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The Therapeutic Potential of Mesenchymal Stem Cell–Derived Exosomes in Treatment of Neurodegenerative Diseases

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Abstract
Neurologic complications are commonly regarded as irreversible impairments that stem from limited potential of regeneration of the central nervous system (CNS). On the other side, the regenerative potential of stem cells has been evaluated in basic research, as well as in preclinical studies. Mesenchymal stem cells (MSCs) have been regarded as candidate cell sources for therapeutic purposes of various neurological disorders, because of their self-renewal ability, plasticity in differentiation, neurotrophic characteristics, and immunomodulatory properties. Exosomes are extracellular vesicles which can deliver biological information over long distances and thereby influencing normal and abnormal processes in cells and tissues. The therapeutic capacity of exosomes relies on the type of cell, as well as on the physiological condition of a given cell. Therefore, based on tissue type and physiological condition of CNS, exosomes may function as contributors or suppressors of pathological conditions in this tissue. When it comes to the therapeutic viewpoint, the most promising cellular source of exosomes is considered to be MSCs. The aim of this review article is to discuss the current knowledge around the potential of stem cells and MSC-derived exosomes in the treatment of neurodegenerative diseases.

Keywords Stem cell · Central nervous system · Regeneration · Neuroprotection · Exosome · Neurodegenerative diseases

Introduction

The increasing prevalence of central nervous system (CNS) disorders has been attributed to neurodegeneration, as characterized in diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), and multiple sclerosis (MS) [1]. A common characteristic among such disorders is the accumulation of misfolded proteins over time, such as tau, amyloid-β, or α-synuclein [2]. Over the past decades, the knowledge of etiopathogenesis of neurodegenerative diseases has been improved; however, a precise and reliable treatment has not yet been accomplished [3]. There are several factors which have contributed to this, for example, the inability to put experimental outcomes into prosperous practice in clinical treatments. In addition, the complexity of the CNS, as well as the multi-facet nature of CNS diseases, has been regarded as the greatest challenge for researchers to overcome the difficulties in diagnosis and therapy of such diseases [4].

Intercellular communication through extracellular vesicles (EVs) may prove to be an effective therapeutic strategy for a variety of diseases [5]. EVs are classified based on their origin and size, and include exosomes, microvesicles, and apoptotic bodies. Exosomes have a diameter size of 30–100 nm and originate in endosomal compartments, known as multivesicular bodies. Nowadays, there is a consensus that exosomes are involved in communications between cells through the transfer of lipids, proteins, RNA and microRNA (miRNA), and membrane receptors [6]. These properties

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allow the exosomes to transfer biological messages to various cells within the body, in order to modulate their physiological function [7]. Human mesenchymal stem cells (MSCs) have an important role in tissue homeostasis and they are able to modulate cell activity through direct cell-to-cell interactions or by secreting soluble biological mediators, such as exosomes. Exosomes derived from MCS have an important role in tissue repair, immunosuppression, and immunomodulation; therefore, they are a very promising tool for targeting neuronal regeneration in neurodegenerative diseases.

An additional challenge for treating neurodegenerative diseases is that most drugs are unable to cross the blood–brain barrier (BBB), which is problematic for neuropharmacological drug delivery. Interestingly, exosomes can be transferred across the BBB; therefore, they offer a solution to this drug delivery problem by improving the transport of drugs into the brain. Furthermore, using sophisticated techniques, it is possible to engineer exosomes to target a desired tissue or location more precisely [8].

In this review, we will present the current literature on exosomal signaling and how these biological signaling molecules can be utilized as a therapeutic tool. Additionally, this review will discuss advances in the field of MSC-derived exosomes, particularly their ability to reduce neuroinflammation while promoting neuronal repair and regeneration. This review will outline how these unique properties of MSC-derived exosomes suggest they should be strongly considered in the search for new treatment strategies of neurodegenerative disorders.

