Advances for the Use of MSCs as Therapeutic Alternatives in Various Pathologies: Induced Pluripotent Stem Cells

Another type of stem cells that has been studied for use in cell therapy are the induced pluripotent stem cells (IPSCs). These cells are derived from adult somatic stem cells by a pluripotent state transformation similar to an embryo [49, 59]. This reprogramming is done by introducing specific transcription factors that are known to increase pluripotency (sex determining region Y (SRY)-box 2 (Sox2), octamer-binding transcription factor 4 (Oct4), Kruppel-like factor 4 (Klf4), and c-Myc) [51, 60, 61] and has appeared as a key advance in cell therapy due to its ability to differentiate into cells of any of the three germ layers and the non-immune rejection of cells in transplant therapies through the development of patient-specific cell therapy protocols [25, 62]. Another advantage of pluripotent cells is that they are not subject to special regulation like MSCs, but they have a high similarity at the molecular and functional level with these embryonic cells [25, 63]. However, among these similarities with ESCs are the problems associated with the capacity of pluripotency, as in some cases the formation of teratomas by the uncontrolled proliferation [62, 64, 65]. Indeed, they do not have a uniform characteristic related to the gradient of the induced pluripotency factors and genetic heterogeneity of the donor [49].

IPSCs are cells that go through a genetic modification in the conversion of pluripotency, related to the appearance of mutations or reactivation of the embryonic gene program and have a

residual epigenetic memory related to the donor's imprint, age, immunogenic specificity, and somatic as well as variations related particularly with the extraction tissue [62, 66–68]. Although pluripotent stem cells (PSCs) offer the possibility of studying a model based on human cells, more importantly they allow the study of the mechanisms of the disease in a cell that has a relevant genetic background when extracting the somatic cells of patients with diseases of unknown molecular origin or unknown pathological pathways [52, 62, 69]. For example, some research has focused on the evaluation of genetic factors and the epigenetic changes that occur during the reprogramming of IPSC to improve the effectiveness of cell replacement therapies for the treatment of neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), and Parkinson's disease (PD) [70, 71].

Interestingly, it has been found that human IPSCs use the same transcription network to generate neuroepithelial and functional neuronal types in the same evolutionary course that ESCs do but with reduced efficacy and some variability [72]. On the other hand, it has been found that the neurospheres derived from the IPSC of humans promote motor functional recovery in mice with spinal cord injury [73] and that a modeling of human cortical development in vitro using this type of cells has facilitated the study of the development of the human brain as well as disorders of the human cerebral cortex [74]. This contributes to the study of the early stages of a human neurodevelopmental disease and provides a cellular tool in the detection of drugs and possible applications for diagnostic purposes and personalized treatment [75]. The progress in the study of IPSCs has allowed advances in the search of therapeutic alternatives or more precise and opportune diagnosis. Other stem cells that have also been studied are the fetal and adult stem cells. These stem cells are also considered multipotent whose characteristics are described in the following section.

Characteristics and Therapeutic Benefits of Fetal and Adult Stem Cells

FSCs originate from the trophectoderm and partly from the mesoderm. The usual fetal tissues used are the Wharton gelatin stem cells (WJSCs) of the placenta, the perivascular umbilical cord stem cells (UCPVCs), and the amniotic fluid stem cells (AFSCs). UCPVCs are cells extracted from the inner layer of the umbilical cord. Originally, they form the extra-embryonic allantois layer that differs from the mesoderm. In adults, MSCs can be isolated from a large number of sources such as bone marrow, spleen, muscle cells, pancreas, and dermis [76–78].

The MSCs derived from the bone marrow (BM-MSC) and the MSCs derived from the adipose tissue (A-MSC) are the most studied stem cells, and they can also be found in dental plug stem cells (DPSCs), HSC, and MSC derived from peripheral blood. There is little or no difference between BM-MSC and A-MSC niches [79, 80]. The bone marrow niche comprises osteoblasts, HSCs, ESC, and their progeny [34], and they clearly differ from the niches of stem cells of hair follicles and teeth [33]. The expression of proteins and molecules of A-MSC has been described as similar with BM-MSC, but it has been observed to have more similarities with WJSCs of placental tissue [81, 82]. From the origin of the endoderm and the ectoderm, some of the stem cells are used for research and therapeutic processes. Among them, we can highlight the epithelial stem cells (EpSCs) and the neural stem cells (NSCs) of ectoderm origin and the HSCs [83] of endoderm origin. In the case of EpSCs, the vast majority of studies refer to what are the intrinsic molecular mechanisms to maintain the state of pluripotency, niche-dependent differentiation, and induction/maintenance of the resting state [84–87]. NSCs are cells located in the subventricular zone and brain regions of the hippocampus of the mammalian brain [88].

Knowledge of the existence of ASC and their various usage have developed a variety of treatment possibilities, but one of the biggest problems experienced is the difficulty of obtaining any of the tissues with stem cells because they all involve invasive procedures for extraction [89]. Also, not all the extraction provides the volume needed to work. For example, extracting MSCs from the bone marrow is not profitable due to the low percentage obtained from MSCs (0.001–0.01%), and this has limited its application and research [33, 90]. On the other hand, adipose tissue is abundant and the frequency of MSCs in adipose tissue is 100 times higher than that found in the bone marrow [80]. Recent studies have shown that MSCs isolated from adipose tissue are more abundant and have a greater viability in the culture (98–100% of the cells of weaves are viable after the extraction) [90–92]. Moreover, adipose-derived MSCs are isolated easily and safely [91, 92] via obtaining up to 500 ml of adipose mass per patient. Other adipose MSCs are obtained in smaller quantities, their extraction volume is less than 10% of the original tissue, and their therapeutic use will depend on the intrinsic individual characteristics of the cells, that is to say, difficulty of isolation, cellular power, crop management, immune response to transplants, and tumor induction capacity, among others [25].

Characterization and Composition Secretome of MSCs

Initially, self-regeneration and tissue replacement capacity by MSCs was the focus of action in regenerative medicine. The ability to generate differentiated cells of a wounded tissue was the main research interest focused on the use of MSCs. In this regard, one of the main lines of study of the MSCs is based on transplants and in differentiation studies of grafts of these cells in co-cultures in vitro [27, 35, 93–95]. This line of research

has given promising results in most cases, differentiation in vivo, but is uncommon partly because the lifetime of the MSCs in the transplanted tissue is between 48 h and 3 months [96, 97]. In addition, the transplanted cells cannot function normally in the organs, because immune rejection may occur [51, 58] or they may promote tumor growth and metastasis [45, 98, 99].

Recently, another research course has emerged in which the type of rescue and repair of MSCs is given by the paracrine activity of secreted factors (growth factors, cytokines, and hormones), cell-to-cell interactions, and the release of extracellular vesicles that include proteins, mRNA, and microRNA [25, 27, 100]. In vivo, the MSCs present a stage of predifferentiation in which they begin to express molecules of target tissue that is also determined by the microenvironment where the MSCs will be transplanted. In the target organ, the MSCs begin to respond to the environmental signals that impose a regulatory action [101] that in most cases is related to specific tissue pathology, generating an immune/inflammatory suppression most likely by paracrine activity [96]. This main action of MSCs to the injury or response to the disease is the secretion of different functional biomolecules, molecules of paracrine secretion, and molecules stored in extracellular vesicles that generate important actions in homeostasis, immune response, development, angiogenesis, trophic action, anti-inflammatory action, pro-regenerative action, proteolysis, adhesion, and organization of the extracellular matrix [32, 34, 102–104].

The MSC secretome dynamically changes its composition depending on the stimuli and microenvironment. Therefore, depending on the environment or the pathology they face, once they are in an affected tissue, they activate different pathways to generate a particular molecular expression response [103]. Generally, the MSC secretome has also been reported in regulating inflammatory responses. The modulation of the immune system also has regenerative effects promoting proliferation and inhibiting the apoptosis of damaged target cells, among other benefits that can serve as a therapeutic alternative in various diseases of both the CNS and other tissues [99, 105]. As mentioned, these effects may be directly or indirectly related to the presence of bioactive molecules, including proteins, mRNA and miRNAs, cytokines, chemokines, growth factors, hormones, extracellular matrix proteins, matrix remodeling enzymes, and vesicle proteins produced by the cell comprise the secretome [19, 103]. These molecules can be involved in the communication from one cell to another that leads to the exchange of genetic information and reprogramming of recipient cells or even the presence, for example, of miRNA-133b involved in the recovery of brain tissue [106–108]. In addition to the active molecules and miRNAs that have been reported in the secretome of MSCs, it has been found that these cells also release extracellular vesicles, known as exosomes, that contain molecules and essential factors to maintain intracellular signaling and communication. The main characteristics of exosomes and advances as a possible therapeutic alternative are presented in the following sections.

