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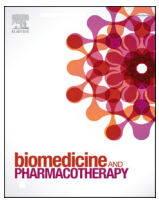

Neural stem cell-derived extracellular vesicles: The light of central nervous system diseases

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ABSTRACT

Central nervous system (CNS) diseases are the leading cause of death worldwide. By performing compensatory functions and improving the inflammatory microenvironment, the transplantation of neural stem cells (NSCs) can promote functional recovery from brain injury, aging, brain tumours, and other diseases. However, the ability of NSCs to differentiate into neurons is limited, and they are associated with a risk of tumourigenicity. NSC-derived extracellular vesicles (NSC-EVs) can modulate the local microenvironment of the nervous system as well as distant neuronal functions. Thus, cell-free therapy may be a novel remedy for CNS disorders. This article reviews the characteristics, contents, and mechanisms of action of NSC-EVs as well as their roles and application prospects in various CNS diseases.

1. Introduction

Neural stem/progenitor cells (NSCs/PCs), which are the most primitive undifferentiated cells in the central nervous system (CNS), can self-renew and differentiate into neural cells (neurons, astrocytes, and oligodendrocytes) [1]. Mammalian endogenous NSCs (eNSCs), which are important for neurogenesis, exist in the subventricular zone, subgranular zone of the hippocampal dentate gyrus (DG), and ependymal zone of the spinal cord [2]. Normally, they are in a quiescent, undifferentiated, and dormant state; thus, are referred to as quiescent NSCs (qNSCs). However, they can be activated, after which they can proliferate, differentiate into neural cells, migrate to repair damaged tissues in response to external stimuli, such as brain injury or cerebral ischaemia [3,4]. Their small number and limited proliferation and differentiation potential prevent them from effectively replenishing damaged neural tissues, and to some extent, this limits their functioning in restoring severe neurological dysfunction [5,6]. Therefore, maximising the neuro regenerative effects of eNSCs has been a major research topic in CNS diseases.

Exogenous NSCs include neural stem cell lines isolated, cultured, and established from human, murine, and other primate embryos. Pluripotent stem cells can also be programmed to generate NSCs. Several preclinical studies have shown that the transplantation of exogenous NSCs can also have a therapeutic effect in neurological diseases [7,8], and possibly, this effect is related to cytokines, such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), or nerve growth factor (NGF). However, exogenous NSC transplantation is controversial given that the engraftment efficiency is very low (less than 5%) and that transplantation often results in a severe inflammatory immune microenvironment [9,10]. Recent studies have shown that the paracrine effect of stem cells may be a safer and more effective therapeutic strategy for replacing cellular therapies [11]. Additionally,

Abbreviations: ASD, autism spectrum disorder; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CSF, cerebrospinal fluid; DG, dentate gyrus; EAE, experimental autoimmune encephalomyelitis; ECs, endothelial cells; eNSCs, endogenous neural stem cells; ESCRT, endosomal sorting complex required for transport; EVs, Extracellular vesicles; GBM, glioblastoma; GSCs, glioma stem cells; GSC-Exos, glioma stem cell-derived exosomes; HGG, high-grade gliomas; hNSC-Exos, human neural stem cell-derived exosomes; HS, heat shock; htNSCs, hypothalamic neural stem/progenitor cells; iNSCs, induced neural stem cell-like cells; iPC, induced pluripotent stem; MCAO, middle cerebral artery occlusion; MSC, mesenchymal stem cell; ncRNAs, noncoding RNAs; NPC, neural progenitor cell; NSCs, neural stem cells; NSC-EVs, neural stem cell-derived extracellular vesicles; NSC-Exos, neural stem cell-derived exosomes; OGD, oxygen-glucose deprivation; PD, Parkinson's disease; piRNA, P-element-induced wimpy testis-interacting RNA; PIWI, P-element-induced wimpy testis; PCs, progenitor cells; PTX3, pentraxin 3; qNSCs, quiescent neural stem cells; SN, substantia nigra; VEGF, vascular endothelial growth factor; VD, vascular dementia.

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extracellular vesicles (EVs), with greater advantages and therapeutic potential, are emerging as a new research topic [12].

EVs are membrane-bound organelles released by all cell and tissue types and contain cargo, such as cytosolic proteins, mRNA, miRNA, and even DNA [13,14]. They are also composed of exosomes (40–100 nm diameter), microvesicles (100–1000 nm diameter), and apoptotic bodies. The exosomes and microvesicles are derived from resting or stimulated cells, whereas apoptotic bodies are derived from dying cells [15]. EVs can cross the blood brain barrier (BBB), diffuse into the cerebrospinal fluid (CSF), and fuse with the plasma membranes of target cells [16]. They also have low immunogenicity; thus, are therapeutically useful in their own right and show promise as carriers in the field of small-molecule drug transport [17]. The bottom line is that EVs have properties that reflect the composition of their cells of origin and they can selectively target cells with similar phenotypes [18,19]. C17.2 neural stem cell-derived exosomes (NSC-Exos) can be loaded with cargo and taken up by endothelial cells (ECs); this process is based on dynamin-dependent endocytosis mediated by heparan sulphate proteoglycans, which act as receptors [20] and suggests that neural stem cell-derived extracellular vesicles (NSC-EVs) may have great potential for use in treating CNS disorders.

Recent studies have also suggested that the use of engineered NSC-EVs may be more advantageous in the future. It is challenging to predict and control the expression profiles of NSC-EVs, and direct regulation of the contents or properties of NSC-EVs can help maximize their therapeutic effects. In this review, we provide an overview of studies on NSC-EVs and their current therapeutic role in CNS disorders, and discuss the future of NSC-EV development.

2. NSC-EVs constituents with therapeutic effects

2.1. miRNA

miRNAs constitute a class of noncoding RNAs (ncRNAs) with length approximately 22 nucleotides, and functionally, they target mRNAs by binding to their 3' untranslated regions (3'-UTRs) [21,22]. Among all the constituents of EVs, miRNAs have been studied extensively and in depth the most [23,24], even though the loading of miRNAs is not random. First, Hsa-miR-1246, has-miR-4488, has-miR-4508, has-miR-4492, and has-miR-4516 were identified as the five most abundant exosomal miRNAs [25]. Second, it was reported that exosome samples from glioma stem cells (GSCs) and NSCs show several differentially expressed miRNAs. Studies have also suggested that this miRNA sorting mechanism is based on the identification of distinct miRNA motifs that are preferentially loaded into exosomes [26]. Further, exosomal miRNAs from NSCs originating from different sources also differ. It has also been reported that the expression of miRNAs is higher in the exosomes of mouse hypothalamic NSCs than in hippocampal NSCs. These differences in exosomal miRNA loading may be responsible for the different roles of different NSC sites. The study of adult neurogenesis has been primarily focused on the hippocampus and subventricular zone of the brain's lateral ventricles. It has also been shown that hypothalamic NSCs are critical for the neuroendocrine regulation of systemic physiological homeostasis. Moreover, hypothalamic NSCs play an important role in controlling the rate of aging in part by releasing exosomal miRNAs [27].

Finally, it has been noted that NSC-Exos are also affected by different conditions and environmental stimuli. For example, the expression of miR-98–3p in exosomes produced by hypoxia-preconditioned NSCs is significantly reduced; thus, their therapeutic effect on stroke is enhanced [28]. Hypoxia also induces and enhances the expression of miR-210–3p in exosomes and this is followed by neural progenitor cell (NPC) uptake and enhanced cell viability [29]. Compared with normal NSC-Exos, pro-inflammatory factor interferon-gamma (IFN-γ) enriched hippocampal NSCs exosomes show higher levels of has-miR-206, has-miR-133a-3p, and has-miR-3656, which play a more important role in cell survival following ischaemic stroke [30]. Additionally, insulin-like growth factor regulates mi-219a-2–3p loading in exosomes from rat NSCs to suppress YY1 expression. This partially suppresses neuroinflammation and promotes neuroprotective effects after spinal cord injury (SCI) [31]. In summary, using NSC-Exos as carriers in cell-to-cell communication by carrying and transferring miRNAs has become a hot research topic.

2.2. piRNA

Murine hypothalamic neural stem/progenitor cells (htNSCs) contain multiple P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs) [32], which typically consist of 24–32 nucleotides and are predominantly found in the reproductive and neural tissues in mammals [33–36]. Unlike miRNAs, piRNAs depend on PIWI-like proteins (including PIWIL1 and PIWIL2) to target many other types of RNA/DNA [37]. A study by Ikhlas suggested that murine htNSC exosomes provide innate immunity against SARS-CoV-2 by attacking and degrading the virus. The study also suggested that exosomes derived from induced htNSCs achieve enhanced antiviral effects via exposure to the SARS-CoV-2 virus or several RNA fragments of the viral genome. Moreover, this previous study revealed that the piRNA-PIWI mechanism is implicated in this process. Although miRNAs are present in NSC-Exos, they cannot exert antiviral effects owing to their ability to target only mRNA. Therefore, this study focused on piRNAs and used PIWI1/2 protein as a biomarker to analyse whether the piRNA mechanism is involved in the antiviral effect of NSC Ex/Mv. The strong fluorescent expression of PIWI2 observed in the cytoplasm and exosomes of NSCs corroborated this idea, suggesting that NSC Ex/Mv exerts antiviral effects via a piRNA-PIWI mechanism and that the antiviral properties of htNSC exosomes with PIWI2 knockdown were attenuated. This previous study also identified mouse species with piRNAs targeting the SARS-CoV-2 genome in both sense and antisense sequences. Notably, large antiviral piRNA libraries enhanced and enriched for these specific antiviral piRNAs owing to exposure to specific viruses or RNA fragments have been established for murine NSC-Exos [32,38]. In summary, the antiviral effects of NSC-Exos may provide initial clues for developing RNA-dependent vaccines.

2.3. LncRNA

LncRNAs are involved in various CNS diseases [39,40], mainly via the competitive adsorption of miRNAs, to further regulate mRNAs, affecting various physiological and pathological processes [41,42]. Recent research shows that rat hippocampal NSC exosomes contain lncRNA myocardial infarction-associated transcript (MIAT) and that hippocampal NSC-Exos ameliorate inflammation and injury in the brains of vascular dementia (VD) rats. In vitro experiments have also demonstrated that damage to hippocampal neurons can be reduced via the MIAT/miR-34b-5p/CALB1 pathway. Moreover, it has also been observed that NSC-Exos significantly improve the learning and memory abilities of VD rats [43].

2.4. CircRNA

circRNAs are more abundant in exosomes than in their corresponding cells. Specifically, exo-circRNAs, which may be regulated by the expression of related genes, can transfer biological activity to recipient cells [44]. Peroneal fornix transection (FF) reportedly blocks major cholinergic input to the hippocampus, resulting in the removal of most cholinergic activity from the hippocampus [45]. However, unlike normal hippocampal exosomes, 14 circRNAs in denervated hippocampal exosomes are upregulated, with circAcbd6 acting as an endogenous miR-320–5p sponge. This inhibited miR-320–5p activity increases the expression of oxysterol-binding protein-related protein 2, and subsequently promotes NSC differentiation [46], suggesting that circRNAs in NSC exosomes play important regulatory roles in the survival and

differentiation of NSCs.