Prospects for Stem Cells in Treatment of Neurodegenerative Disease

Among the sophisticated treatment approaches, application of stem cells is of particular interest in the literature. Stem cells have the potential to perform self-renewal and are categorized based on their ability to differentiate into a particular tissue or cell type. Totipotent stem cells can give rise to all human cells, while pluripotent cells can develop into the tissues originating from the three main germinal layers, including mesoderm, endoderm, and ectoderm. However, multipotent stem cells differentiate into specific lineage of cells and are classified as either adult or embryonic stem cells. Researchers have assessed the benefits and limitations of both adult and embryonic stem cells for use in clinical practice [9]. Embryonic stem cells have a major clinical advantage due to their ability to differentiate into a vast variety of cell types. On the other side, there are ethical issues as these stem cells are derived from embryos. Additionally, embryonic stem cells should be used with caution due to the possibility of stimulating an undesirable immune reaction or cancer development. In comparison with embryonic stem cells, adult stem cells have a diminished capability, but they also have a reduced risk of stimulating an immune response or tumor development. Moreover, ethical issues are not raised against using these cells in the clinic [10]. Researchers have managed to successfully reprogram adult somatic cells to develop into pluripotent stem cells, called induced pluripotent stem cells (iPSCs), through activation or transfection of certain transcription factors, such as Oct3/4, Sox2, Klf4, and c-Myc [11, 12]. This intervention could produce patient-matched stem cells, which resolve ethical and immune rejection issues. Currently, these cells are utilized experimentally to model several diseases, including neurological diseases. The reason for tumor development by these cells occurs due to impaired reprogramming or incorrect inhibition of tumor suppressor genes which has limited their application in clinical trial [13].

MSCs are considered multipotent stem cells that possess neuroprotective properties. MSCs are currently isolated from several sources, such as bone marrow (BM), umbilical cord (UC), and adipose tissue [14]. These cells encompass promising cellular characteristics that suggest they will have therapeutic potential in CNS complications. Among these beneficial features is the ease of isolation through almost noninvasive approaches, simplicity of culturing, and proliferation. Moreover, MSCs possess low immunogenic properties, they are less likely to develop tumors, and their utilization has no ethical controversies [15]. Researchers have established the beneficial effects of MSCs in the treatment of CNS diseases, such as PD [16, 17]. Furthermore, human MSCs, producing brain-derived neurotrophic factor (BDNF), delivered these beneficial factors to the site of injury after being transplanted in the brain of a rodent PD model [18]. Moreover, MSCs isolated from UC can improve angiogenesis in the brain following stroke [19], while MSCs isolated from adipose tissue enhance motor neurons in an animal model of amyotrophic lateral sclerosis (ALS) [20]. Interestingly, it has been shown that the conditioned medium from mesenchymal stem cells (isolated from human adipose tissue) preserved mitochondrial function and increased wound closure in astrocytic cells subject to a scratch assay and metabolic injury (glucose withdraw) [21, 22]. These protective actions are thought to be mediated by neuroglobin, a member of the globin family [23, 24]. In this regard, this conditioned medium was able to upregulate neuroglobin expression in astrocytes, while blockade of neuroglobin using iRNA has been shown to dampen the neuroprotection induced by this medium in astrocytes exposed to scratch injury [25]. These previous studies demonstrate that paracrine factors, partly mediated by neuroglobin, released by MSCs might produce neuroprotection by improving mitochondrial dynamics and cell survival following mechanical and metabolic damage.

Identification of MSCs as a Well-suited Cell Source

MSCs possess the capacity to be differentiated into several mesenchymal tissues, such as adipocytes, osteoblasts, and chondrocytes [26]. MSCs can be easily extracted and proliferated in vitro, thus contributing to their popularity in experimental research. Nonetheless, more research is required to unveil the complete profile of the functions and activities of MSCs [27].

There are a number of key issues about MSCs that should be considered. It is important to assign a clear difference between MSCs cultured in vitro and in vivo MSCs. A bulk of data regarding MSCs has obtained from the in vitro experiments which may not necessarily mirror the in vivo characteristics [28]. MSCs obtained from in vitro cultures indicate functionally heterogeneous cells [29]. This observation can be applied when considering the cellular content of MSCs and may contribute to the variations in functional features of secreted components, like exosomes. MSC-like cells obtained from various tissues should be distinguished from MSCs, as MSCs exhibit differentiation properties. For example, when BM-derived MSCs were ectopically transplanted into mouse heterotopic bone or marrow structures were generated, dental pulp–derived MSCs produced dentin and pulp tissue after transplantation [30]. Therefore, MSC-like cells originating from different sources display distinct functional properties, suggesting that they may also secrete EVs with different features.