Exosomes of MSCs: a New Cell-Free Therapeutic Option

The study of exosomes as signal transmitters is recent and has gained ascending attention in current days. The exosomes, together with microsomes, constitute the extracellular vesicles that have lower immunogenicity, absence of cytotoxicity, and non-mutagenic characteristics [109]. In addition, they have a small size with a diameter of 40–100 nm [98] and can be permeable to biological barriers. It is known that the molecules stored in the exosome are defined by different membrane protein complexes, of which the main protagonists are ESCRT-0, ESCRT-III associated with p53, and the ubiquitinated major histocompatibility complex [110].

Exosomes are also known as molecular transporters and show a superior transport system that allows the supply of small proteins and different RNA (mRNA, miRNA, tRNA, and other non-coding RNAs) that intervene in the immunoregulatory response of MSCs [111–113]. In addition, the use of exosomes has a safety profile superior to the use of cells because they are structures that can store and transport molecules safely without losing their function and conserving its properties and cytoprotective benefits with the activation of pathways where they are really necessary [98].

Exosomes have been reported in different types of cells or substrates, among which are cancer cells [114], serum of cancer patients [115], and serous ovarian carcinoma [116] as well as in the MSCs derived from adipose tissue (A-MSC) and bone marrow (BM-MSC) [98, 117, 118]. Other investigations report that MSC exosomes are mediators during inflammation by releasing anti-inflammatory cytokines and influencing the apoptosis of activated T cells [110, 119]. Also, MSCs can participate in the healing of cutaneous wounds by the action of Wnt family member 4 (Wnt4) administered in extracellular vesicles [120]. On the other hand, it has been shown that extracellular vesicles obtained from human umbilical cord Wharton's jelly MSCs (hWJMSCs) reduced the growth of T24 bladder carcinoma cells in vitro and in vivo [121], reduced apoptosis induced by liver disease [122], decreased heart attack induced by cardiovascular diseases [123], and have beneficial effects in pulmonary diseases [124]. In this regard, Harrell et al. found that exosomes derived from MSCs were as efficient as transplanted cells in limiting the extent of injury and ocular inflammation [125]. In this regard, other studies have shown that exosomes derived from MSCs have similar functions like repairing tissue damage, suppressing inflammatory responses, and modulating the immune system without the risks of aneuploidy or immune rejection after

allogeneic administration [126]. However, the mechanisms by which protection is given are not yet fully known and some results remain controversial.

Alternatively, studies of the effects of extracellular vesicles on pathologies of the CNS have been also advanced. Recently, Zhang et al. demonstrated for the first time that exosomes from MSCs can improve brain's functional recovery, promote angiogenesis and neurogenesis, and decrease neuroinflammation in rats subject to traumatic brain injury (TBI) [127]. The positive effects of exosome have been attributed to the action of proteins, lipids, and RNA present in these vesicles and that may have a specific therapeutic role [128]. In regard to these findings, the evaluation of the effect of nanovesicles and exosomes derived from MSC has greatly advanced and appears as a promising future therapeutic alternative for brain pathologies. For example, exosomes of BM-MSCs that are transferred to neurons and astrocytes have not only diminished cognitive deterioration induced by diabetes [129] but when administered intravenously also targeted M2-type macrophages in spinal cord injury [130]. On top of that, it has been reported that stem cells derived from multipotent mesenchymal stromal cells overexpress different microRNAs currently investigated for the treatment of brain pathologies. For example, the microRNA 133b has shown to increase neuronal plasticity and to improve neurological function in a rat model of stroke [131]. Another miR, the miR-26a, present in exosomes, has also been related to axonal regeneration, neurogenesis, synaptic development and plasticity, synaptic transmission, and maintaining neuronal morphology [132]. Similarly, nanovesicles derived from adult stem cells reduce the movement of T lymphocytes and improve chronic experimental autoimmune encephalomyelitis [133].

According to Zhang et al. [127], functional recovery has been demonstrated, possibly promoting angiogenesis that allows renewal of the endogenous endothelium as well as neurogenesis after the use of exosomes derived from MSCs. The authors report that together these effects significantly improve spatial learning and motor recovery in rats with experimental intracerebral hemorrhage [134]. Considering the benefits of exosomes for the treatment of different pathologies, nowadays, different types of culture have been established that allow obtaining exosomes with more protective effects. For example, a previous study [135] determined that 2D and 3D cultures of BM-MSCs allow obtaining exosomes for the treatment of TBI. However, the exosomes obtained in 3D scaffolds gave better results in spatial learning than exosomes grown in 2D and in general terms the exosomes significantly improved functional recovery in rats after a TBI promoting angiogenesis and endogenous neurogenesis and in this particular case also reduced neuroinflammation [135]. It seems to expand the study of MSCs exosomes and the analysis of the possible mechanism of action on the pathologies is considered as a primary objective in the search for effective therapeutic alternatives, low demanding and fast-acting for the treatment of CNS diseases or else at least provide an approximation preventing progression of the injuries. Some advances in this area are presented below, focused in the CNS and the effect of biomolecules in the protection and recovery of the different cells that form the brain tissue.

Action of the Secretome of Mesenchymal Stem Cells on Pathologies

Nowadays, it is a well-known fact that the paracrine action of MSCs is based on the secretion of trophic factors and cytokines [136]. Therefore, proteomic studies of MSCs have been developed, mainly derived from bone marrow, adipose tissue, fetal and embryonic, as well as their secretome or paracrine factors, in order to detect prospective biomarkers, identify molecules in response to the injury, select objectives for the treatment, and study cell signaling [103, 137, 138]. With the accumulating evidence from these studies on the effect of paracrine factors in different tissues and the constant search for therapeutic strategies, effective treatments could be developed for different diseases, including CNS ailments [139–142] (Fig. 1).

An explicit example is that for some time the conditioned medium of MSC (CM-MSCs) derived from different sources has been evaluated in cardiac, renal, bone regeneration, or inflammatory processes, among others [143]. Particularly, for cardiac tissue as reviewed by Gnecchi et al. [144], the mesenchymal ones that are grafted release a wide range of soluble factors that can be used to prevent and reverse the remodeling in the ventricle with ischemic injury [145]. It is possible that the protective effect is given by immunomodulators and antioxidants, the presence of extracellular superoxide dismutase (SOD3) [146], the increase of anti-inflammatory proteins such as the tumor necrosis factor-inducible gene 6 protein (TSG-6) [147, 148], or due to the effect of exosomes derived from MSCs that increased the levels of ATP, NADH, AKT, and phosphorylated GSK3β, in addition to reducing oxidative stress [149].

At the muscular level, CM-MSCs can reduce apoptosis and fibrosis intramuscularly [102] and the factors present in the CM-MSCs may favor the treatment of immune diseases, the rejection of transplants [150], and in the treatment of acute kidney injury through an anti-apoptotic protective effect [151, 152]. To this long list of beneficial effects of the trophic factors produced and secreted by MSCs, they help to improve the proliferation of endothelial cells and angiogenesis in a model of ischemia of the hind limbs in rats [153], accelerate the formation of bony scars [154–156], recovery of rheumatoid arthritis [157], regeneration of jaws in rabbits [18, 158], and they have a therapeutic effect in the treatment of diabetes mellitus [159], among other diseases [160]. Indeed, these secreted factors from MSCs decrease pro-apoptotic markers (caspase-3, α-smooth muscle actin (α-SMA), and