2.5. Proteins

NSC-Exos have a high protein content and these proteins play an important role in the treatment of various systemic diseases (45). Pentraxin 3 (PTX3) is the most abundantly expressed protein in human neural stem cells-derived exosomes (hNSC-Exos), and NSC-Exos with knockdown of PTX3 inhibit anti-inflammatory activity produced by microglia in response to LPS stimulation, suggesting that PTX3 is one of the important cargoes involved in the anti-inflammatory activity of hNSC-Exos [47]. Netrin1 is a functional protein associated with neuronal differentiation that is highly expressed in exosomes derived from NSCs, and it increases the levels of transcription factors, Hand2 and Phox2b in NSCs and BMSCs and promotes the differentiation of BMSCs and NSCs into neurons in vitro. Thus, Netrin1 in NSC-Exos is partially involved in neuronal repair and plays a role in the treatment of spina bifida aperta [48]. Additionally, microvesicles released from human embryonic stem cell-derived NSCs inhibit apoptosis in HL-1 cardiomyocytes by promoting autophagy and regulating AKT and mTOR via the translocation of heat shock protein 70 [49]. All these findings suggest that protein cargo in NSC-Exos plays an important regulatory role in various systems.

Notably, protein encapsulation during NSC exocytosis is not invariable as is the case with miRNAs. According to the results of a proteomics study, there are significant differences in exosomal protein content and enrichment pathways between quiescent and proliferating-stage NSCs. However, exosome inhibitors can delay the transition of proliferating NSCs into quiescence and activate the translation of proteins in quiescent NSCs, such as cell proliferation markers, MCM2, Ki-67, and CycD1, to cause the quiescent NSCs to exit the G0 phase. This suggests that quiescent NSC-Exos discard some proteins, such as ribosomal proteins, to maintain the quiescent state. However, the discard of these proteins does not exclude the possibility of exosomal cargo delivering signals to recipient cells or altering the microenvironment [50]. This implies that the protein cargo of NSC-Exos hides a great deal of information that is worth mining and greatly reflects the altered biological state of parent cells.

Finally, it is noteworthy that NSC-EVs or NSC-Exos carry proteins that mediate the transmission of immune responses between donor and recipient cells. The IFN-γ pathway is highly specifically induced in NPCs exposed to pro-inflammatory cytokines and this effect is mirrored in EVs. Further, the activation of Stat1-dependent signalling in target cells, such as NIH 3T3 cells, results from the intercellular transfer of IFN-γ bound to IFN-γ receptor 1 (Ifngr1) on the surface of EVs, and Ifngr1 on target cell membranes receives IFN-γ delivered by the EV-associated IFN-γ/Ifng1 complex, which activates the Stat1 signalling pathway. This form of EV-mediated communication confirms the ability of NSCs to function as grafts to sense signals and communicate with host immune system. Unmodified or functionalized EVs and exosomes are emerging as promising cell-free anti-inflammatory agents [51].

2.6. Mitochondria

Mitochondrial dysfunction is an established feature of several inflammatory and degenerative CNS diseases. NSC-EVs are enriched in mitochondria with intact activity and conserve mitochondrial membrane potential and respiration. NSC-EVs also favour the release mitochondria that still possess functional properties for transfer to target cells. In vitro assays have also demonstrated that functional mitochondrial transfer from NSCs to L929Rho0 cells successfully restores congenital mitochondrial dysfunction and dystrophy. Further, EV-associated mitochondrial targeting of pro-inflammatory macrophages (Mφ) results in significant gene expression and metabolism changes in the Mφ. It has also been observed that EV treatment with the mitochondrial uncoupling agent, FCCP, or its depletion does not alter Mφ gene expression profile and metabolism. Notably, the immunomodulatory effect of transferred mitochondria on immune cells reflects the activation status of their parent cells. It has also been observed that mitochondria derived from apoptotic cells are potent activators of innate immune response, while mitochondria derived from healthy cells are significantly less inflammatory [52]. In vivo tests have also demonstrated that the intracerebroventricular injection of NSCs consistently releases, and transfers mitochondria via EVs in a mouse model of neuroinflammation and ameliorated clinical deficits [53]. All these findings indicate that NSCs EVs open a new avenue for cell-free therapy to correct mitochondrial dysfunction in the CNS.

3. CNS diseases with NSC-EV implication

3.1. Glioma

Gliomas are common primary tumours of the nervous system [54]. Particularly, high-grade glioma (HGG) shows a poor therapeutic response and has a poor clinical prognosis, with a median survival period of only 15 months, regardless of interventions, including surgery, radiotherapy, and chemotherapy [55]. NSCs can cross the BBB and bring about intrinsic tumour tropism. They can also migrate to hypoxic regions of tumours [56–58]; thus, they have long been expected to function as carriers of anti-tumour drugs. Recent studies have suggested that the ability of NSCs to target drug delivery may be related to the exosomes they produce. STAT3 is an important transcription factor involved in the progression of malignant gliomas [59], and STAT3 antisense oligonucleotides (STAT3ASO) for rapid internalisation by hNSCs upon binding to CpGs, have also been established. Internalised CpG-STAT3ASO fractions have also been co-labelled with CD63-positive intracellular vesicles in the cytoplasm via confocal microscopy, indicating that CPG-STAT3ASO is present in exosome-producing compartments and CpG-STAT3ASO-encapsulated exosomes can effectively knock down STAT3 in target cells and enhance the immunostimulatory properties of CpG-STAT3ASO. This study also showed that glioma-associated myeloid cells (e.g., microglia and macrophages) are activated by CpG-STAT3ASO delivered by NSC-EVs.

Further, the growth of mouse GL262 subcutaneous gliomas is significantly inhibited by NSCs containing CpG-STAT3ASO [60]. NSC-Exos can also load therapeutic miRNAs (antimiRNA-21 and miRNA-100) and deliver them to brain cancer cells. Specifically, the intranasal administration of miRNA-loaded CXCR4-engineered exosomes in an orthotopic glioblastoma (GBM) mouse model showed that the delivered miRNA sensitized GBM cells to temozolomide, resulting in dramatic tumour regression and improved overall survival in mice [61].

The migration of NSCs is affected by the chemokines and growth factors produced by glioma cells. However, studies have shown that this chemotaxis may be attributed to glioma stem cells (GSCs), which constitute a small subset of cells with stem-like characteristics in malignant gliomas. They originate from mutated NSCs, which are primarily responsible for chemoradio therapy resistance in brain tumours. Further, the exosome-sorted miRNAs produced by glioma cells are very different from those produced by NSCs.

Tűzesi explored the miRNA expression profiles of paediatric glioblastoma-derived GSCs and their exosomes. First, the results obtained indicated that there are very few differentially expressed cellular miRNAs between GSCs and NSCs; however, the more differentially expressed cellular miRNAs were detected in the exosome samples. These miRNAs have been validated or predicted to be involved in cancer-related signalling pathways, suggesting that they may play a role in tumourigenesis. Therefore, it was inferred that the exosomal miRNA burden of GSCs is similar to that of NSCs, and both cell types are affected by well-defined mechanisms. Notably, glioma cells are regulated by NSC-Exos, and NSCs are also affected by glioma stem cell exosomes (GSC-exos). In particular, GSC-exo miRNAs alter gene expression in NSCs that are enriched in nervous system development, neurogenesis, or

neuronal differentiation [62]. This evidence is sufficient to confirm that the relationship between the occurrence and development of gliomas and NSCs is intricate. Thus, it is necessary to pay more attention to exosomes that play an important role in the communication between NSCs and GBMs.

3.2. Stroke

Stroke is one of the leading causes of death and disability worldwide, and the role of NSC-Exos in enhancing neural repair after stroke has been extensively studied. First, NSC-Exos tail vein injection reduces the area of cerebral infarction in a thromboembolic mouse model. This therapeutic effect was found to be superior to that of mesenchymal stem cell (MSC)-derived exosomes [63]. Next, the therapeutic effect of NSC-Exos in a porcine middle cerebral artery occlusion (MCAO) model has also been confirmed. The porcine ischaemic stroke model was found to be more predictive of outcomes between preclinical rodent models and human clinical trials [64]. Additionally, the intravenous administration of NSC-Exos exerted a dose-dependent therapeutic effect in a mouse MCAO model, stimulating neural regeneration and synaptic plasticity [65]. This previous study also showed that NSC-Exos reverse immunosuppression in the periphery 7 days after ischemia, while bringing about significant increases in B and T lymphocyte levels, with central immunity unaffected. Another study showed that femoral vein injection of NSC-Exos enhances brain tissue B lymphocyte and T lymphocyte levels 24 h after ischaemia given that the NSC-Exos treatment reduced the activation of the pro-inflammatory signalling pathway NF-κB/P65 after ischaemia. In vitro experiments have also demonstrated the reversal of ABCB1 overexpression in brain endothelial cells after oxygen-glucose deprivation (ODG), and this appeared to enhance the stability of the BBB and reduced the recruitment of inflammatory cells in the early (24-h) ischaemic brain [66].

However, NSC-Exos do not only affect BBB permeability, but also seem to play a complex communication role between various neurocytes. First, NSC-Exos significantly reduce neuronal apoptosis after OGD and promote neuronal proliferation via the miR-150–3p/CASP2 pathway [67]. Additionally, hNSC-EVs play a protective role by promoting Nrf2 nuclear translocation to upregulate antioxidant expression and reduce ROS levels to protect neurons from oxidative stress damage [68]. NSC-Exos also strongly and significantly affect inflammatory signalling in microglia. It has also been observed that the intracerebroventricular injection of NSC-Exos can co-label microglia [69] and reduce microgliosis after stroke [70]. Neonatal subventricular NSC-Exos transplantation also targets and modifies microglia and affects the release of inflammatory factors from microglia. Additionally, the Let-7 miRNA family activates endosomal TLR-7 receptors, and synthetic EV containing Let-7 strongly stimulate inflammatory factor release from microglia. Notably, cultures of EV-treated microglia appear to act back on NSCs to inhibit their division [71].