Exosomes

Characterization of Exosomes

Several types of EVs have been characterized over the past three decades. Among such EVs are exosomes which are characterized by a diameter of 30–100 nm, apoptotic bodies that have a 1-μm diameter, and finally ectosomes with the size of 100 nm–1 μm in diameter. Ectosomes include microparticles, microvesicles, and shedding vesicles [31, 32]. Exosomes stem from the endocytic-exocytic pathway, while other EVs originate directly from the plasma membrane of cells. It has been shown that the molecules that are involved in exosomes biogenesis are highly conserved among various eukaryotes. Experimental research has demonstrated that all types of cells, as well as extracellular fluids, possess exosome-like EVs in spite of their differences [33, 34]. It should be noted that assigning an exact distinction among other kinds of EVs is hard due to the complex nature and functions of these different vesicles [35]. However, all EVs share common properties that confer them the potential to function in intercellular communications that result in various kinds of responses in the target cells.

Biogenesis of Exosomes

Characterization of exosomes as an extracellular vesicle first occurred when multivesicular bodies (MVBs) were observed to fuse with the plasma membrane during the maturation process of reticulocytes to red blood cells (RBCs), during which intraluminal vesicles (ILVs) were secreted into the extracellular environment [36, 37]. MVBs then became popular due to their extracellular location and their organization characteristics, where the lumen is considered as same to the cytosolic part of the cell and its membrane is considered as the plasma membrane. Afterwards, a great deal of attempts has been carried out to clarify the biogenesis mechanism of MVBs [38, 39]. The two major biogenesis pathways, endosomal sorting complex required for transport (ESCRT)–dependent and ESCRT-independent pathways, have been identified which orient the cellular content toward ILVs. The machinery components of ESCRT include four compartments, encompassing ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, which are involved in sequestering the ubiquitinated proteins that originate from the membrane toward an endosomal microdomain and then modulate the integration process that finally produces ILVs containing this content. In contrast, the ESCRT-independent pathway integrates cellular content into exosomes which occurs through the budding of ceramide-induced ILVs [40, 41]. The sorting of other proteins is mediated through variations in the canonical ESCRT-dependent approach [42, 43]. In the context of integrating nucleic acids into ILVs, it has been demonstrated that miRNAs possessing the GGAG motif conjugate to sumoylated proteins like hnRNPA2B1 before its integration into ILVs [44].

Content of Exosomes

Researches have demonstrated that exosomes possess proteins that are involved in different cellular activities, including heat shock proteins (such as HSP70), vesicular transport proteins (such as Rab GTPases and annexins), MVB biogenesis components (such as TSG 101 and Alix), integrins (such as CD63, CD9, CD81, and CD82) [45, 46], signal transduction factors (like kinase proteins), cytoskeletal proteins (such as actin and moesin), and enzymes involved in cell metabolism (such as GAPDH, LDHA, PGK1, and PKM) [47]. Markers related to exosomes are commonly found in MVBs. A number of important markers are found in the cytosol, including HSP70 which is involved in microautophagy processes of cytosolic proteins into MVBs [48]. Additionally, the cytosol contains proteins involved in exosome biogenesis, like tumor susceptibility gene 101 protein (TSG101) and Alix which is also

known as programmed cell death 6 interacting protein (PDCD6IP). Conversely, there are some non-cytosolic markers that are membrane-related proteins, such as CD63 (also known as LAMP-3) [49], MHC-II, CD8 [50], and CD9 [51], and proteins found in lipid raft, including flotillin-1 [52] and flotillin-2 [53]. However, it has been demonstrated that there are some proteins that are unique among exosomes, such as Gp96, calnexin, cytochrome C, and GM130 [54]. Exosomal membranes have a lipid profile that shows a lipid raft complex and may contain ceramide, cholesterol, phosphatidylserine, and sphingomyelin [55].