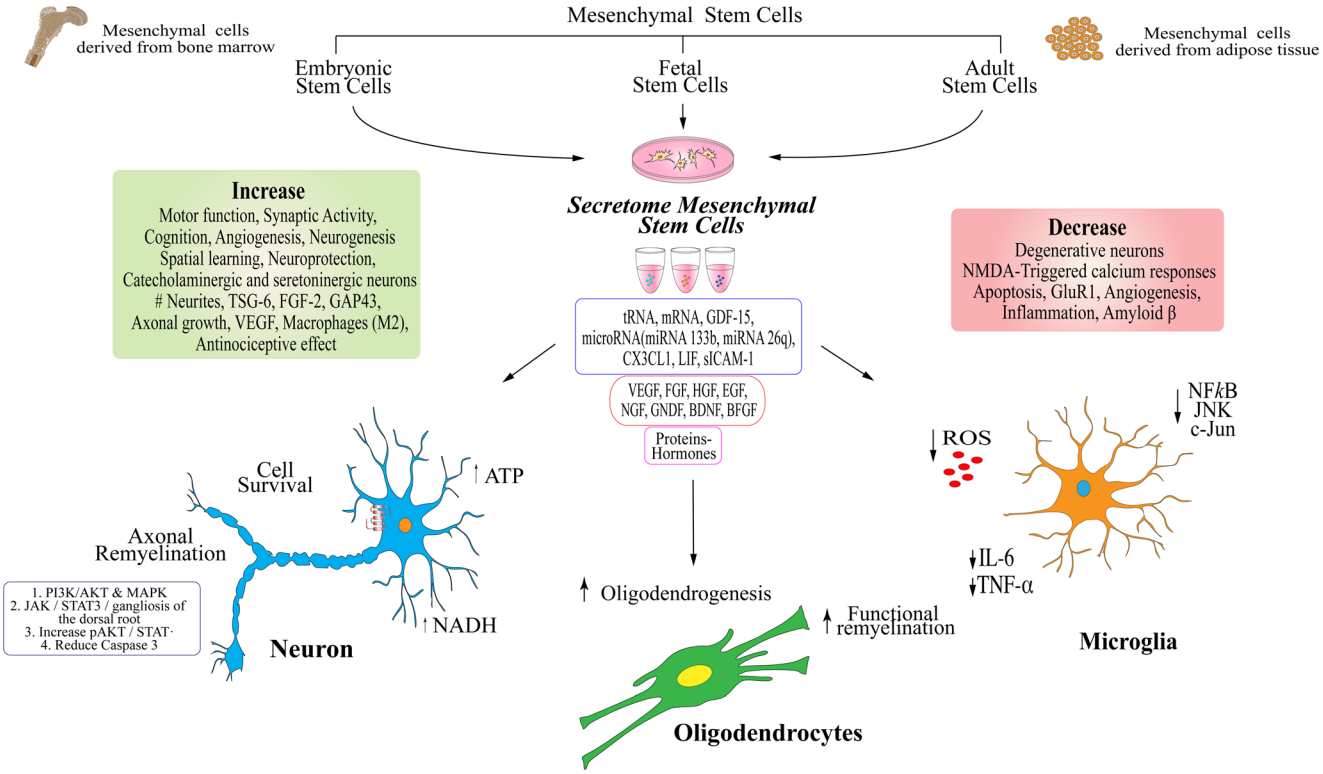

Fig. 1 Effects of the conditioned medium of mesenchymal stem cells (MSCs) in brain cells. The conditioned medium (CM) of MSC can be obtained from adipose tissue (CM-A-MSC), bone marrow (CM-BM-MSC), dental pulp (CM-hDPSC), and umbilical cord blood (CM-HUCPVC). It contains bioactive molecules and/or trophic factors such as VEGF-FGF-HGF-EGF with anti-apoptotic properties, decreases neurodegeneration, increases the number of neurites and angiogenesis, promotes the recovery of the spinal cord, increases the levels of ATP-NADH, and activates different survival pathways at the neuronal level, such as phosphorylation of Akt, Jak/STAT3. The conditioned medium also decreases the activation of microglia and promotes the oligodendrogenesis and functional remyelination, making it as suitable therapeutic strategy to counteract different pathologies at the CNS

proliferating cell nuclear antigen (PCNA)) in rats subjected to unilateral ureteral obstruction [161]. In addition, it has been demonstrated using in vivo paracrine models that exosomes decrease apoptosis and the formation of pulmonary fibrosis mediated by an anti-inflammatory mechanism provided by the MSCs [162] and with similar results [163] reported in the rats with periodontal defects, a periodontal tissue regeneration linked with cytokines present in the CM-MSCs.

The benefits of the use of the secretome in various pathologies that affect humans are widely reported. A significant number of studies support the protective and beneficial effect of the secretome on brain tissue, especially in pathologies that are currently considered a public health problem in different countries such as AD. A detail of the molecules and factors with beneficial effects is discussed below in the context of different animal models of pathologies of the CNS.

The Secretome of MSCs as a Therapeutic Alternative for CNS Pathologies

The brain tissue is formed by complex and integrated relationships between different types of cells. The neurons are responsible for neurotransmission, and the glia, mainly astroglia, function as the cerebral administration system playing a leading role in neuronal survival by maintaining cerebral homeostasis, controlling secreted trophic factors, buffering extracellular K^+ concentrations, recycling glutamate, metabolizing glucose into lactate, and forming the blood-brain barrier. The physiology of these cell types is strongly altered during cerebral pathologies, causing the loss of their functions and the protection they provide to the tissue. Brain pathologies have common characteristics of cell damage. For example, they increase the production and accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), denaturation, and protein aggregation, besides secreting apoptotic factors, generally related to the mitochondrial malfunction and alterations in the metabolism.

Because of the sequelae and cell death left by physiological changes at the cellular level, cerebral pathologies have had an impact on the quality of life of the world population, and given its complexity, it has been difficult to establish effective therapies or treatments for some pathologies that prevent the progress of the injury with cognitive and motor sequelae [6, 7]. In this regard, CM-MSCs have also been evaluated in the search for protective or restorative agents in different cerebral

pathologies such as TBI, neurodegenerative diseases, spinal cord injury, ischemia, and stroke (Table 3).

Traumatic Brain Injury

TBI is often caused by accidents [182, 183]. It is known as an impairment of brain function caused by stroke, laceration, or rapid and strong movement of the brain inside the skull, producing neurological damage [184]. The initial manifestations of TBI include an altered level of consciousness, convulsions, coma, and confusion. The final results of the injury are disorders affecting memory, attention, language, reading, and writing as well as spatial orientation tasks [185]. There are different physiopathological phases that compromise changes in different types of brain cells. The first phase includes mechanical damage causing the rupture of the blood-brain barrier and a diffused axonal injury [186], and the second phase is a cascade of events caused by mitochondrial breakdown and free radical release [187–189]. In addition, the functions of neurons are affected by the loss of other brain cells such as astrocytes [190, 191].

The complexity of TBI has sought different therapeutic alternatives for the treatment or prevention of progression of injury. One of these therapeutic alternatives is the use of MSCs from different sources. To mention, studies have revealed that endothelial progenitor cells migrate from the bone marrow into the bloodstream in response to injury or inflammation [192, 193]. In other studies, it was found that medullary stromal cells applied intravenously [194] and the transplantation of fetal neural stem cells [195, 196] help in improving the functional cognitive outcome after acute brain injury. Recently, Otsuka et al. [197] reported that transplantation of MSCs derived from human cranial bone, cultured under microgravity conditions, had exceptional neuroprotective effects in mice with TBI.

A topic that is often debated nowadays is the nature of metabolites secreted by MSCs that can be used for the treatment of TBI. The BM-MSCs have been widely studied and the results showed beneficial effects for various brain diseases [15, 198]. For example, intravenous injection of BM-MSCs secretome reduced neuronal loss and apoptosis, promoted the production of VEGF, and induced functional improvements in a rat model of TBI [164]. More importantly, they found that the factors secreted by BM-MSCs modulated the inflammatory response by decreasing the profile of cytokines expression on the brain tissue. Also, in a model of experimental TBI, the secretome from BM-MSCs promoted the survival and proliferation of neural stem cells and the subsequent repair of the injured tissue [9] whereby suggesting that they could be a promising strategy for recovery from TBI.