Additionally, NSC-Exos are phagocytosed by astrocytes and exert a protective effect on astrocytes after OGD [72,73]. Astrocytes are important neurocytes that constitute the BBB in addition to brain endothelial cells. Astrocyte activation and release of MMP9 contribute to the degradation of basal membranes, thereby promoting BBB breakdown [74–76]. Therefore, the role of NSC-Exos and astrocytes in stroke requires further investigation. Finally, NSC-Exos can act on NSCs themselves and promote neurogenesis after stroke. miR-9 is abundantly expressed in mouse NSC-Exos, and targeting Hes1 transcripts promotes the differentiation of NSCs and the maturation of neurons and glial cells [77]. A recent study showed that NSC-Exos loaded with BDNF are more beneficial for the differentiation of NSCs into neurons in vitro as well as in vivo relative to normal NSC-Exos. Moreover, these engineered exosomes reduce microglia expression and greatly attenuate neuroinflammatory response after ischemic stroke in rats [78]. In summary, NSC-Exos play a complex role in ischaemic stroke by significantly reducing brain infarct volume and improving neurological function, and may be an important repair strategy in stroke.

3.3. Alzheimer's disease

Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterised by BBB damage, mitochondrial dysfunction, and the accumulation of misfolded proteins in the brain, including age-related plaques (Aβ) and neurogenic fibre tangles [79,80]. The pathogenesis of AD is complex, and there is no effective therapy to block or reverse its progression; NSC-Exos are a potentially effective treatment for AD. First, as discussed above, NSC-Exos reduces brain injury after stroke by enhancing the stability of the BBB, thereby reducing inflammatory infiltration into brain tissue. Similarly, AD induces BBB dysfunction and disrupts the BBB in the 5 ×FAD mouse model aged 4 months. Meanwhile, exosomes from hNSCs can reverse leakage in the in vitro BBB model from primary EC of 5 ×FAD mice [81]. Notwithstanding, the specific mechanism by which NSC-Exos exert a protective effect on the BBB requires further investigation. It has also been demonstrated that intracerebroventricular injection of EVs derived from mouse embryonic hippocampal NSCs for 5 weeks can rescue cognitive deficits in 9-month--old AD mice. Compared with the vehicle group, the NSCs-EV group showed improved mitochondrial function, accelerated SIRT1 activation, enhanced synaptic activity and integrity, reduced inflammatory responses, and rescued cognitive deficits; however, this treatment did not alter the number of Aβ plaques. In summary, the therapeutic role and potential molecular mechanisms of NSC-Exos in AD are worthy of further in-depth study [82].

It has also been observed that a special group of patients with AD who are non-demented but have the similar pathological changes can resist cognitive impairment [83]. Additionally, the hippocampal DG of non-cognitively impaired patients exhibits an increased number of NSCs [84]. Thus, Micci et al. showed that local signalling generated by exosomes released from endogenous NSCs can modulate the ability of synapses to bind Aβo. Further, intracerebroventricular injection of NSC-Exos also renders neuronal synapses less susceptible to toxic Aβo binding, protects synapses from the Aβo-induced inhibition of LTP expression, and prevents Aβo-driven memory deficits. The study of pathological mechanisms in patients who are non-demented can facilitate the further exploration of issues related to cognitive dysfunction in patients with AD [85]. A very recent study showed that NSC-Exos pretreated with herbal Catalpol encapsulated more miR-138–5p, and this inhibited apoptosis in AD model cells. Additionally, the intragastric administration of Catalpol has also shown the ability to alleviate AD progression and improve cognitive ability in mouse [86]. All these findings highlight the great potential of NSC-Exos in the treatment of AD.

3.4. Parkinson's disease

Parkinson's disease (PD) is another degenerative disease of the CNS, characterised by progressive loss of dopaminergic neurons in the substantia nigra (SN) due to mitochondrial dysfunction, neuroinflammation, defective protein clearance, and the accumulation of alpha-synuclein [87–89]. The pathogenesis of PD is unknown, and current therapeutic options do not completely reverse its progression. A recent study has shown that NSC-EVs show high potential for application in the development of PD therapies. Further, in a previous study, 6-OHDA toxin was used to induce ROS accumulation and thus PD-like effects in both in vitro and in vivo models. F3 cells are immortalised human neural stem cell lines obtained from the foetal telencephalon. Their derived EVs reduce the levels of 6-OHDA-mediated intra-neuronal oxidative stress and 6-OHDA-induced release of inflammatory factors from microglial cell sites.

Furthermore, intracerebral injection of F3-EVs in the SN region of PD model mice significantly reduced neuroinflammation at this site, and this was accompanied by significant decreases in the release of reactive

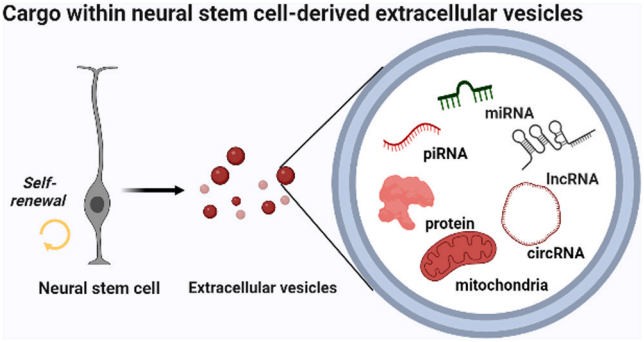

Fig. 1. NSC-EVs constituents with therapeutic effects.

astrocytes, activated microglia, and pro-inflammatory factors in the striatum and SN region. Most importantly, F3-EVs ultimately increased the activity of dopamine neurons in the SN region, which is important for PD treatment. The isolation of F3-EVs revealed several specific miRNAs associated with cell survival or neurogenesis, such as has-mir-182–5p, has-mir-183–5p, has-mir-9, and has-let-7, which are potentially active components for their role in PD models [90]. In conclusion, NSC-EVs are a promising direction for PD treatment. However, the associated mechanisms involved need to be further explored.

3.5. Spinal cord injury

Spinal cord injury (SCI) is a common and harmful injury to the spinal cord resulting from spinal surgery that causes sensory and motor dysfunction [91]. Primary injury in SCIs includes the mechanical destruction of spinal microvascular endothelial cells and neurons, while secondary injuries include inflammation, hypoxia, ischaemia, and neuronal apoptosis [92–94]. The recovery of neurological function after SCI remains very challenging. Recent studies have shown that NSC-Exos preconditioning can exert anti-inflammatory and anti-apoptotic effects by promoting autophagy [95]. NSC-EVs can also reduce the apoptosis of spinal neurons, microglial activation, and the expression levels of pro-inflammatory factors in vitro and in vivo. Importantly, it has been observed that the intrathecal injection of NSC-EVs mediates the activation of autophagy and increases the expression of autophagy markers, LC3B and Beclin1, which are essential for its anti-inflammatory and anti-apoptotic effects after SCI to prevent tissue damage [96]. Moreover, 14–3–3 is induced and regulates autophagy under oxidative stress conditions, possibly as a cell survival mechanism in CNS diseases. Rong et al. demonstrated that 14–3–3 t within NSC-EVs increases the induction of autophagy by increasing the expression of Beclin-1 and facilitating its recruitment to autophagosomal precursors. NSC-EVs overexpressing 14–3–3 t show further enhanced anti-apoptotic and anti-inflammatory effects in vitro and in vivo, unlike 14–3–3 t knockdown in NSC-EVs in vitro, which attenuate this protection [97]. The positive role of NSC-Exos in the subventricular zone of the brain in rat SCI has also been discussed by Mohammed et al. Notably, the intrathecal injection of NSC-EVs inhibits the formation of NLRP3 inflammatory vesicle complexes, and significantly restores spinal cord tissue neuronal cell survival and motor function in rats within 14 days [98]. Finally, NSC-Exos also exert potential therapeutic effects on SCI by enhancing the angiogenic capacity of SCMECs, and this can be partly attributed to the presence of the angiogenesis-related protein, VEGF-A. In vivo

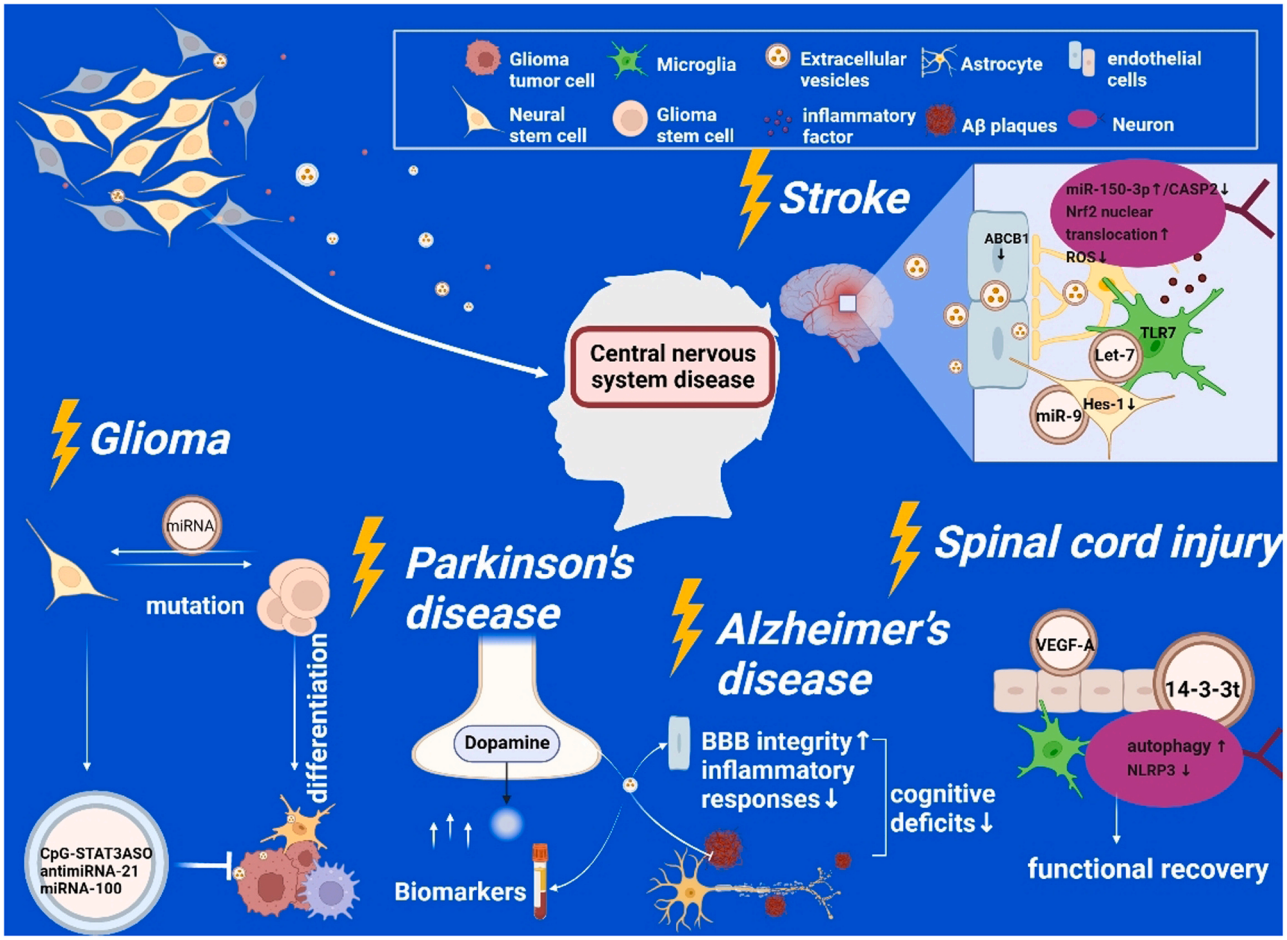

Fig. 2. CNS diseases with NSC-EV implication.