Interaction of Exosomes

A tremendous amount of data suggests that exosomes are involved in transferring biomolecules which participate in a wide range of functions in the target cells [56–58]. Several mechanisms have been hypothesized for uptaking a vesicle inside a cell, which are mainly conducted through endocytosis [59], phagocytosis [60], macropinocytosis [61], and internalization by lipid rafts [62]. Furthermore, a direct incorporation through the plasma membrane has also been demonstrated [63]. Prior to vesicle entry into the target cell, exosomes may attach to the molecules on the plasma membrane by integrins like CD81, CD9, CD51, and CD61, as well as molecules found in extracellular space like heparan sulfate [64]. Upon the orientation of exosomes into endocytic organelles, the decrease in pH enhances fusion of vesicles to the cell membrane [65]. As a result, exosomal lipids can indirectly approach the plasma membrane when they follow through the endocytic way.

Studies show that MVBs prefer to be in close proximity with the RNA-induced silencing complex (RISC). RISC is involved in the incorporation of miRNAs and participates in miRNA biogenesis [66]. It has also been suggested that endocytosed exosomes could act as ILVs and play a role in fusion with internal membrane of MVBs [56]. This process allows RNAs present in the lumen of ILVs to be direct toward the RISC complex in the cytosol. Other content of lumen can also be transferred to the cytosol through this approach.

Evidence also suggests that exosomes may be involved in cellular biology through a ligand-mediated signaling transduction mechanism. Exosomes are involved in delivering the major histocompatibility complex (MHC)–bound antigens to be presented to T lymphocytes for maintaining long-term immune memory or tolerance [67]. Moreover, dendritic cells can deliver exosomes containing MHC-II loaded tumoral antigens and stimulate anti-tumoral responses by T lymphocytes [68]. Subsequently, a bulk of investigations has been carried out utilizing exosome-mediated cargos to deliver various molecules or drugs to the site of injury in diseases, such as PD or AD.

Exosomes and Suppression of Neuroinflammation

Studies have indicated that chronic inflammation in the CNS is involved in the etiopathogenesis of neurodegenerative disorders. Thus, devising new therapies that combat the players of neuroinflammation may provide a potentially sophisticated approach [69]. Numerous studies have indicated that exosomes show anti-neuroinflammatory characteristics in the CNS. It has been shown that exosome-encapsulated curcumin suppresses neuronal inflammation and autoimmune responses induced by myelin oligodendrocyte glycoprotein (MOG) in a mouse model of MS [70]. Moreover, when exosomes were nasally administered, they were quickly delivered to the mouse brains. In addition, exosomes caused a decrease in the frequency of inflammatory microglial cells in the involved sites [70]. It has been shown that dendritic cell–derived exosomes suppress neuroinflammation in vitro and in vivo [71, 72]. Therefore, exosomes may conduct their beneficial effects through modulation of CNS microglial cells through suppression of neuroinflammatory feedbacks.

Exosomes Cross Through Blood–Brain Barrier

The BBB is a physical barrier between the circulating blood and the brain that has an important role in controlling the brain microenvironment. It has been suggested that there are two mechanisms for the entering of exosomes into the CNS. First, exosomes may be uptaken by endothelial cells, then cross into the cell through transcytosis, and finally transferred into the recipient cell [73]. Secondly, exosomes may cross intercellular junctions between endothelial cells and enter into the CNS. It has been shown that exosome-associated miR-105 can downregulate the expression of ZO-1, a critical molecular component of tight junctions, and, therefore, demolish the barrier action of endothelial cells [74]. Exosomal miR-181c has been shown to down-modulate 3-phosphoinositide-dependent protein kinase-1 (PDPK1) expression, resulting in lower levels of phosphorylated cofilin and aberrant polymerization of actin in endothelial cells from the brain [75]. Furthermore, exosomes can enhance vascular leakiness, allowing exosomes to enter the target tissue. Additionally, exosomes secreted by pathologic cells can alter vascular permeability and sometimes the integrity of BBB contributing to the development of neurodegenerative diseases. A similar effect is observed with human dental pulp stem cell–derived exosomes which enhanced vascular permeability in BALB/c mice [76]. However, it should be recognized that increased permeability of the BBB has been associated with several neurological diseases and, thus, further research is still needed to disclose the possible advantages and disadvantages of the therapeutic potential of exosomes with respect to modulation of BBB permeability.