It is noteworthy that not only the factors of the BM-MSCs are related to the protection against TBI, but also other CM-MSCs have shown encouraging beneficial effects in this neurological condition. Firstly, several studies have reported the presence of bioactive factors and cytokines with powerful long-lasting antinociceptive effect on neuropathic pain developed after a nerve injury [199] as well as other compounds such as HGF and VEGF have been reported to be present in the CM-MSCs. Nevertheless, when pre-conditioning of MSCs with hypoxia was performed before the isolation of exosomes, the rats with TBI treated with these specific secretomes showed better results in the motor and cognitive functional tests and enhanced neurogenesis and less brain damage after injury [167]. The potential benefits of using CM-MSCs for treating neurological disorders have encouraged the use of the new genetics technologies to design MSCs with enhanced tropism and paracrine secretion of cytokines and growth factors for localization and recovery of TBI, including the use of the chemokine receptor interaction involving the CXC4 (CXCR4)-SDF1α (factor 1α derived from stromal cells) factors [120].

Several studies with different experimental approaches, but related to the TBI, have also shown an effect of MSC on restoration of tissue structure and function. One of these cases is the crush injury of the optic nerve. In this study, they showed that MSC therapy was associated with an increase in the expression of FGF-2 in the ganglion cell layer of the retina, suggesting a beneficial result mediated by trophic factors from mesenchymal stem cells [168]. Other studies also showed that the CM-MSC is involved in the protection of neurons against glutamate excitotoxicity as the reduction of NMDA-triggered calcium responses and surface GluR1, and these results suggest that the CM-MSC involves reduced activity of the NMDAR and GluR1-containing AMPAR and TNF-mediated neuroprotection [169].

Neurodegenerative Diseases

Neurodegenerative diseases, such as AD, PD, and HD, are another type of brain pathologies that have higher incidence in the world's population [200, 201] and for which the CM-MSCs have also been evaluated as alternative for treatment [139, 202]. These therapies will take advantage of the innate trophic support of the MSCs by using genetically engineered MSCs as delivery vehicles of growth factors, such as BDNF and GDNF, to support injured neurons [142]. Clinical trials for the administration of MSCs and application of their secretome in the CNS to treat different nerve pathologies are currently underway. In search for alternative treatments for neurodegenerative diseases, the differentiation capacity of MSCs into functional dopaminergic neurons [203] and also the anti-apoptotic effect on dopaminergic neurons of factors secreted by MSCs has been evaluated [170]. However, although the mechanisms of action of MSCs factors are not clear, recent

studies support the hypothesis that the therapeutic effect of MSCs is mediated by the receptors MAPK-1 and PDGFRB [204]. There is evidence compelling that monoaminergic fibers are affected in PD [205, 206]. Interestingly, BM-MSCs have shown to protect both catecholaminergic and serotonergic neuronal perikarya structure and transporter function from oxidative stress by the secretion of GDNF [207]. New perspectives for therapeutic strategies have led to carrying out tests with genetic modifications on MSCs in such a way that they overexpressed GDNF, finding protection and budding of the dopaminergic terminals induced by secreted GDNF [208]. With all this evidence, it is reasonable to postulate that MSC therapy can reduce the risk of PD, based on the fact that MSCs can act as a ROS detoxification system and as a provider of neurotrophic factors as reported previously [209]. Despite this progress, further investigation and broader approaches to investigate the scientific scope of these potential therapeutic alternatives are still needed.

Another neurodegenerative pathology that has attracted great interest in the search for therapeutic alternatives is the multiple sclerosis. Also, in this condition, BM-MSCs and the CM-MSC have been evaluated for therapy. In a first study by Dahbour et al. (2017), it was found that there is a correlation between a lower number of lesions and a higher content of molecules such as interleukin-6 (IL-6), interleukin-8 (IL-8), and VEGF present in the CM-MSCs at the beginning of the study [171]. This correlation may explain the magnitude of improvement in the state of disability [171]. Alternatively, it was reported in in vivo murine demyelinating model that intraventricular injections of MSCs provide paracrine effects on the oligodendrogenesis of the subventricular zone, suggesting that MSC-secreted factors may be an effective method to promote oligodendrogenesis and functional remyelination [210]. To date, phases 1, 2, and 2a pre-clinical trials conducted to assess the safety and efficacy of MSCs in humans showed that an intravenous and/or intramuscular injection of MSC and neurotrophic factors have synergistic clinical benefits in amyotrophic lateral sclerosis [211, 212].

AD has a high prevalence in the elderly [213, 214] and abundant research have been done about its pathophysiology and possible treatments including the use of factors secreted by the MSC of the human umbilical cord (hUCB-MSC), such as the GDF-15 factor, that promotes endogenous neurogenesis of the adult hippocampus and synaptic activity in a model of AD [215]. In addition, the use of these cells reduced β-amyloid ($A\beta x\text{-}_{42}$) plaques in the hippocampus and other regions. These data suggest that sICAM-1 derived from hUCB-MSC decreases Aβ plaques by inducing NEP expression in microglia via the sICAM-1/LFA-1 signaling pathway [216]. At the same time, clinical trials have shown that the cerebral stereotactic injection of hUCB-MSC in patients with dementia due to mild to moderate AD was feasible, safe, and well tolerated [217]; however, still more studies are needed to evaluate their efficacy to be used in the treatment of AD. Finally, a previous study [218] showed that several transplants of MSCs in the striatal zone regulated factors such as FGF-2 and significantly reduced the number of degenerating neurons in the striatum. This effect was probably mediated by factors secreted by MSCs since these were visible up to 60 days after transplantation that may be involved in the reduction in brain damage under neurodegenerative conditions [218].

Spinal Cord Injury

In comparison to other pathologies, the effect of CM-MSCs on spinal cord injury (SCI) showed greater progress in the discovery of possible mechanisms of neuroprotection. In this regard, studies at the neuronal level in an excitotoxicity model revealed that the expression of GAP-43, an essential protein for axonal and dendritic growth, increased considerably in parallel to an increase in the levels of ATP, $NAD^{(+)}$, and NADH after the treatment of neurons with conditioned medium mesenchymal cells derived from adipose tissues (CM-A-MSCs). These results can be relevant since excitotoxicity occurs in many neurological conditions including after SCI or TBI.

Previous studies have shown that trophic factors and bioactive molecules present in the CM-MSCs are regulating survival signaling pathways. A first study [172] pointed out that BDNF and GDNF found in the CM-BM-MSCs are involved in the growth of neurons of the spinal cord and that the protection exercised by BDNF is mediated in part by the activation of Akt. These findings correlate with studies in cerebellar neurons where they show that BM-MSCs are able to protect cerebellar neurons from cultured rodents by modulating the phosphatidylinositol 3-kinase (PI3K)/Akt and MAPK pathways, and the antioxidant effect of superoxide dismutase 3, a protein synthesized and secreted by the BM-MSCs [146]. Similar results in axonal growth of neurons mediated by BDNF from hUCB-MSCs were reported previously [173].

Another signaling mechanism of action of CM-MSCs is the activation of the Jak/STAT3 pathway to maintain the well-being of the embryonic dorsal root ganglia [174]. As previously reported [174], neurogenesis and neurite growth are not only mediated by BDNF, but also influenced by IL-6 and the leukemia inhibitory factor (LIF) produced by PSC [174]. Besides, another study showed that intrathecal infusion of exosomes derived from mesenchymal stem cells of the human umbilical cord (UC-MSC) suppressed nerve ligation-induced upregulation of c-Fos, CNPase, GFAP, and Iba1 and also reduced the level of TNF-α and IL-1β in male rats with pain induced by nerve lesions [165]. The above suggests that this signaling pathway stimulated by the CM-MSCs can be targeted by alternative therapies against peripheral nerve injury.

Interestingly, the characterization of the CM-BM-MSCs revealed a series of molecules such as cytokines and IGF-1, HGF, VEGF, and TGF-β1 that contribute to neuronal survival and the growth of neurites in vitro and that can probably be used for the treatment of SCI, as previously demonstrated in rats [219]. Impressively, current pieces of evidence from studies using secretomes from A-MSC, BM-MSC, and HUCPVCs highlight the fact that these cells induce the same degree of differentiation of human neural progenitor cells and neurite growth in dorsal root ganglion explants [220].

These new perspectives in regenerative medicine research have encouraged the testing of new forms of application of trophic factors from MSCs. One of the new approaches includes the intrathecal administration of trophic factors secreted by mesenchymal stromal cells. The results showed that these factors improved the functional recovery and decreased the expression of IL-2, IL-6, and TNF-α in a rat model of SCI [221]. On top of that, recent research shows that a new 3D biomimetic hydrogel designed to administer the factors secreted by the MSCs significantly immunomodulated the proinflammatory environment in an SCI mouse model, increasing the M2 macrophage population and promoting a pro-regenerative environment in situ [222].