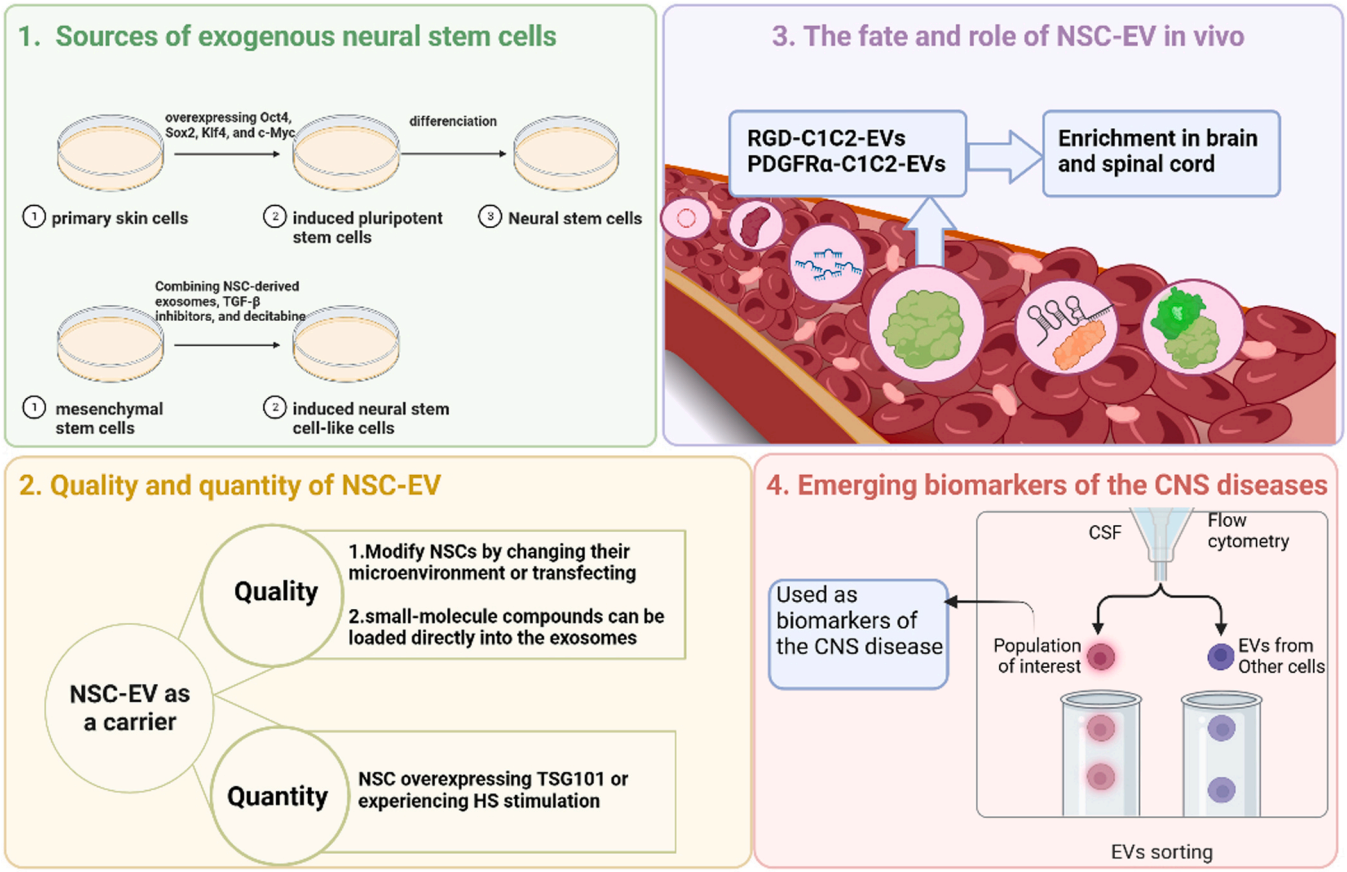

Fig. 3. Limitations and breakthroughs of NSC-EVs.

experiments in SCI mice have also shown that treatment with NSC-Exos results in increased local microvascular density, spinal cord lumen atrophy, and recovery of motor function. In addition, VEGF-A inhibition in NSC-Exos suppresses therapeutic effects, suggesting that exosomal VEGF-A promotes microvascular regeneration and functional recovery after SCI [99].

4. Limitations and breakthroughs of NSC-EVs

Ongoing advances in the field of NSC-EVs have increasingly shown that NSC-EVs have multiple functions and utility, from serving as diagnostic and prognostic markers to explaining disease progression to functioning as therapeutic targets for reversing disease. Thus, they hold great promise in the treatment of neurological disorders. However, there are still many limitations and problems in the application of NSC-EVs.

4.1. Increase NSC-EV number

4.1.1. Sources of NSC-Exos

NSCs are found in the deep brain; therefore, their acquisition requires high-risk invasive procedures. Thus, scientists have focused on the use of exogenous NSCs derived from somatic cell reprogramming. A Japanese research group generated induced pluripotent stem (iPS) cells from primitive skin cells by overexpressing Oct4, Sox2, Klf4, and c-Myc [100]. These iPS cells were then be used to generate tissues, including NSCs. Further, EVs secreted by iPSC-derived NSCs are rich in the miRNAs and proteins involved in neuroprotection, anti-apoptosis, antioxidant, anti-inflammatory, and BBB repair. Thus, they can promote synaptogenesis, synaptic plasticity, and also enhance cognitive function. The intranasal administration of EVs adulterates neurons, microglia, and astrocytes in almost all adult rat and mouse brain regions and enhances hippocampal neurogenesis [101]. This suggests that iPSC-derived NSC-EVs have great potential for use in the treatment of CNS disorders. Using iPSC-derived NSCs from normal and idiopathic autism spectrum disorder (ASD), Moore et al. found that miR-1290 is the main cause of the differentiation defect in ASD-NSCs and that introducing EV-like particles into miR-1290 can significantly promote ASD-NSC neuronal differentiation [102]. Recent studies have also demonstrated the possibility of generating iPSC-derived NPC neurospheres, allowing retroviral (e.g., HIV-1 and HTLV-1) replication and subsequently highlighting the potential of stem cell EVs in rescuing cellular damage induced by HIV-1 infection [103].

Recently, researchers have also attempted to bypass the pluripotent state and avoid the risks associated with iPS cells by directly transforming somatic cells into induced NSC-like cells (iNSCs); this greatly circumvents the risk of viral transfection and tumourigenesis involved in cell programming. It also reduces the lengthy steps and time required to obtain NSCs. Further, MSCs are the most promising reprogramming materials in this regard because they are pluripotent and self-renewing, and are abundantly expressed in several tissues; thus, are readily available. Combining NSC-Exos, TGF-β inhibitors, and decitabine is the most effective strategy for transdifferentiating MSCs into iNSCs. The resulting iNSCs can be expanded in suspension cultures via multiple passages and under such conditions, they can differentiate into cells expressing neural markers [104]. However, whether iNSC can secrete extracellular vesicles and exert therapeutic effects in this way remains unknown and needs further exploration.

4.1.2. Promotion of exosome secretion from NSCs

The secretion of exosomes is related to their biogenesis mechanism, and the endosomal sorting complex required for transport (ESCRT) plays a prominent role in this process. The tumour susceptibility gene 101

Table 1
Contents carried by neural stem cell extracellular vesicles.

Species	Sources	Vesicle types	Treatment	Contents	Reference
Human	CTX0E03 cell line	Exosome	/	miR-1246, miR-4488, miR-4508, miR-4492, and miR-4516	[23]
Mouse	Hypothalamus	Exosome	/	miR-106a-5p, miR-20a-5p, miR-30e-5p, miR-9–5p	[25]
Human	/	Exosome	Hypoxia	miR-98–3p	[26]
Rat	Mesencephalic	Exosome	Hypoxia	miR-210	[27]
Human	Fetal brain	Exosome	IFN-γ	miR-206, miR-133a-3p, miR-4677–5p, miR-205–5p, miR-3656, miR-34c-3p, miR-3151–5p	[28]
Rat	Cortex	Exosome	IGF-1	miR-219–2a-3p	[29]
Mouse	Hypothalamus	Ex/Mv	/	PiRNA	[30,36]
Human	Hippocampal	Exosome	/	LncMIAT	[41]
rat	Hippocampal	Exosome	/	CircAcbd6	[44]
Human	iPSC-NSC	EV	/	miRs-320a，320b，103a-3p，21–5p，26a-5p，30a-3p，181a-5p，191–5p; Agrin, PTX3, Hemopexin, Gal-3BP and Nidogen-1	[45,46]
Rat	hippocampal	exosome	/	Netrin1	[47]
Human	hESC-NSC	Mv	/	HSP70	[48]
Mouse	SVZ	EV	Th-1 like cytokines	IFN-γ	[50]
Mouse	SVZ	EV	/	Mitochondria	[51,52]

IFN-γ, inflammatory factor interferon gamma, IGF-1, insulin growth factor-1; Ex/Mv, exosomes/microvesicles; iPSC, induced pluripotent stem cells; EV, extracellular vesicles; NSC, neural stem cell; hESC, human embryonic stem cell; SVZ, subventricular zone

(TSG101) is an integral part of the ESCRT machinery and plays an important role in identifying and sorting ESCRT cargo [105,106]. Eun-Jung et al. showed that NSCs overexpressing TSG101 upregulate the cellular components associated with exosome biogenesis. This greatly enhances exosome secretion, which prevents ischaemia-induced exosome secretion via anti-inflammatory effects and growth/trophic factor production to prevent ischaemia-induced brain injury [107]. In addition to the transfection of NSCs, Christa et al. isolated EVs secreted by rat NSCs in response to heat shock (HS) stimulation. Nanoparticle tracking analysis has also confirmed that HS-derived EVs are more abundant and have larger diameters than non-HS (NHS)-derived EVs. GO enrichment analysis has shown that the proteins in HS-derived EVs are primarily involved in the negative regulation of the apoptotic process and in the positive regulation of DNA repair. Importantly, compared to NHS-derived EVs, HS-derived EVs exhibit greater neuroprotection against oxidative stress and Aβ-induced neurotoxicity in a cell culture model of AD. They can also significantly attenuate Aβ-induced apoptosis and oxidative stress. These results suggest that NSCs respond to HS by increasing EV production and altering EV morphology and cargo to provide better neuroprotection [108]. In addition, stem cells can stimulate exosome secretion when cultured in three-dimensional spheroids. They can also enhance exosome production via hypoxia-inducible factors during hypoxia [109,110]. Hence, understanding the mechanism of exosome secretion and promoting exosome secretion can be another direction for NSC-Exos therapy.