Manipulation of Exosomes

Several approaches have been devised to engineer exosomes. A bulk of investigations have focused on modulation of exosomes to increase the integration capacity of vesicles with the target cell via improved ligand/receptor binding, resulting in increased endocytosis potential. In a previous study, exosomes containing GE11-conjugated platelet-derived growth factor receptor (PDGFR) was evaluated. The GE11 peptide has a high affinity for the epidermal growth factor receptor (EGFR), which is abundantly found on various human cancerous cells with an epithelial origin. It was shown that GE11-conjugated PDGFR exosomes harboring let-7 could suppress the tumor development [77]. In addition, engineered EVs expressing the neuron-specific rabies viral glycoprotein (RVG) peptide conjugated with LAMP2b were used to transfer a small interference RNA (siRNA) to target μ-opioid receptors in the mouse brain. Importantly, this experiment indicated successful outcomes in the treatment of addiction to morphine [78]. This data suggests that modulation of exosomes could provide a novel target for enhancing the differentiation potential of a specific cell type or to decrease neuroinflammation. Furthermore, investigation of specific molecular stem cell characteristics could contribute to the enhancement of targeting via exosomes. In spite of some studies in this context [79], there is still paucity of data in this area.

Despite the limited research on specific markers for characterization of neurogenic niches, there are some studies showing that engineered proteins expressing an extracellular domain enhance integration of exosomes into target cells. Current research has demonstrated that gap junction proteins, such as connexin 43 and connexin 26, are expressed within the neurogenic niche. Connexin 43 has also been shown to be overexpressed by astrocytes [80], while connexin 26 is enriched in the neurogenic niche of the subependymal layer [81]. Such enrichment is probably beneficial since connexin 43 has been demonstrated to mediate exosomal integration, as well as internalization of target cells [82]. Therefore, the extracellular domain of a tetraspanins, such as CD63, could be conjugated to a peptide that binds to connexins [83, 84]. Using an extracellular protein, like tenascin C, also enhances the potential to attain specificity. Tenascin C has been shown to be highly enriched in the subependymal layer obtained from embryonic and adult mouse samples [85, 86]. Tenascin C is chiefly expressed in astrocytes; however, neurons can also express it in some settings [87]. Although tenascin C is found in the extracellular matrix, it has the potential to be targeted in order to integrate exosomes and contribute to its endocytosis into cells located in the niche. It has been shown that a mimetic peptide, which is specifically conjugated to tenascin C, has already been developed with the aim of targeting specific microenvironments [88]. Nonetheless, little has been disclosed regarding enriched proteins in the cells originating from other neurogenic niches, like the spinal cord and subependymal layer. Approaches like transgenic animals and cell sorting methods will continue to contribute to our knowledge in this field [89, 90].

A number of proteins have been recognized that play a role in maintaining precursor differentiation and enriching neurogenic niche, like Notch, EGFR [91], and Noggin/bone morphogenetic protein (BMP) [92]. This is an interesting area in neuroscience and there are various studies attempting to characterize the key phases in maintaining neural stem cells and their differentiation into phenotypically functional CNS cells [93–95]. Therefore, the favorable approach to modulate the neurogenic niche to gain a therapeutic potency is highly challenging.

A number of studies have engineered exosomes by miRNAs to modulate neurogenesis [96, 97]. It has been shown that Notch receptors and ligands that are transmembrane proteins are pertinent to developing an exosome targeted therapy. Hairy and enhancer of split 1 (Hes1) is a well-known target of Notch which has been indicated to suppress neurogenesis. It has been demonstrated that traumatic brain injury triggers Hes1 downregulation in order to enhance neurogenesis. Therefore, targeting downregulation of Hes1 in the subgranular zone in the hippocampus through administration of RNA interference (RNAi) leads to a remarkable enhancement in neuronal growth and improved cognitive abilities following traumatic brain injury in rodents [98]. Additionally, a number of miRNAs have been recognized to target Notch and related signaling factors, such as Hes1. miR-9 is one of the most assessed factors involved in neurogenesis [99] which has been shown to downregulate Hes1 levels and enhance neuronal differentiation [100], while miR-124 is upregulated in the subventricular zone after stroke, implying that it has a role in functional recovery of neuronal injuries [101]. It has also been indicated that expression of let-7 increases the glial fate and that downregulation of let-7 leads to neuronal differentiation [102]. Inhibition of the protein deacetylase Sirtuin 1 (SIRT1, also known as NAD-dependent deacetylase sirtuin-1) increases the generation of new oligodendrocyte precursor cells in the brain and ameliorates clinical symptoms in animal models of demyelination injuries [103]. As a consequence, miRNAs which regulate SIRT1, such as miR-204-5p, could be targeted to beneficially load exosomes to the subventricular zone in the treatment of CNS disorders [104]. Let et al. [105] used exosomes derived from MSCs to deliver exogenous miRNAs to neural progenitor cells and astrocytes in order to correct the expression of target genes. Therefore, considering the potential of engineered MSC-derived exosomes in targeting a specific cell through modification of pertinent surface molecules in exosomes may contribute to a beneficial approach for delivering miRNAs to target cells in the neurogenic niche in diseases.