Ischemia and Stroke

Ischemia and stroke are another type of pathologies that are often affecting the well-being of humans as TBI affects the family, social, and professional life of patients who suffer from cognitive sequelae and other long-term negative effects after the traumatic event [223, 224]. In vivo models of cerebral ischemia induced by hypoxia and ischemic stroke models have been used to evaluate the effects of the trophic factors from BM-MSCs and A-MSCs, finding for both cases that these factors have a neuroprotective effect in stroke/induced injury [146, 175].

Similar results were found when using MSCs, which have shown in organotypic cortical brain slices subject to oxygen-glucose deprivation to release human amniotic factors that may protect the brain from acute injury. Surprisingly, in this study, they also found that fractions rich in metabolites of less than 700 kDa were protective, but this fraction did not contain protein or ribonucleic molecules. Interestingly, another study reported equal effectiveness of the CM-BM-MSCs subjected to ischemia and those under normoxygenic conditions. Both cells effectively increased neuronal connection and survival in mixed glia/neuron cultures. Besides, an intravenous infusion in in vivo models of stroke significantly improved functional recovery, increased neurogenesis, and attenuated the infiltration of microglia/macrophages in the brains, revealing a therapeutic potential of the factors present in CM-BM-MSCs [175]. All these studies have reported an improvement in functional recovery in different models in vivo with ischemic stroke, opening a plethora of possibilities and new alternatives for the treatment of this type of pathologies.

About the mechanism of action by which the CM-MSCs is exerting its neuroprotective effects in stroke, BDNF and HGF have been reported to have protective effects on damaged neonatal cortical neurons in a model of oxygen and glucose deprivation (a cellular model of stroke-related conditions), decreasing the signs of apoptosis/necrosis [176]. However, the presence of these two factors does not discard the possibility that there are other paracrine molecules with neurotrophic potential contributing to these effects. In this regard, the secretome analysis of MSC derived from the limbus (L-MSCs) showed that molecules including the human growth factor, cytokines, and other factors such as VEGF, VEGFR3, BDNF, IGF-2, and HGF not only stimulate the growth of neurites, but also protect the hypoxic neurons in vitro and in vivo in models of focal cerebral ischemia in rats [177].

To date, several studies support the presence of BDNF secreted by transplanted MSCs. A previous study showed how BDNF was one of the critical paracrine factors that play a fundamental role in the attenuation of severe brain lesions induced by intraventricular hemorrhage in newborn rats. BDNF knockdown worsens posthemorrhagic hydrocephalus, behavioral performance, astrogliosis, TUNEL+ cells, ED-1+ cells, and inflammatory cytokines in severe IVH-induced brain injury [178, 179]. A second study showed that treatment with UC-MSCs attenuated the cerebral reactive gliosis and hypomyelination and elevated BDNF and HGF levels in the cerebrospinal fluid, serum, and brain tissue of a mouse model of neonatal IVH [180]. Ultimately, it is important to note that paracrine factors secreted by MSCs protect neurons from apoptotic cell death in the cerebral ischemia model by glucose and oxygen deprivation by activating and increasing the phosphorylation of STAT3 and Akt in neuronal cultures after treatment with CM-MSCs [181].

Effect of the Secretome of Mesenchymal Cells Derived from Adipose Tissue in Brain Pathologies

Adipose tissue is a vascularized connective tissue with important functions in protection, as an insulator and energy reservoir, and it can even act as an endocrine organ. When it is enzymatically disintegrated, the adipose tissue can produce A-MSCs [225]. Secretory studies have revealed that A-MSCs release molecules that can mediate processes of repair in different tissues including the nervous system (Table 1). Although the proinflammatory cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, IL-7, IL-8, IL-11, and TNF-α play a fundamental role in attracting phagocytic cells for the cleaning of waste in the injured area [17], it has been reported that trophic factors such as VEGF [241], HGF, TGF-β, FGF-2 [242–244], and other

Table 1 Regenerative potential of the secretome from adipose-derived mesenchymal stem cells (CM-A-MSC)

Transplanted cells/medium condition	Proteins and secreted factors	Effect	References
Transplant/secretion	BDNF	Regeneration of tissues through growth of blood vessels, nerves, and myelination	[226]
Transplant/secretion	HGF-HA	Vocal cord regeneration	[227]
Conditioned medium	FGF, HGF, VEGF, SOD3, SOD2	Increase in the activity of SOD and GPX protect human dermal fibroblasts from oxidative damage, reducing cell death by apoptosis	[228]
Conditioned medium	IGF, HGF, TGF-β1, VEGF	Angiogenesis, epithelization and remodeling	[63]
Conditioned medium	TGF-b1, VEGF, bFGF, KGF, PDGF-A, HGF	Decrease in the rate of erythema, melanin, and transepidermal water loss	[229]
Conditioned medium	HGF, G-CSF, GM-CSF, IGFBPs, IL-12, PDGF-AA, PEDF, SODs	Antioxidant and reparative effect mediated by the activation of dermal fibroblasts and keratinocytes	[230]
Transplant/secretion	TGF-b1, VEGF, bFGF, KGF, PDGF-A, HGF	Angiogenesis, anti-apoptosis, increased vascular growth, and immunomodulatory effects	[231]
Conditioned medium	No information	Protection of cortical neurons against apoptosis and excitotoxicity of glutamate; increased GAP-43, ATP, NAD^+, and NADH	[232]
Conditioned medium	IGF-1, BDNF	Neuropathological recovery and increased cognitive and motor ability in the long term after a hypoxic-ischemic lesion	[21]
Conditioned medium	TNF-α	Accelerates wound closure, induces angiogenesis, proliferation, and infiltration of immune cells in a cutaneous wound	[233]
Secretome	bFGF, VEGF, NGF, SCF, HGF	Improve the metabolic viability of hippocampal cultures and neuronal cell density	[82]
Conditioned medium	TIMP-1, SPARC	Neuroprotective effects against retinal damage; used as a treatment for retinitis pigmentosa and macular degeneration	[234]
Transplant/secretion	IL-6, VEGF, Angiogenin, MCP3, MCP1, IGF1, TGF-ß, PDGF-BB, bFGF, EGF	Induce bone regeneration in lesions created surgically in the rabbit's jaws	[18]
Secretome	PEDF, CADH2, IL-6, SEM7A, GDN	Improve neurite/axonal growth in an in vitro model of axonal regeneration based on DRG explants	[220]
Secretome	VEGF, HGF, FGF2	Increase neovascularization and improve wound healing in injured tissues	[235]
Exosomes	Neprilysin protein (NEP)	Reduction of secretion and intracellular levels of β-amyloid	[236]
Secretome/pre-conditioning with deferoxamine	Pro-angiogenic, neuroprotective and anti-inflammatory factors	Treatment of diabetic neuropathy	[237]
Conditioned medium	BDNF, TGFβ	Axonal morphological recovery, electrophysiological characteristics, and normal cell viability	[238]
Conditioned medium	NGF	Increase neuritogenesis of PC12 cells	[239]
Conditioned medium	VEGF, HGF, BDNF	Activation PI3-K/Akt y MAPK; reduction of caspase-3 levels	[240]

immunosuppressive molecules that contribute to controlling inflammation such as prostaglandin E2 (PGE2) [245, 246] and IL-10 [247], are essential for wound healing. In addition, extracellular matrix molecules, hormones, and some lipid mediators have been also reported to have beneficial neuroprotective effects [244, 248]. A comparative study of the effects of CM-A-MSCs and CM-HUCPVC indicated that the latter promoted the strongest effect on neuronal survival [82]. The trophic factors present in the CM of these two cell types were bFGF, NGF, SCF, HGF, and VEGF with only bFGF absent in the CM-A-MSCs and a small expression of NGF in CM-HUCPVC [82]. In another study, BDNF and adipokines have been reported inside A-MSC secretome [246]. Furthermore, some studies have concluded that A-MSCs show similar cytokine secretory abilities than BM-MSCs [17]. On the other hand, there is evidence indicating that CM-A-MSC-derived factors are responsible for protecting neurons against excitotoxicity induced by increased expression of GAP-43, inhibition of neuronal damage and apoptosis, and stimulation of the regeneration and repair of nerves and the tissue bioenergetics. These effects have been suggested to be exerted through increasing the levels of high energy molecules and cellular metabolism [20]. Currently, several studies evaluated the effect of CM-A-MSCs as a possible therapeutic alternative in various pathologies. For example, in a rat model of hypoxic-ischemic brain injury (HI), it was found that the CM-A-MSCs prevented the loss of cortical and hippocampal volume [21]. After 2 months of ischemia, the behavioral and learning tests showed that the subjects treated with CM-A-MSCs presented significantly better results in the functional maze water tests than controls [21]. A previous study [21] compared the effects of secretome of MSCs from different