4.2. Application of engineered NSC-EVs in CNS diseases

4.2.1. Use of NSC-EVs as carriers of specific cargoes

The content of NSC-Exos is considerably heterogeneous. The encapsulation of exosomal content is influenced by parent cell characteristics, and as mentioned above, it is possible to modify parent cells by changing their microenvironment [111] or transfecting them with shRNA. Thus, the exosomal content is modified and the exosomes are functionally altered. However, the differences in the release of cargo contained in NSC-Exos induced by environmental changes are complex and difficult to accurately control. A more straightforward approach is to directly modify exosomal cargo so that NSC-Exos become direct carriers of some small-molecule compounds. FTY720 is a functional antagonist of sphingosine 1-phosphate receptor-1 (S1P1), which has a long half-life in vivo and can exert immunomodulatory functions [112]. FTY720-labeled exosomes were obtained by co-incubating purified NSC-Exos with an FTY720 solution after ultrasonication. Thereafter, it was observed that FTY720-NSC-Exos treatment after SCI promotes

Table 2
Role of NSC-derived extracellular vesicles in CNS diseases.

Species	Sources	Administration	Mechanism	Disease Model	Reference
Human	F3 cell line	Intratumorally	CpG-STAT3ASO	Mouse Glioblastoma	[59]
/	/	Intranasal	AntimiR-21, miR-100	Mouse Glioblastoma	[60]
Human	PSC-NSC	Tail vein	/	Mouse TE	[62]
Human	H9-NPC	Peripheral ear vein	/	Porcine MCAO	[63]
Mouse	SVZ	Femoral vein& Retroorbital injection	/	Mouse MCAO	[64]
Mouse	SVZ	Femoral vein	ABCB1, MMP9, NF-κB	Mouse MCAO	[65]
Rat	Cortex	Tail vein	miR-150–3p/CASP2	Rat MCAO	[66]
Rat	/	ICV	/	Rat MCAO	[69]
Mouse	Brain	Intravenous	/	Mouse MCAO	[72]
Mouse	Hippocampal	ICV	Mitochondrial function-related factors, SIRT1, Synaptic proteins	Mouse AD	[80]
Rat	Hippocampal	ICV	miR-485, miR-17, miR-322	Mouse AD	[83]
Human	F3 cell line	Intracerebral	miR-9, Let-7	Mouse 6-OHDA induced PD	[87]
Mouse	Forebrain	Intrathecal	miR-374–5p/STK4	Mouse SCI	[92]
Rat	Spinal cord	Tail vein	Autophagy: Beclin1, LC3B	Rat SCI	[93]
Rat	Spinal cord	Tail vein	14–3–3 t/Beclin1	Rat SCI	[94]
Rat	SVZ	Intrathecal	NLRP3, ASC and Caspase-1	Rat SCI	[95]
Mouse	Cortex	Tail vein	VEGF-A	Mouse SCI	[96]

ASO, antisense oligonucleotides; STAT3, signal transducer and activator of transcription 3; PSC, pluripotent stem cell; TE, thromboembolic; MCAO, middle cerebral artery occlusion; NPC, Neural progenitor cell; NSC, Neural stem cell; SVZ, subventricular zone; ICV, intracerebroventricular; AD, Alzheimer's disease; SCI, spinal cord injury; ASC, apoptosis-associated speck-like protein.

neuronal morphology and thus improves hindlimb motor behaviour compared with NSC-Exo- or FTY720-only treatments.

Meanwhile, FTY720-NSC-Exos treatment improves endothelial function and alleviated apoptosis as well as spinal cord oedema, thereby improving function and behaviour after SCI by modulating the PTEN/AKT signalling pathway [113]. BDNF is the most abundant and widely distributed neurotrophic factor in the nervous system. It exhibits several neuroprotective properties after ischaemic and traumatic brain injury [114]. In a previous study, BDNF protein was co-incubated with hNSC-Exos for 24 h. Thereafter, the BDNF was loaded into exosomes using Exo-Fect reagent to obtain BDNF-hNSC-Exos, which exhibited stronger anti-apoptotic and pro-differentiation abilities than hNSC-Exos. In a rat ischaemic stroke model, BDNF-hNSC-Exos effectively inhibited microglial expression and promoted endogenous NSC differentiation into neurons, resulting in reduced infarct volume and improved neurological function [78]. These findings demonstrate that changes in exosome quality can significantly enhance their efficacy in the treatment of CNS diseases.

4.2.2. Engineered NSC-EVs show higher targeting in vivo

NSC-EVs have emerged as novel therapeutic agents for the treatment of CNS diseases. However, the intravenous delivery of EVs into the ischaemic brain remains challenging owing to the poor targeting of unmodified EVs and dilution after systemic administration. Intravenous NSC-Exos can cross the BBB and effectively exert some therapeutic effects. However, a relatively large number of exosomes remain in the peripheral organs, such as the liver and lungs, and may be effectively eliminated by the liver [65,115,116]. Thus, enhancing targeting capability has become an inevitable trend to maximise the potential of NSC-Exos in the future.

Tada et al. generated a recombinant fusion protein containing an arginine-glycine-aspartate (RGD)− 4 C peptide (ACDCRGDCFC) fused to the phosphatidylserine (PS)-binding structural domain of lactic acid mucin (C1C2). RGD peptides are well-known ligands, and RGD-based nanomaterials have been shown to successfully deliver therapeutic or contrast agents to the ischaemic brain [117], and the C1C2 structural domain enhances the affinity to PS on EV membranes [118]. Subsequently, RGD-C1C2-bound EV (RGD-EVs) were injected intravenously through the tail vein in a MCAO. Thus, it was observed that the RGD-EVs targeted diseased regions of the ischaemic brain after intravenous administration, resulting in a strong suppression of inflammatory response [119].

Xing et al. recently developed engineered NSC-Exos that could effectively target oligodendrocytes in demyelinating CNS diseases, such as multiple sclerosis. PDGFRα expression was enhanced in the brain and spinal cord of the experimental autoimmune encephalomyelitis (EAE) mice, suggesting that this receptor could be a potential target for drug delivery. Further, the group fused the C1C2 structural domain with PDGFA (a ligand for PDGFRα), a polypeptide anchored to the surface of EV, to form an engineered EV called EVP. Thereafter, the EAE mice were injected intravenously with the obtained EVPs, and it was observed that they showed low distribution in the spleen and liver, but were more abundant in the brain and spinal cord, and targeted oligodendrocyte lineages to a greater extent than EV. These results suggest that EVPs can significantly improve the efficient delivery of drugs to spinal cord lesions. Further, these engineered EVPs were then used as carriers for drug delivery. For example, the engineered EVPs loaded with triiodothyronine (T3) exerted a significant myelin regenerative effect, relative to treatment with EVPs or T3 alone, EVPs + T3 treatment exerted a more significant therapeutic effect and inhibited disease progression [120]. Bryostatin-1 (Bryo-1) is a natural compound with significant anti-inflammatory capacity, and the use of EVPs as a targeting carrier for its encapsulation results in it showing greater stability and concentration than is the case with natural Bryo-1 in the CNS. Specifically, compared with EVP or Bryo-1-only administration, EVPs + Bryo-1 significantly improved clinical disease progression, reduced pro-inflammatory cell infiltration and astrogliosis, protected BBB integrity, altered the pro-inflammatory phenotype of microglia in the CNS of EAE mice, and significantly improved myelin protection and promoted myelin regeneration [121,122]. In conclusion, the enormous translational potential of NSC-Exos is clear, and the future of CNS disease treatment may be partially dominated by engineered exosomes.

4.3. Emerging biomarkers of CNS diseases

The analysis of changes in RNA or protein levels within EVs of a specific population is a non-invasive strategy of forecasting and making novel diagnostic and prognostic markers for various diseases [123]. As mentioned earlier, the sorting process of NSC-EV cargo is induced by specific conditions. For example, the expression of exosomal miRNAs is altered by environmental factors, and the loading of protein cargo is indicative of quiescent or activated NSCs. Thus, NSC-EVs are likely to be sensitive and can function as specific indicators of CNS disease. In a recent study, Candelario et al. highlighted the possibility of using EMV release from iPSC-derived NPCs as a new biomarker of PD [124]. Furthermore, 90% of PD cases are idiopathic (non-genetic), with the remainder involving known LRRK2 genetic mutations associated with the PD phenotype and behaviour. The gene expression of EMV cargoes from iPSC-derived NPCs from patients with LRRK2 mutations is altered compared to gene expression in EMVs released from adult NPCs with idiopathic PD. In contrast, iPSC-derived NPCs from patients with LRRK2 mutation correction reverses this alteration. The above findings have significant implications for the early diagnosis of PD and the monitoring of response to treatment. These findings also suggest that to some extent NSC-EVs are very promising as emerging biomarkers for reflecting neurological disease status and monitoring their prognosis.

However, at present, much is still required for the clinical application of NSC-EVs in this regard. First, CSF collection and NSC acquisition from the deep brain are extremely invasive compared to the traditional use of CSF-EVs as biomarkers for CNS disease prediction [125]. In the above studies, NPCs induced and differentiated from skin fibroblast from patients were utilized, and this reduced the complexity of clinical sample collection to a certain extent. However, iPSC-NPCs are not influenced by the microenvironment under pathological conditions and appear inferior to CSF-EVs. Second, both CSF-EVs and EVs from various body fluids originate from numerous sources, including almost all the cells through which the fluid passes [126]. Therefore, the advantage of NSC-EVs is that they may be more specific for predicting pathological diseases. In conclusion, the future of NSC-EVs is promising and at the same time challenging. Combining the two previously mentioned aspects, identifying cells based on origin via the specific phenotypic characteristics of the EVs, sorting EVs in CSF or blood, and extracting EVs produced by NSCs are most suitable for better interpreting the information carried by EVs.

5. Conclusion

As the origin of various nervous system cell types, NSCs have unique advantages in CNS diseases. In particular, NSC-EVs play a pivotal role as messengers, therapeutic agents, cargo carriers, and biomarkers in various physiological and pathological settings in CNS diseases. However, studies on the principles of their dynamic regulation during brain development are limited, and this may have significant implications in guiding the diagnosis and treatment of CNS diseases. Additionally, engineered EVs have been extensively studied for application in the management of other systemic diseases, particularly cancer, and combining these technologies with NSC-EVs may enhance their application. In conclusion, NSCs-EVs could be developed as a new therapeutic strategy that sheds light on the diagnosis and treatment of CNS diseases. Figs. 1–3 and Tables 1–2.

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NA.