Prospects for MSC-Derived Exosomes in Treatment of Neurodegenerative Disorders

Since exosomes can be obtained from different cells sources to mediate neuroprotective and neurotherapeutic functions (Fig. 1), investigations have been focusing on the best cell source to generate and deliver exosomes to the CNS niche. It has long been postulated that MSCs isolated from dental pulp originate from the cranial neural crest cells, a precursor that can develop into neural cells [106]. Nonetheless, it has recently been shown that dental pulp–derived MSCs also originate from peripheral glial cells [107]. Therefore, unlike other mesodermal tissues such as bone marrow and adipose tissue, dental pulp–derived MSCs could be appropriate for stimulating the neural differentiation and should be clinically utilized to ameliorate neurodegeneration. This hypothesis is supported by researchers who have demonstrated that dental pulp–derived MSCs can be developed into neurons [108–110]. Dental pulp–derived MSCs were transplanted into rats with spinal cord complications, which produced positive outcomes and ameliorated injury symptoms [111]. Conversely, treatment with bone marrow–derived MSCs failed to produce the same beneficial outcomes. A paracrine mechanism was suggested as the major mechanism of improvement of neuroregeneration [111]. This was supported by Nosrat et al. [112] who reported that dental pulp–derived MSCs secreted neurotrophic factors that recovered motor neurons following spinal cord injury. These studies clearly demonstrate that dental pulp–derived MSCs possess specific neurogenic characteristics which could be therapeutically applied in the treatment of neurodegenerative diseases.

Exosomes have produced beneficial effects in a variety of models of neurodegenerative diseases, such as Parkinson's disease. 6-Hydroxydopamine (6-OHDA) is commonly used as an in vivo and in vitro model of Parkinson's disease because it triggers selective apoptosis of dopaminergic neurons. Jarmalavičiūtė et al. [113] reported that exosomes obtained from human dental pulp stem cells were able to suppress apoptosis of dopaminergic neurons following treatment with 6-OHDA. However, if the same stem cells were cultured through normal settings, the exosomes failed to inhibit apoptosis, demonstrating that culture situation has an important impression on the properties of the exosomes. 6-OHDA induces apoptosis through the generation of reactive oxygen species (ROS) [114], suggesting that exosomes can decrease the sensitivity of dopaminergic neurons to oxidative stress. Future investigations are required to determine the specific proteins or miRNAs which account for theses neuroprotective characteristics of exosomes.

Further investigations have utilized exosomes obtained from MSCs to improve cell survival during oxidative stress. Human UC-MSC-derived exosomes were observed to decrease cisplatin-mediated renal oxidative stress, as well as apoptosis [115]. Additionally, it has been shown that