sources such as adipose tissue, bone marrow cells, and umbilical cord cells and found that the A-MSC secretome has a greater potential to promote axonal growth than secretomes of all the other sources [220]. However, a previous study compared the property of different MSCs to migrate to tumor sites, to be used as tumor vectors. One of these studies [249] reported that UC-MSCs were more efficient than A-MSC for the induction of apoptosis but being unable to distinguish differences in their capacity of inducing cell differentiation [249]. Additionally, similar to other CM-MSCs, the presence of in vitro and in vivo angiogenic cytokines in CM-A-MSCs such as VEGF, HGF, and FGF2, which increase neovascularization and improve wound healing in injured tissues, has been reported [235]. This data suggests that therapy with A-MSCs and/or CM-A-MSCs could accelerate wound healing through differentiation and vasculogenesis and other repair processes.

The effects of CM-A-MSCs were evaluated in pathological conditions such as SCI, ischemia, and stroke. In an in vitro model of inflammation due to SCI, the exposure to CM-A-MSCs stabilized the neuronal population but had no effect on astrogliosis, which suggests that the effect was due to the neuroprotective and trophic factors [250]. Similar results were found in the recovery of neurological deficits in a stroke model in rats with a faster and more pronounced improvement compared to A-MSC injection [251].

While there are very few studies in relation to neurodegenerative diseases, in AD, the secretome of the CM-A-MSCs also showed an effect when they were associated with neprilysin. This association gave exosomes the characteristic of being enzymatically active, with the property of acting on N2 cells to decrease the levels of the intracellular and secreted forms of Aβ peptide [236]. These results suggest that this approach can be tested in other studies as a possible treatment for AD.

The evidence presented so far has shown that the beneficial effect of CM-A-MSCs on different pathologies is probably due to different bioactive molecules synthesized and released by the MSCs. This has encouraged the use of CM-A-MSC after some pre-conditioning of the MSCs. For example, it is known that MSCs that were pre-conditioned with deferoxamine increase the production of pro-angiogenic, neuroprotective, and anti-inflammatory factors, which can be utilized as a possible treatment for diabetic neuropathy [237]. Another approach used to enhance the beneficial effects of secretomes from A-MSCs was the pre-stimulation of MSCs with TNF-α and IFN-γ. This pre-stimulus caused the cells to release TSG-6, a protein that may be mitigating the visual deficits induced by a blast lesion through its anti-inflammatory properties on activated microglia and endothelial cells [252]. On top of that, it was recently found that the neuroprotective effect of CM-A-MSCs is affected by *N*-acetyl-cysteine supplementation. The study suggests that neuronal restorative effect of CM-A-MSCs is associated with not only the release of essential neurotrophic factors but also the maintenance of the redox state to preserve neuronal function [238].

Finally, another study described an additional mechanism of action by which the CM-A-MSCs exert their protective effect [239]. In this study, it was found that the activation of the AMP-activated kinase pathway (AMPK) was induced by the presence of NGF, which has been reported in the A-MSC secretome and involved in the neuritogenesis of PC12 cells [239]. In correlation with these findings [240], the authors found that CM-A-MSCs protected PC12 cells from apoptosis caused by glutamate excitotoxicity. However, in this case, other factors were found such as VEGF, HGF, and BDNF that are believed to be related to the activation of pathways such as PI3K/Akt and MAPK or in the reduction of caspase-3 levels [240]. Taken together, these results may be useful for the treatment of stroke or neurodegenerative diseases.

Effect of the Secretome of Mesenchymal Stem Cells on Glial Cells and Their Involvement in Cerebral Pathologies

After brain injury, astrocytes are the first to respond generating a physiological action, either releasing proinflammatory and/or anti-inflammatory factors [253]. Despite these and other beneficial effects of astrocytes for the nervous tissue, therapeutic alternatives as well as the search for possible treatments for various brain pathologies have been focused mainly at the neuronal level, forgetting other cells and their protective properties [254]. In this sense, some studies have recently focused their interest on evaluating the effect of CM-A-MSCs, CM-BM-MSCs, or CM from another type of MSCs in the protection or stability of glial cells such as microglia and astrocytes.

In relation to microglia, a study carried out using the immortalized cell line of murine microglia BV2 and primary postnatal brain cell cultures activated by lipopolysaccharides (LPS) revealed that treating the cells with CM-MSCs reduced the mRNA and protein expression of proinflammatory cytokines (IL-6 and TNF-α), JNK, NF-κB, and c-Jun. These results suggested that MSCs can modulate microglial activities through paracrine effects [255]. Similar results, though more focused on the release of neuroprotective substances, were reported in murine microglia N9 through an interaction of these cells with MSC. It was suggested that MSCs, via releasing of paracrine factors, can change the phenotype of the microglia from harmful to neuroprotective, increase autophagy and and release of substances associated with CX3CR1, thus favoring the protection of the tissue [256]. The previous results are supported by studies carried out in co-cultures with MSCs suggesting that MSCs could restore the homeostatic functions of the retinal microglia against light damage, mainly through activation of CX3CL1/CX3CR1 axis [257].

Torrente and colleagues using a model of glucose deprivation and mechanical injury (*scratch*) reported that the CM-A-

MSCs enhance viability, increase wound closure, and reduce the production of ROS in an astrocytic model (T98G) under glucose deprivation [258]. This result was consistent with the decrease in the percentage of fragmented and condensed nuclei found using the same model previously reported [259]. Also, in the case of astrocytes, some studies have reported additional effects of CM-A-MSCs on viability, morphology, mitochondrial protection, and ROS regulation, among others (Tables 2 and 3). On the other hand, it has been reported that another type of cells (HUCPVC) releases neuroregulatory factors that have an impact on cell density. For example, CM-HUCPVC not only influenced the density of neurons, but also protected the viability and increased the proliferation of astrocytes and oligodendrocytes; however, they had no effect on microglial cells. These results suggest that the paracrine factors of MSCs can mediate not only cell density, but also can be used in brain tissue recovery and reparative therapy of glial cells in different pathologies [262, 263]. It is well-known that mitochondria are one of the main organelles that are affected in response to pathological change in the brain environment [265]. Considering this evidence, several studies have evaluated the effect of CM-MSCs on the protection of the mitochondria and their functions. This investigation has revealed that bioactive factors and molecules secreted by different MSCs can reduce the production of ROS. Specifically, the CM-A-MSCs had an effect in decreasing the production of superoxide (O^{2-}) [258]. In another study, the authors found that the CM-A-MSCs also protected against oxidative stress damage, reducing DNA oxidation, lipid peroxidation, and nitration of proteins caused by glucose deprivation and mechanical injury (*scratch*) in an astrocyte cell model (T98G) [259]. Interestingly, in an in vitro model of ischemic human astrocytes, the pre- and post-ischemia treatment with CM-hDPSCs and CM-BM-hMSCs attenuated the expression of GFAP, nestin, and musashi-1 induced by oxygen-glucose deprivation (OGD), blocked ROS production, and positively regulated IL-1. This finding supports the hypothesis that trophic factors produced by hDPSCs and BM-hMSCs represent an alternative source of cell therapy for ischemic stroke [264].