Ethics approval and consent to participate

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Consent for publication

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Author contributions

Bo Fang and Yuanyuan Li drafted the manuscript or revised it critically for important intellectual content. All authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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References

[1] A.M. Bond, G.L. Ming, H. Song, Adult mammalian neural stem cells and neurogenesis: five decades later, Cell stem Cell 17 (4) (2015) 385–395.
[2] L.C. Fuentealba, K. Obernier, A. Alvarez-Buylla, Adult neural stem cells bridge their niche, Cell stem Cell 10 (6) (2012) 698–708.
[3] L. Zhang, X. Han, X. Cheng, X.F. Tan, H.Y. Zhao, X.H. Zhang, Denervated hippocampus provides a favorable microenvironment for neuronal differentiation of endogenous neural stem cells, Neural Regen. Res. 11 (4) (2016) 597–603.
[4] H. Kumamaru, K. Kadoya, A.F. Adler, Y. Takashima, L. Graham, G. Coppola, M. H. Tuszynski, Generation and post-injury integration of human spinal cord neural stem cells, Nat. Methods 15 (9) (2018) 723–731.
[5] A. Kriegstein, A. Alvarez-Buylla, The glial nature of embryonic and adult neural stem cells, Annu. Rev. Neurosci. 32 (2009) 149–184.
[6] K.M. Christian, H. Song, G.L. Ming, Functions and dysfunctions of adult hippocampal neurogenesis, Annu. Rev. Neurosci. 37 (2014) 243–262.
[7] L. Chen, G. Zhang, Y. Gu, X. Guo, Meta-analysis and systematic review of neural stem cells therapy for experimental ischemia stroke in preclinical studies, Sci. Rep. 6 (2016) 32291.
[8] H.J. Lee, K.S. Kim, I.H. Park, S.U. Kim, Human neural stem cells over-expressing VEGF provide neuroprotection, angiogenesis and functional recovery in mouse stroke model, PloS One 2 (1) (2007), e156.
[9] H. Sakata, P. Narasimhan, K. Niizuma, C.M. Maier, T. Wakai, P.H. Chan, Interleukin 6-preconditioned neural stem cells reduce ischaemic injury in stroke mice, *Brain: a J. Neurol.* 135 (Pt 11) (2012) 3298–3310.
[10] G.L. Zhang, Z.H. Zhu, Y.Z. Wang, Neural stem cell transplantation therapy for brain ischemic stroke: Review and perspectives, World J. stem Cells 11 (10) (2019) 817–830.
[11] S.I. van de Wakker, F.M. Meijers, J.P.G. Sluijter, P. Vader, Extracellular vesicle heterogeneity and its impact for regenerative medicine applications, Pharmacol. Rev. (2023).
[12] H. Ruan, Y. Li, C. Wang, Y. Jiang, Y. Han, Y. Li, D. Zheng, J. Ye, G. Chen, G. Y. Yang, et al., Click chemistry extracellular vesicle/peptide/chemokine nanocarriers for treating central nervous system injuries, Acta Pharm. Sin. B 13 (5) (2023) 2202–2218.
[13] P. Sharma, L. Schiapparelli, H.T. Cline, Exosomes function in cell-cell communication during brain circuit development, Curr. Opin. Neurobiol. 23 (6) (2013) 997–1004.
[14] G. van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat. Rev. Mol. Cell Biol. 19 (4) (2018) 213–228.
[15] V. Budnik, C. Ruiz-Cañada, F. Wendler, Extracellular vesicles round off communication in the nervous system, Nat. Rev. Neurosci. 17 (3) (2016) 160–172.
[16] Q. Long, D. Upadhya, B. Hattiangady, D.K. Kim, S.Y. An, B. Shuai, D.J. Prockop, A.K. Shetty, Intranasal MSC-derived A1-exosomes ease inflammation, and prevent abnormal neurogenesis and memory dysfunction after status epilepticus, Proc. Natl. Acad. Sci. USA 114 (17) (2017). E3536-e3545.
[17] S.K. Reddy, A.R. Ballal, S. Shailaja, R.N. Seetharam, C.H. Raghu, R. Sankhe, K. Pai, T. Tender, M. Mathew, A. Aroor, et al., Small extracellular vesicle-loaded bevacizumab reduces the frequency of intravitreal injection required for diabetic retinopathy, Theranostics 13 (7) (2023) 2241–2255.
[18] X. Xie, S. Lian, Y. Zhou, B. Li, Y. Lu, I. Yeung, L. Jia, Tumor-derived exosomes can specifically prevent cancer metastatic organotropism, J. Control. Release: Off. J. Control. Release Soc. 331 (2021) 404–415.
[19] H. Wei, F. Chen, J. Chen, H. Lin, S. Wang, Y. Wang, C. Wu, J. Lin, G. Zhong, Mesenchymal Stem Cell Derived Exosomes as Nanodrug Carrier of Doxorubicin for Targeted Osteosarcoma Therapy via SDF1-CXCR4 Axis, Int. J. Nanomed. 17 (2022) 3483–3495.
[20] B.S. Joshi, I.S. Zuhorn, Heparan sulfate proteoglycan-mediated dynamin-dependent transport of neural stem cell exosomes in an in vitro blood-brain barrier model, Eur. J. Neurosci. 53 (3) (2021) 706–719.
[21] L. Stevanato, J.D. Sinden, The effects of microRNAs on human neural stem cell differentiation in two- and three-dimensional cultures, Stem Cell Res. Ther. 5 (2) (2014) 49.
[22] A.J. Enright, B. John, U. Gaul, T. Tuschl, C. Sander, D.S. Marks, MicroRNA targets in drosophila, Genome Biol. 5 (1) (2003) R1.
[23] G. Camussi, M.C. Deregibus, S. Bruno, V. Cantaluppi, L. Biancone, Exosomes/microvesicles as a mechanism of cell-to-cell communication, Kidney Int. 78 (9) (2010) 838–848.
[24] J. Lotvall, H. Valadi, Cell to cell signalling via exosomes through esRNA, Cell Adhes. Migr. 1 (3) (2007) 156–158.
[25] L. Stevanato, L. Thanabalasundaram, N. Vysokov, J.D. Sinden, Investigation of content, stoichiometry and transfer of miRNA from human neural stem cell line derived exosomes, PloS One 11 (1) (2016), e0146353.
[26] C. Villarroya-Beltri, C. Gutiérrez-Vázquez, F. Sánchez-Cabo, D. Pérez-Hernández, J. Vázquez, N. Martin-Cofreces, D.J. Martinez-Herrera, A. Pascual-Montano, M. Mittelbrunn, F. Sánchez-Madrid, Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs, Nat. Commun. 4 (2013) 2980.
[27] Y. Zhang, M.S. Kim, B. Jia, J. Yan, J.P. Zuniga-Hertz, C. Han, D. Cai, Hypothalamic stem cells control ageing speed partly through exosomal miRNAs, Nature 548 (7665) (2017) 52–57.
[28] G. Zhang, L. Chen, X. Guo, H. Wang, W. Chen, G. Wu, B. Gu, W. Miao, J. Kong, X. Jin, et al., Comparative analysis of microRNA expression profiles of exosomes derived from normal and hypoxic preconditioning human neural stem cells by next generation sequencing, J. Biomed. Nanotechnol. 14 (6) (2018) 1075–1089.
[29] M. Zhao, Y. Gao, F. Wang, X. Cheng, T. Zhao, Y. Zhao, M. Fan, L. Zhu, Neural progenitor cells-secreted exosomal miR-210 induced by hypoxia influences cell viability, Neuroreport 31 (11) (2020) 798–805.
[30] G. Zhang, Z. Zhu, H. Wang, Y. Yu, W. Chen, A. Waqas, Y. Wang, L. Chen, Exosomes derived from human neural stem cells stimulated by interferon gamma improve therapeutic ability in ischemic stroke model, J. Adv. Res. 24 (2020) 435–445.
[31] K. Ma, H. Xu, J. Zhang, F. Zhao, H. Liang, H. Sun, P. Li, S. Zhang, R. Wang, X. Chen, Insulin-like growth factor-1 enhances neuroprotective effects of neural stem cell exosomes after spinal cord injury via an miR-219a-2-3p/YY1 mechanism, Aging 11 (24) (2019) 12278–12294.
[32] B. Yu, S. Ikhlas, C. Ruan, X. Zhong, D. Cai, Innate and adaptive immunity of murine neural stem cell-derived piRNA exosomes/microvesicles against pseudotyped SARS-CoV-2 and HIV-based lentivirus, iScience 23 (12) (2020), 101806.
[33] C.D. Malone, G.J. Hannon, Small RNAs as guardians of the genome, Cell 136 (4) (2009) 656–668.
[34] C. Goriaux, E. Théron, E. Brasset, C. Vaury, History of the discovery of a master locus producing piRNAs: the flamenco/COM locus in Drosophila melanogaster, Front. Genet. 5 (2014) 257.
[35] V.N. Kim, Small RNAs just got bigger: piwi-interacting RNAs (piRNAs) in mammalian testes, Genes Dev. 20 (15) (2006) 1993–1997.
[36] K.W. Kim, PIWI proteins and piRNAs in the nervous system, Mol. Cells 42 (12) (2019) 828–835.
[37] Y. Ghosheh, L. Seridi, T. Ryu, H. Takahashi, V. Orlando, P. Carninci, T. Ravasi, Characterization of piRNAs across postnatal development in mouse brain, Sci. Rep. 6 (2016) 25039.
[38] S. Ikhlas, A. Usman, D. Kim, D. Cai, Exosomes/microvesicles target SARS-CoV-2 via innate and RNA-induced immunity with PIWI-piRNA system, Life Sci. Alliance 5 (2022) 3.
[39] Y. Zhang, Q. Xia, J. Lin, LncRNA H19 attenuates apoptosis in MPTP-induced Parkinson's disease through regulating miR-585-3p/PIK3R3, Neurochem. Res. 45 (7) (2020) 1700–1710.
[40] H. Li, C. Yang, J. Zhang, W. Zhong, L. Zhu, Y. Chen, Identification of potential key mRNAs and LncRNAs for psoriasis by bioinformatic analysis using weighted gene