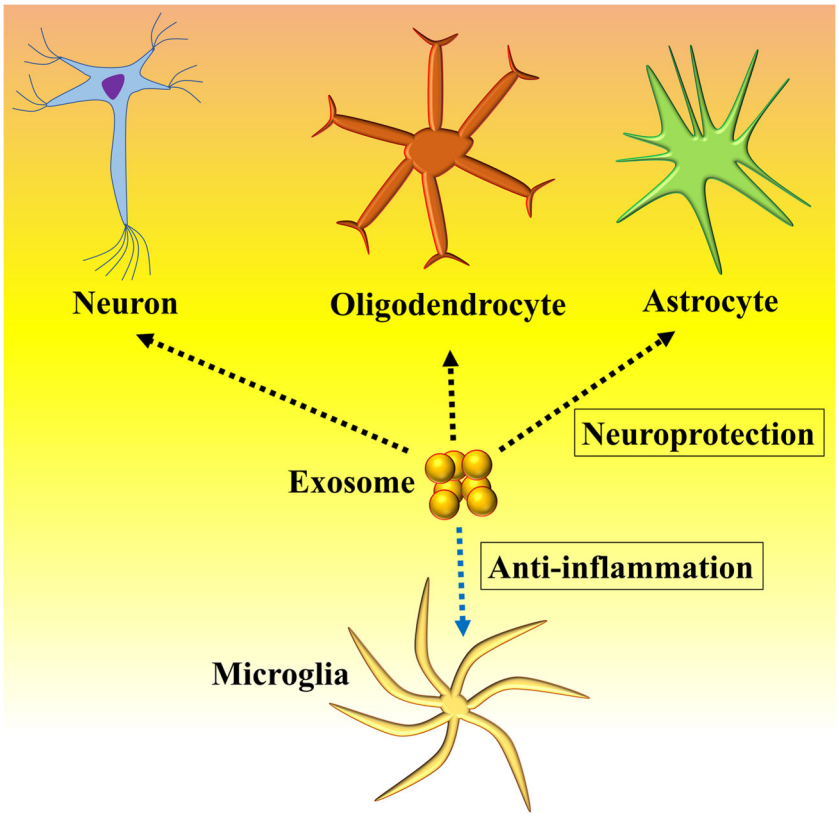

Fig. 1 Schematic illustration of the possible capabilities of exosomes derived from various mesenchymal stem cells in ameliorating neurodegenerative settings. Exosomes may have neuroprotective activity through protecting neurons from different injuries and supporting the oligodendrocytes. Exosomes may convey anti-inflammatory messages to the CNS niche by imposing neuroinflammatory behaviors to microglial cells and modulating them to produce neurotherapeutic factors

chaperone αB crystalline, a pigment produced by the human retinal epithelium, is found inside exosomes and this may play a protective role against oxidative stress in retinal cells [116]. Exosomes produced by mouse macrophages remarkably enhanced the cell survival against 6-OHDA-induced injury [71]. Interestingly, exosomes caused a reduction in ROS levels in activated macrophages regardless of whether they were loaded with catalase, implying that exosomes might function in a similar way in microglial cells under neuroinflammatory conditions. Moreover, in vivo studies suggested that exosomes loaded with catalase caused a decrease in microgliosis and improved the survival of dopaminergic neurons in mice which were treated with 6-OHDA [71]. As a result, it is suggested that stem cell–derived exosomes produce neuroprotective through reduced oxidative stress. These anti-oxidant effects are particularly important in neurodegenerative diseases as oxidative stress is a large contributor to disease progression.

Several studies have suggested a neurotherapeutic behavior of exosomes obtained from adipose tissue–derived MSCs. Adipose tissue–derived MSCs can produce neprilysin-bound exosomes [117]. As a type II membrane–associated metalloendopeptidase, neprilysin is reportedly a critical proteolysis in the cleavage of β-amyloid. It was demonstrated that the expression and function of neprilysin were reduced in patients with AD [118]. Exosomes derived from adipose tissue–derived MSCs were shown to display neprilysin-related enzyme activity and were involved in reducing β-amyloid levels in neuroblastoma cells. In addition, exosomes obtained from adipose tissue–derived MSCs expressed greater amounts of neprilysin in comparison with bone marrow–derived MSCs, emphasizing functional and activity differences in exosomes obtained from different sources [117]. Considering these observations, it would be interesting to evaluate the in vivo therapeutic power of exosomes obtained from adipose tissue–derived MSCs by applying them to animal models of neurodegeneration. It was also been indicated that exosomes obtained from murine adipose tissue–derived MSCs improved the survival of human neuroblastoma cells and protected the murine hippocampal neurons with oxidative damage [119]. These exosomes displayed an inverse dose-dependent effect with regard to the viability of cell. Additionally, this study by Farinazzo et al. [119] found that exosomes obtained from murine adipose tissue–derived MSCs enhanced remyelination and stimulated the progression of oligodendroglial progenitors. These beneficial effects are supported by Bonafede et al. [120] who demonstrated the neuroprotective effects of exosomes obtained from murine adipose tissue–derived MSCs in an in vitro model of ALS. In this study, a motor neuron–like cell line which highly expressed human superoxide dismutase 1 (SOD1) mutants and was therefore under oxidative stress was used to demonstrate that exosomes protect motor neuron–like cells from oxidative injury. These results highlight the potential for exosomes to be applied as a therapeutic tool to treat motor neuron disorders [120].