This extensive research approach on the effect of CM-MSCs in the protection of mitochondrial functions in astrocytes has also been confirmed in models of glucose deprivation and mechanical injury (*scratch*), where cells treated with the CM-A-MSC regained the potential of mitochondrial membrane [259, 260]. Besides, the conservation of the cellular

Table 2 Effects of the secretome of mesenchymal stem cells on glial cells

Cells	Source	Protective effect	Reference
Microglia	CM-MSC	Decreases in mRNA expression (IL-6 and TNF-α)	[255]
		Reduction in expression of proteins NF-κB, JNK, and c-Jun	
	CM-MSC	Change of proinflammatory reactive phenotype to neuroprotective phenotype	[256]
Astrocytes	CM-A-MSC	↑ Cell viability protection	[258]
			[260]
		↑ Nuclear fragmentation and condensation	[258]
			[260]
		↑ Preservation of mitochondrial membrane potential	[258]
			[260]
		↓ Lipoperoxidation	[259]
		↓ Nitration of proteins	[259]
		↓ DNA damage	[259]
		↑ Number of mitochondria and mitochondrial crests	[259]
		↑ GFAP	[259]
			[261]
		↓ Reactive oxygen species	[259]
			[260]
	CM-HUCPVC	↑ Proliferation of glial cells	[262]
			[263]
	CM-A-MSC	↑ Polarity index	[260]
	CM-BM-MSC	↓ Interleukin-1 (IL-1)	[264]
	CM-DPSC-MSC		
	CM-A-MSC	Positive regulation of neuroglobin	[259]
	CM-MSC	Inhibition of p38 MAPK and JNK	[261]
		Regulation of p53 and STAT1	

Table 3 Therapeutic strategies of CM-hMSC in different CNS pathologies

Source	Pathology	Model	Application	Advances	References
Secretome normoxia--preconditioned human mesenchymal stem cells	TBI	Adult male rats	Intravenously	Attenuation of motor deficit and cerebral infarction of rats by reducing neuronal cell loss and apoptosis and promoting VEGF production	[164]
BM-MSC and CM-BM-MSC	TBI	Adult (12-week-old) C57BL/6 male mice	Both MSC transplantation and MSC conditioned medium	Decreased a broad cytokine profile in tissue in vivo models, increased the proliferation of NSC, and induced a higher expression of GFAP in vitro models	[9]
Exosomes derived from human umbilical cord mesenchymal stem cell (UC-MSC)	Nerve injury-induced pain	Male rats	Intrathecal infusion of exosomes	Suppressed nerve ligation-induced upregulation of c-Fos, CNPase, GFAP, and Iba1 also inhibited the level of TNF-α and IL-1β	[165]
Conditioned medium of bone marrow-derived mesenchymal stromal cells	Neuropathic pain	Male C57Bl/6 mice	Both MSC transplantation and CM-BM-MSC by endovenous injection	The levels of IL-1β, TNF-α, and IL-6 were found reduced	[166]
Normoxic-preconditioned BM-MSC secretome	TBI	Adult male rats	Administered intravenously of normoxic--preconditioned BM-MSC secretome	Greater number of newly forming neurons and significantly less than the controls in brain-damaged volume and apoptosis	[167]
Bone marrow-derived mesenchymal stem cells	Optic nerve crush	Adult rats	MSC transplantation	Significant increase IN the number of Tuj1 and Brn3a positive cells in the retina and the expression of FGF-2 and interleukin-1β in the ganglion cell layer of the retina stimulated the axon regeneration	[168]
Bone marrow- derived mesenchymal stem cells	Glutamate excitotoxicity	Mice embryos	MSC conditioned medium	MSC-mediated neuroprotection against glutamate excitotoxicity involved a reduced AMPAR function containing NMDAR and GluR1 and a TNF-mediated neuroprotection	[169]
Bone marrow- derived mesenchymal stem cells	Parkinsonian model	Adult female rats	Intravenous MSC administration	Neuroprotection of dopaminergic neurons at least partly through anti-apoptotic effects of SDF-1α.	[170]
Human bone marrow mesenchymal stem cells	Neurodegenerative disorders	Brain stem neuronal cell cultures	MSC conditioned medium	Protected catecholaminergic and serotonergic neuronal perikarya and transporter function from oxidative stress through the secretion of glial-derived neurotrophic factor	[25]
Bone marrow-derived mesenchymal stromal cells (BM-MSCs) Mesenchymal stromal cell-conditioned media (MSC-CM)	Multiple sclerosis	Phase I/IIa clinical study human patients	BM-MSCs and CM-BM-MSC were injected intrathecally into patients	A decrease of 4 and 3.5 points on the EDSS was achieved in two patients; decreased lesion	[171]
Bone marrow-derived mesenchymal stromal cells (BM-MSCs)	Chronic demyelinating murine model	6-week-old C57BL/6 mice	Intraventricular injections of MSCs	Increased neural stem progenitor cell (NSPC) proliferation also activated endogenous functional remyelination	[50]
Bone marrow-derived mesenchymal stem cells	Variety of neurological diseases	Neuronal cultures were prepared from the cortices of E16 rat embryos	MSC conditioned medium	Promoted neuronal survival in vitro and activated the PI3kinase/Akt pathway and reduced p38 signaling	[172]
HUCPVC conditioned medium (CM)	Axonal injury	Primary cultures of rat embryonic hippocampal neurons	MSC conditioned medium	Promoted axonal outgrowth in CNS neurons and this effect was mediated by BDNF	[173]

Table 3 (continued)

Source	Pathology	Model	Application	Advances	References
Induced mesenchymal progenitor cells (MiMPCs)	Peripheral nerve injuries	Dorsal root ganglia from chick embryos	MSC conditioned medium	Promoted neurite outgrowth via neurotrophin and cytokine production	[174]
Bone marrow-derived mesenchymal stem cells (MSCs)	Neurological disorders involving the cerebellum	Neuronal cultures from the cerebella of E18 rat embryos	MSC conditioned medium	Protected cerebellar neurons against toxic insults via modulation of both the phosphatidylinositol 3-kinase/Akt and MAPK pathways	[146, 175]
Human umbilical cord-derived mesenchymal stromal cells (UC-MSCs)	Oxygen-glucose deprivation	Cortical neuron primary cultures from embryonic day 16 fetuses	Co-cultured with UC-MSCs	Neuroprotection in neonatal cortical neurons Reduction in the number of neurons displaying signs of apoptosis/necrosis	[176]
Limbus stroma-derived mesenchymal stromal cells (L-MSCs)	Brain ischemic injury	Both rat primary cortical neuron culture (1–2 days old) and adult male rats	Normoxic and hypoxic conditioned media	Neurotrophic factors stimulated neurite outgrowth and protected neurons against brain ischemic injury	[177]
hUCB-MSCs	Intraventricular hemorrhage (IVH)-induced brain injury	Newborn rats	MSC transplantation	BDNF secreted by transplanted MSCs play a seminal role in attenuating severe IVH-induced brain injuries in newborn rats	[178, 179]
Umbilical cord-derived mesenchymal stromal cells (UC-MSCs)	Neonatal intraventricular hemorrhage model	Neonatal mouse model at postnatal day 5	Intravenously administered UC-MSCs	Attenuated periventricular reactive gliosis, hypomyelination, and periventricular cell death	[180]
Bone marrow-derived mesenchymal stem cells (MSCs)	Oxygen-glucose deprivation (OGD) model of cerebral ischemia	Primary rat cortical neurons from the cerebral cortex rats at embryonic day 17	MSC conditioned medium	Rescued cortical neurons from apoptotic cell death Increased phosphorylation of STAT3 and Akt	[181]

ultrastructure not only preserves the number of mitochondria, but also the integrity of their crests [259].

It has been reported that the polarity index, considered as a parameter related to cell migration and cell morphology, is positively affected by the CM. A-MSCs improved cell morphology and the polarity index of the astrocytic cell line T98G [259] and in human-like astrocytes subjected to glucose deprivation and mechanical injury (*scratch*) [260]. These results not only provide evidence of the protective effect of biomolecules, but also open the venue of a possible new therapeutic alternative in the protection of astrocytes against TBI. A plethora of evidence shows that the protective effect of MSCs can be exerted through regulation of oxidative stress, improvement of cell migration, and mitochondrial protection mediated by factors and/or bioactive neuroregulatory molecules. However, the mechanism by which these molecules present in CM-MSCs exert protection on glial cells has not been fully elucidated and more studies are still needed. In an in vitro ischemic human astrocyte model, it was found that CM-MSCs positively regulated IL-1β as a cytoprotective factor against cell death [264]. Also, in an astrocytic (T98G) model subjected to glucose deprivation and mechanical injury (*scratch*), the genetic silencing of neuroglobin, a protein that is considered to have neuroprotective effects, prevented the protective action of CM-A-MSC [259]. Finally, another study [261] showed that the paracrine factors of MSCs promote the survival of astrocytes by a mechanism associated with inhibition p38 MAPK and JNK, regulation of p53 and STAT1, and downregulation of GFAP after ischemic stroke in vitro [261] (Fig. 2). Therefore, it is believed that the factors present in the CM may mediate the expression or signaling cascade of molecules that favor the survival and recovery of important cellular functions in the face of different insults.