co-expression network analysis, Mol. Genet. Genom.: MGG 295 (3) (2020) 741–749.
[41] Y. Gao, N. Zhang, C. Lv, N. Li, X. Li, W. Li, lncRNA SNHG1 knockdown alleviates amyloid-β-induced neuronal injury by regulating ZNF217 via sponging miR-361-3p in Alzheimer's disease, J. Alzheimer'S. Dis.: JAD 77 (1) (2020) 85–98.
[42] J. Zhuang, P. Cai, Z. Chen, Q. Yang, X. Chen, X. Wang, X. Zhuang, Long noncoding RNA MALAT1 and its target microRNA-125b are potential biomarkers for Alzheimer's disease management via interactions with FOXQ1, PTGS2 and CDK5, Am. J. Transl. Res. 12 (9) (2020) 5940–5954.
[43] D. Qi, X. Hou, C. Jin, X. Chen, C. Pan, H. Fu, L. Song, J. Xue, HNSC exosome-derived MIAT improves cognitive disorders in rats with vascular dementia via the miR-34b-5p/CALB1 axis, Am. J. Transl. Res. 13 (9) (2021) 10075–10093.
[44] Y. Li, Q. Zheng, C. Bao, S. Li, W. Guo, J. Zhao, D. Chen, J. Gu, X. He, S. Huang, Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis, Cell Res. 25 (8) (2015) 981–984.
[45] L. Liu, S. Ikonen, T. Tapiola, H. Tanila, T. van Groen, Fimbria-fornix lesion does not affect APP levels and amyloid deposition in the hippocampus of APP+PS1 double transgenic mice, Exp. Neurol. 177 (2) (2002) 565–574.
[46] W. Li, B. Shan, X. Cheng, H. He, J. Qin, H. Zhao, M. Tian, X. Zhang, G. Jin, circRNA Acbd6 promotes neural stem cell differentiation into cholinergic neurons via the miR-320-5p-Osbpl2 axis, J. Biol. Chem. 298 (4) (2022), 101828.
[47] R. Upadhya, L.N. Madhu, S. Rao, A.K. Shetty, Proficiency of extracellular vesicles from hiPSC-derived neural stem cells in modulating proinflammatory human microglia: role of pentraxin-3 and miRNA-21-5p, Front. Mol. Neurosci. 15 (2022), 845542.
[48] L. Ma, X. Wei, W. Ma, Y. Liu, Y. Wang, Y. He, S. Jia, Y. Wang, W. Luo, D. Liu, et al., Neural stem cell-derived exosomal netrin1 contributes to neuron differentiation of mesenchymal stem cells in therapy of spinal bifida aperta, Stem Cells Transl. Med. 11 (5) (2022) 539–551.
[49] L. Zhang, J. Gao, T. Chen, X. Chen, X. Ji, K. Ye, J. Yu, B. Tang, Y. Wei, H. Xu, et al., Microvesicles derived from human embryonic neural stem cells inhibit the apoptosis of HL-1 cardiomyocytes by promoting autophagy and regulating AKT and mTOR via transporting HSP-70, Stem Cells Int. 2019 (2019), 6452684.
[50] J. Zhang, J. Uchiyama, K. Imami, Y. Ishihama, R. Kageyama, T. Kobayashi, Novel roles of small extracellular vesicles in regulating the quiescence and proliferation of neural stem cells, Front. Cell Dev. Biol. 9 (2021), 762293.
[51] C. Cossetti, N. Iraci, T.R. Mercer, T. Leonardi, E. Alpi, D. Drago, C. Alfaro-Cervello, H.K. Saini, M.P. Davis, J. Schaeffer, et al., Extracellular vesicles from neural stem cells transfer IFN-γ via Ifngr1 to activate Stat1 signaling in target cells, Mol. Cell 56 (2) (2014) 193–204.
[52] M. Zhu, A.S. Barbas, L. Lin, U. Scheuermann, M. Bishawi, T.V. Brennan, Mitochondria released by apoptotic cell death initiate innate immune responses, ImmunoHorizons 2 (11) (2018) 384–397.
[53] L. Peruzzotti-Jametti, J.D. Bernstock, C.M. Willis, G. Manferrari, R. Rogall, E. Fernandez-Vizarra, J.C. Williamson, A. Braga, A. van den Bosch, T. Leonardi, et al., Neural stem cells traffic functional mitochondria via extracellular vesicles, PLoS Biol. 19 (4) (2021), e3001166.
[54] T.R. Smith, M.M. Hulou, J. Abecassis, S. Das, J.P. Chandler, Use of preoperative FLAIR MRI and ependymal proximity of tumor enhancement as surrogate markers of brain tumor origin, J. Clin. Neurosci. Off. J. Neurosurg. Soc. Australas. 22 (9) (2015) 1397–1402.
[55] J. Miska, M.S. Lesniak, Neural stem cell carriers for the treatment of glioblastoma multiforme, EBioMedicine 2 (8) (2015) 774–775.
[56] A. Carpentier, P. Metellus, R. Ursu, S. Zohar, F. Lafitte, M. Barrié, Y. Meng, M. Richard, C. Parizot, F. Laigle-Donadey, et al., Intracerebral administration of CpG oligonucleotide for patients with recurrent glioblastoma: a phase II study, Neuro-Oncol. 12 (4) (2010) 401–408.
[57] K.S. Aboody, A. Brown, N.G. Rainov, K.A. Bower, S. Liu, W. Yang, J.E. Small, U. Herrlinger, V. Ourednik, P.M. Black, et al., Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas, Proc. Natl. Acad. Sci. USA 97 (23) (2000) 12846–12851.
[58] R. Mooney, M. Hammad, J. Batalla-Covello, A. Abdul Majid, K.S. Aboody, Concise review: neural stem cell-mediated targeted cancer therapies, Stem Cells Transl. Med. 7 (10) (2018) 740–747.
[59] J. Huynh, A. Chand, D. Gough, M. Ernst, Therapeutically exploiting STAT3 activity in cancer - using tissue repair as a road map, Nat. Rev. Cancer 19 (2) (2019) 82–96.
[60] T. Adamus, C.Y. Hung, C. Yu, E. Kang, M. Hammad, L. Flores, S. Nechaev, Q. Zhang, J.M. Gonzaga, K. Muthaiyah, et al., Glioma-targeted delivery of exosome-encapsulated antisense oligonucleotides using neural stem cells, Mol. Ther. Nucleic Acids 27 (2022) 611–620.
[61] K. Wang, U.S. Kumar, N. Sadeghipour, T.F. Massoud, R. Paulmurugan, A. Microfluidics-Based, Scalable approach to generate extracellular vesicles with enhanced therapeutic MicroRNA loading for intranasal delivery to mouse glioblastomas, ACS nano (2021).
[62] Á. Tűzesi, T. Kling, A. Wenger, T.R. Lunavat, S.C. Jang, B. Rydenhag, J. Lötvall, S. M. Pollard, A. Danielsson, H. Carén, Pediatric brain tumor cells release exosomes with a miRNA repertoire that differs from exosomes secreted by normal cells, Oncotarget 8 (52) (2017) 90164–90175.
[63] R.L. Webb, E.E. Kaiser, S.L. Scoville, T.A. Thompson, S. Fatima, C. Pandya, K. Sriram, R.L. Swetenburg, K. Vaibhav, A.S. Arbab, et al., Human neural stem cell extracellular vesicles improve tissue and functional recovery in the murine thromboembolic stroke model, Transl. Stroke Res. 9 (5) (2018) 530–539.
[64] R.L. Webb, E.E. Kaiser, B.J. Jurgielewicz, S. Spellicy, S.L. Scoville, T. A. Thompson, R.L. Swetenburg, D.C. Hess, F.D. West, S.L. Stice, Human neural stem cell extracellular vesicles improve recovery in a porcine model of ischemic stroke, Stroke 49 (5) (2018) 1248–1256.
[65] X. Zheng, L. Zhang, Y. Kuang, V. Venkataramani, F. Jin, K. Hein, M.P. Zafeiriou, C. Lenz, W. Moebius, E. Kilic, et al., Extracellular vesicles derived from neural progenitor cells–a preclinical evaluation for stroke treatment in mice, Transl. Stroke Res. 12 (1) (2021) 185–203.
[66] L. Zhang, I. Graf, Y. Kuang, X. Zheng, M. Haupt, A. Majid, E. Kilic, D.M. Hermann, M.N. Psychogios, M.S. Weber, et al., Neural progenitor cell-derived extracellular vesicles enhance blood-brain barrier integrity by NF-κB (Nuclear Factor-κB)-dependent regulation of ABCB1 (ATP-binding cassette transporter b1) in stroke mice, Arterioscler., Thromb., Vasc. Biol. 41 (3) (2021) 1127–1145.
[67] H. Luo, G. Ye, Y. Liu, D. Huang, Q. Luo, W. Chen, Z. Qi, miR-150-3p enhances neuroprotective effects of neural stem cell exosomes after hypoxic-ischemic brain injury by targeting CASP2, Neurosci. Lett. 779 (2022), 136635.
[68] Q. Liu, Y. Tan, T. Qu, J. Zhang, X. Duan, H. Xu, Y. Mu, H. Ma, F. Wang, Therapeutic mechanism of human neural stem cell-derived extracellular vesicles against hypoxia-reperfusion injury in vitro, Life Sci. 254 (2020), 117772.
[69] M.C. Morton, V.N. Neckles, D.M. Feliciano, Isolation of extracellular vesicles from subventricular zone neural stem cells, Methods Mol. Biol. (Clifton, NJ) 2019 (2002) 75–85.
[70] M. Mahdavipour, G. Hassanzadeh, E. Seifali, K. Mortezaee, H. Aligholi, F. Shekari, P. Sarkoohi, Z. Zeraatpisheh, A. Nazari, S. Movassaghi, et al., Effects of neural stem cell-derived extracellular vesicles on neuronal protection and functional recovery in the rat model of middle cerebral artery occlusion, Cell Biochem. Funct. 38 (4) (2020) 373–383.
[71] M.C. Morton, V.N. Neckles, C.M. Seluzicki, J.C. Holmberg, D.M. Feliciano, Neonatal subventricular zone neural stem cells release extracellular vesicles that act as a microglial morphogen, Cell Rep. 23 (1) (2018) 78–89.
[72] A. Yoshimura, N. Adachi, H. Matsuno, M. Kawamata, Y. Yoshioka, H. Kikuchi, H. Odaka, T. Numakawa, H. Kunugi, T. Ochiya, et al., The Sox2 promoter-driven CD63-GFP transgenic rat model allows tracking of neural stem cell-derived extracellular vesicles, Dis. Models Mech. 11 (2018) 1.
[73] X. Sun, J.H. Jung, O. Arvola, M.R. Santoso, R.G. Giffard, P.C. Yang, C.M. Stary, Stem cell-derived exosomes protect astrocyte cultures from in vitro ischemia and decrease injury as post-stroke intravenous therapy, Front. Cell. Neurosci. 13 (2019) 394.
[74] R.G. Rempe, A.M.S. Hartz, B. Bauer, Matrix metalloproteinases in the brain and blood-brain barrier: Versatile breakers and makers, J. Cereb. Blood Flow. Metab.: Off. J. Int. Soc. Cereb. Blood Flow. Metab. 36 (9) (2016) 1481–1507.
[75] R.J. Turner, F.R. Sharp, Implications of MMP9 for blood brain barrier disruption and hemorrhagic transformation following ischemic stroke, Front. Cell. Neurosci. 10 (2016) 56.
[76] J. Song, C. Wu, E. Korpos, X. Zhang, S.M. Agrawal, Y. Wang, C. Faber, M. Schäfers, H. Körner, G. Opdenakker, et al., Focal MMP-2 and MMP-9 activity at the blood-brain barrier promotes chemokine-induced leukocyte migration, Cell Rep. 10 (7) (2015) 1040–1054.
[77] P. Yuan, L. Ding, H. Chen, Y. Wang, C. Li, S. Zhao, X. Yang, Y. Ma, J. Zhu, X. Qi, et al., Neural stem cell-derived exosomes regulate neural stem cell differentiation through miR-9-Hes1 axis, Front. Cell Dev. Biol. 9 (2021), 601600.
[78] Z.H. Zhu, F. Jia, W. Ahmed, G.L. Zhang, H. Wang, C.Q. Lin, W.H. Chen, L.K. Chen, Neural stem cell-derived exosome as a nano-sized carrier for BDNF delivery to a rat model of ischemic stroke, Neural Regen. Res. 18 (2) (2023) 404–409.
[79] G.L. Bowman, L. Dayon, R. Kirkland, J. Wojcik, G. Peyratout, I.C. Severin, H. Henry, A. Oikonomidi, E. Migliavacca, M. Bacher, et al., Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults, Alzheimer'S. Dement.: J. Alzheimer'S. Assoc. 14 (12) (2018) 1640–1650.
[80] S. Dewanjee, P. Chakraborty, H. Bhattacharya, L. Chacko, B. Singh, A. Chaudhary, K. Javvaji, S.R. Pradhan, J. Vallamkondu, A. Dey, et al., Altered glucose metabolism in Alzheimer's disease: Role of mitochondrial dysfunction and oxidative stress, Free Radic. Biol. Med. 193 (Pt 1) (2022) 134–157.
[81] Liu Y., Huber C.C., Wang H.: Disrupted blood-brain barrier in 5×FAD mouse model of Alzheimer's disease can be mimicked and repaired in vitro with neural stem cell-derived exosomes. *Biochemical and biophysical research communications* 2020.
[82] B. Li, J. Liu, G. Gu, X. Han, Q. Zhang, W. Zhang, Impact of neural stem cell-derived extracellular vesicles on mitochondrial dysfunction, sirtuin 1 level, and synaptic deficits in Alzheimer's disease, J. Neurochem. 154 (5) (2020) 502–518.
[83] O. Zolochevska, N. Bjorklund, R. Woltjer, J.E. Wiktorowicz, G. Taglialatela, Postsynaptic proteome of non-demented individuals with Alzheimer's disease neuropathology, J. Alzheimer'S. Dis.: JAD 65 (2) (2018) 659–682.
[84] D. Briley, V. Ghirardi, R. Woltjer, A. Renck, O. Zolochevska, G. Taglialatela, M. A. Micci, Preserved neurogenesis in non-demented individuals with AD neuropathology, Sci. Rep. 6 (2016) 27812.
[85] M.A. Micci, B. Krishnan, E. Bishop, W.R. Zhang, J. Guptarak, A. Grant, O. Zolochevska, B. Tumurbaatar, W. Franklin, C. Marino, et al., Hippocampal stem cells promotes synaptic resistance to the dysfunctional impact of amyloid beta oligomers via secreted exosomes, Mol. Neurodegener. 14 (1) (2019) 25.
[86] S. Meng, H. Chen, C. Deng, Z. Meng, Catalpol mitigates Alzheimer's disease progression by promoting the expression of neural stem cell exosomes released miR-138-5p, Neurotox. Res. 41 (1) (2023) 41–56.
[87] W. Poewe, K. Seppi, C.M. Tanner, G.M. Halliday, P. Brundin, J. Volkmann, A. E. Schrag, A.E. Lang, Parkinson disease, Nat. Rev. Dis. Prim. 3 (2017) 17013.
[88] B.G. Trist, D.J. Hare, K.L. Double, Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease, Aging Cell 18 (6) (2019), e13031.