Currently, bone marrow is the most prevailing source of MSCs. A majority of research has evaluated the therapeutic potential of exosomes obtained from bone marrow–derived MSCs in ameliorating the clinical course of various neurologic disorders. In one study, it was indicated that exosomes obtained from MSCs were accompanied with a significant improvement of outcomes in the treatment of therapy-refractory graft versus host disease [121]. Furthermore, MSC-derived exosomes and MSCs were both beneficial in improving the post-stroke generation of neurons in mice. Additionally, exosomes were reported to improve peripheral immune responses following ischemic stroke by alleviating the quality and quantity of immunosuppression [122]. Studies have also found that exosomes obtained from rat bone marrow–derived MSCs produce enhanced recovery and neurovascular plasticity following traumatic brain injury and stroke [123, 124]. Exosomal miR-133b is suggested to be the primary mechanism involved in enhancement of brain recovery in stroke. Xin et al. [125] reported that MSCs rebalanced the expression of miR-133b in ischemic brain tissues. Ischemic settings were observed to cause an overexpression of miR-133b in exosomes obtained from MSCs and, therefore, resulted in enhancement of neuron growth through suppression of RhoA expression. miR-133b is expressed in the midbrain dopaminergic neurons and is downregulated in the midbrain tissue of PD patients [126]. As a result, miR-133b should be used for designing therapeutic approaches in the future through MSC-derived exosomes. Exosomes can also be derived from MSCs isolated from umbilical cord tissue that has demonstrated significant therapeutic capacity in preclinical investigations [127]. Nonetheless, no clinical study has been conducted to date to evaluate the neuroprotective potential of exosomes obtained from umbilical cord–derived MSCs.

The research presented here clearly demonstrates the promising therapeutic potential of exosomes derived from MSCs in the treatment of neurodegenerative disorders. Nonetheless, a number of caveats and flaws should be considered. Further research is required to profile the proteins, mRNA, and miRNAs in exosomes derived from MSC-like cells obtained from various sources. Additionally, MSCs which have been differentiated into proinflammatory and anti-inflammatory phenotypes need to be compared for the content of exosomes they produce [128]. In addition, it is necessary to systematically compare the therapeutic characteristics of exosomes obtained from various MSCs. The first step to meet these insufficiencies should be in vitro investigations, followed by experimental models of neurodegenerative disorders to assess the functionality of the various exosomes. Such outcomes will pave the way toward simplified choosing of well-suited exosomal sources to battle neurodegenerative disorders.

Conclusions and Future Directions

Currently, there is an accumulating amount of evidence showing that exosomes obtained from various sources of stem cells, especially MSCs, have neurotherapeutic potential and can be successfully applied for the therapy of several neurodegenerative diseases. Furthermore, the development of genetically modified exosomes derived from MSCs might illuminate a new horizon for devising therapeutic strategies for the treatment of neurodegenerative diseases. Nonetheless, we should first overcome the issues in the way toward extended application of exosomes in clinical practice. Different studies commonly apply different approaches in isolation, quantification, and characterization of exosomes which may contribute to some of the controversies arising about certain experimental conclusions. Oftentimes, a standard protein quantification approach is employed to quantify exosomes, while nanoparticle tracking analysis and empirical activity units have also been carried out to quantify exosomes derived from the soluble phase of cell sources. Therefore, it is tough to put the results next to each other and perform a reliable comparison of the findings. Furthermore, it is currently imperative to conduct an extensive profiling of the proteins and RNAs, in order to test the functionality of exosomes through a standardized setting to achieve reliable and reproducible results which will improve our understanding of the exosomes therapeutic potency. It should also be noted that exosomes are complicated compartments and possess a variety of functional proteins and RNA molecules that may contribute to safety concerns. As a consequence, future studies should proceed with caution and focus on modulating exosomes in a way to specifically orient them toward the desired target, as this may result in favorable therapeutic outcomes with little side effects.

Authors' Contributions Designed and conceived the idea: AMG, NK, HAT, AS

Wrote the manuscript: AMG, NK, GEB, HAT, AS

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Compliance with Ethical Standards

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

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