Differences of the Secretome of the Mesenchymal Stem Cells Depend on Cell Type, Location, and Methodology Used in the Analysis

For the study of MSCs, different techniques have been developed. Some studies are based on the approach of shotgun

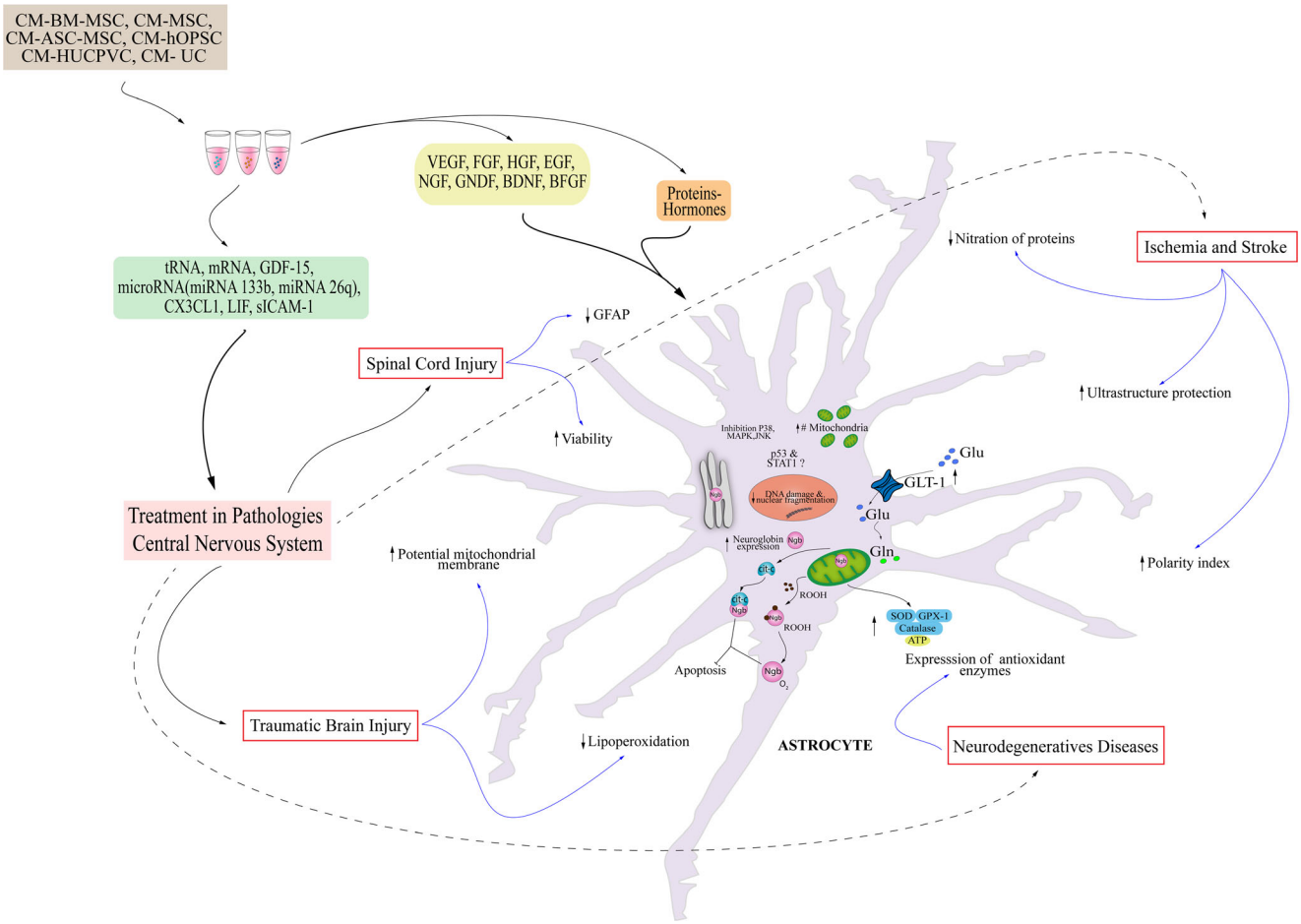

Fig. 2 Effect of the conditioned medium of mesenchymal stem cells (CM-MSC) on glial cells and diseases of the central nervous system. There are very few studies that evaluate the effect of CM-MSC on glial cells. Among the reported findings are the protection of cell viability, mitochondrial functions and ultrastructure, as well as the increase in the polarity index related to cell migration capacity. Similarly, ROS reduction, GFAP expression, modulation of p38/MAPK and JNK, and regulation of p53 and STAT have been described as well. All these protective characteristics mentioned above convert the secretome of mesenchymal stem cells into an alternative therapeutic treatment in diseases that affect the CNS. As an example, an improvement in the integrity of proteins and lipids in models of ischemia and TBI has been described through the reduction of oxidative stress. In fact, the conditioned medium of mesenchymal stem cells derived from adipose tissue increases the expression of the neuroglobin protein

proteomics and on the proteomic approach based on candidate proteins. Other methodologies have focused on changes of protein expression using MSCs cells, their conditioned medium, or only from extracellular vesicles in models of response to the disease, cellular differentiation, or undifferentiated cell states [26, 96]. Considering the above, the great variety of approaches in proteomics studies and MSC secretome analysis do not yet provide a clear, complete, and reliable knowledge of the molecules secreted from MSCs. One instance could be the study conducted by Kapur and Kats [244] and Makridakis et al. [26], who assessed a compilation of the proteins observed in the shotgun proteomics of the CM-A-MSC and CM-BM-MSC studies. The authors point out how the characterization and detection of the secretome protein differ with respect to the type of cells that are analyzed, secretome preparation method, and detection method [26, 244].

Most proteomic studies are based on the analysis of different stages of differentiation (e.g., from the adipose stem cell to a fully differentiated adipocyte) and then the comparison of the up- or downregulation of genes. Nevertheless, each study is circumstantial and shows only a few proteins similarly expressed. Altogether, this evidence implies a great need for a more biology-related data. The increasing number of proteins that are identified in different environments (pathological or non-pathological) is creating a great understanding of protein functions by placing them in different locations at different levels of expression. However, some precautious must be noticed because some of these proteins may be misplaced and represent artifacts, while others may be misinterpreted [26, 96, 103, 244]. For example, differences that can be found between the proteomic analysis between CM-MSCs and MSCs, depending on the location of the protein in the cell could represent different related functions with the moment depending on

its subcellular location (peri/extracellular) [103]. This represents a key obstacle in what is the function of each molecule in response to a pathological event and therefore will be related to the potential action upon a therapeutic use. However, it is essential to perform a thorough and careful characterization of the secreted molecules as well as to establish complete and uniform guidelines and criteria for obtaining them so that it can be applied and produced for clinical use.

Conclusions and Further Directions

It is a fact that not only the MSCs but also the paracrine factors and/or bioactive molecules synthesized and secreted by these cells have great benefits on the nervous system by relieving and reducing the impact of the lesions on brain functions. MSCs' secretome holds a promise for the regeneration of neurites, neuronal protection, preservation of astrocytic functions, repair of cell bioenergetics, reduction of excitotoxicity and ROS generation, and promotion of axon growth. Recent research has revealed different actions induced by MSCs' secretome including inhibition of pathways related to cell death and activation of cell survival and protection signals. It is important to further study the effects of MSCs' secretome on glial cells since previous studies focused exclusively on neurons.

It is also important to extend the studies on the mechanism of action of the factors and/or bioactive molecules present in the MSCs' secretome on pathologies such as TBI, ischemia, and stroke. Pre-clinical and clinical studies may allow advances in the knowledge of the effectiveness of CM-MSCs; therefore, these agents may become a promising treatment in neurological conditions for which there are not still effective therapies. This approach may solve the problems of differentiation and migration of the MSCs to the site of injury and would add the advantages that the use of exosomes or specific biological factors secreted by from MSCs will facilitate the transport, storage, and management of the therapeutic preparations at the clinical stage.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

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