[89] A.K. Reeve, J.P. Grady, E.M. Cosgrave, E. Bennison, C. Chen, P.D. Hepplewhite, C. M. Morris, Mitochondrial dysfunction within the synapses of substantia nigra neurons in Parkinson's disease, NPJ Park. 'S. Dis. 4 (2018) 9.
[90] E.J. Lee, Y. Choi, H.J. Lee, D.W. Hwang, D.S. Lee, Human neural stem cell-derived extracellular vesicles protect against Parkinson's disease pathologies, J. Nanobiotechnol. 20 (1) (2022) 198.
[91] R. Kumar, J. Lim, R.A. Mekary, A. Rattani, M.C. Dewan, S.Y. Sharif, E. Osorio-Fonseca, K.B. Park, Traumatic spinal injury: global epidemiology and worldwide volume, World Neurosurg. 113 (2018) e345–e363.
[92] C. Chen, Y.P. Zhang, Y. Sun, W. Xiong, L.B.E. Shields, C.B. Shields, X. Jin, X. M. Xu, An in vivo duo-color method for imaging vascular dynamics following contusive spinal cord injury, J. Vis. Exp. JoVE (130)) (2017).
[93] H.M. Bramlett, W.D. Dietrich, Long-term consequences of traumatic brain injury: current status of potential mechanisms of injury and neurological outcomes, J. Neurotrauma 32 (23) (2015) 1834–1848.
[94] X. Yuan, Q. Wu, P. Wang, Y. Jing, H. Yao, Y. Tang, Z. Li, H. Zhang, R. Xiu, Exosomes derived from pericytes improve microcirculation and protect blood-spinal cord barrier after spinal cord injury in mice, Front. Neurosci. 13 (2019) 319.
[95] L. Zhang, P. Han, Neural stem cell-derived exosomes suppress neuronal cell apoptosis by activating autophagy via miR-374-5p/STK-4 axis in spinal cord injury, J. Musculoskelet. Neuron Interact. 22 (3) (2022) 411–421.
[96] Y. Rong, W. Liu, J. Wang, J. Fan, Y. Luo, L. Li, F. Kong, J. Chen, P. Tang, W. Cai, Neural stem cell-derived small extracellular vesicles attenuate apoptosis and neuroinflammation after traumatic spinal cord injury by activating autophagy, Cell death Dis. 10 (5) (2019) 340.
[97] Y. Rong, W. Liu, C. Lv, J. Wang, Y. Luo, D. Jiang, L. Li, Z. Zhou, W. Zhou, Q. Li, et al., Neural stem cell small extracellular vesicle-based delivery of 14-3–3 t reduces apoptosis and neuroinflammation following traumatic spinal cord injury by enhancing autophagy by targeting Beclin-1, Aging 11 (18) (2019) 7723–7745.
[98] I. Mohammed, S. Ijaz, T. Mokhtari, M. Gholaminejhad, M. Mahdavipour, B. Jameie, M. Akbari, G. Hassanzadeh, Subventricular zone-derived extracellular vesicles promote functional recovery in rat model of spinal cord injury by inhibition of NLRP3 inflammasome complex formation, Metab. Brain Dis. 35 (5) (2020) 809–818.
[99] D. Zhong, Y. Cao, C.J. Li, M. Li, Z.J. Rong, L. Jiang, Z. Guo, H.B. Lu, J.Z. Hu, Neural stem cell-derived exosomes facilitate spinal cord functional recovery after injury by promoting angiogenesis, Exp. Biol. Med. 245 (1) (2020) 54–65.
[100] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (4) (2006) 663–676.
[101] R. Upadhya, L.N. Madhu, S. Attaluri, D.L.G. Gitaí, M.R. Pinson, M. Kodali, G. Shetty, G. Zanirati, S. Kumar, B. Shuai, et al., Extracellular vesicles from human iPSC-derived neural stem cells: miRNA and protein signatures, and anti-inflammatory and neurogenic properties, J. Extracell. Vesicles 9 (1) (2020), 1809064.
[102] D. Moore, B.M. Meays, L.S.V. Madduri, F. Shahjin, S. Chand, M. Niu, A. Albahrani, C. Guda, G. Pendyala, H.S. Fox, et al., Downregulation of an evolutionary young miR-1290 in an iPSC-derived neural stem cell model of autism spectrum disorder, Stem Cells Int. 2019 (2019), 8710180.
[103] H. Branscome, P. Khatkar, S. Al Sharif, D. Yin, S. Jacob, M. Cowen, Y. Kim, J. Erickson, C.A. Brantner, N. El-Hage, et al., Retroviral infection of human neurospheres and use of stem Cell EVs to repair cellular damage, Sci. Rep. 12 (1) (2022) 2019.
[104] L.S.A. Valerio, K. Sugaya, Xeno- and transgene-free reprogramming of mesenchymal stem cells toward the cells expressing neural markers using exosome treatments, PloS One 15 (10) (2020), e0240469.
[105] S. Saksena, J. Sun, T. Chu, S.D. Emr, ESCRTing proteins in the endocytic pathway, Trends Biochem. Sci. 32 (12) (2007) 561–573.
[106] B. McDonald, J. Martin-Serrano, Regulation of Tsg101 expression by the steadiness box: a role of Tsg101-associated ligase, Mol. Biol. Cell 19 (2) (2008) 754–763.
[107] E.J. Yoon, Y. Choi, T.M. Kim, E.K. Choi, Y.B. Kim, D. Park, The neuroprotective effects of exosomes derived from TSG101-overexpressing human neural stem cells in a stroke model, Int. J. Mol. Sci. 23 (2022) 17.
[108] C.C. Huber, E.A. Callegari, M.D. Paez, S. Romanova, H. Wang, Heat shock-induced extracellular vesicles derived from neural stem cells confer marked neuroprotection against oxidative stress and amyloid-β-caused neurotoxicity, Mol. Neurobiol. 59 (12) (2022) 7404–7412.
[109] M. Kim, H.W. Yun, D.Y. Park, B.H. Choi, B.H. Min, Three-dimensional spheroid culture increases exosome secretion from mesenchymal stem cells, Tissue Eng. Regen. Med. 15 (4) (2018) 427–436.
[110] W. Zhang, X. Zhou, Q. Yao, Y. Liu, H. Zhang, Z. Dong, HIF-1-mediated production of exosomes during hypoxia is protective in renal tubular cells, Am. J. Physiol. Ren. Physiol. 313 (4) (2017). F906-f913.
[111] Fan Y., Li J., Fang B.: A. Tale of Two: When Neural Stem Cells Encounter Hypoxia. *Cellular and molecular neurobiology* 2022.
[112] D.T. Bowers, C.E. Olingy, P. Chhabra, L. Langman, P.H. Merrill, R.S. Linhart, M. L. Tanes, D. Lin, K.L. Brayman, E.A. Botchwey, An engineered macroencapsulation membrane releasing FTY720 to precondition pancreatic islet transplantation, J. Biomed. Mater. Res. Part B, Appl. Biomater. 106 (2) (2018) 555–568.
[113] J. Chen, C. Zhang, S. Li, Z. Li, X. Lai, Q. Xia, Exosomes derived from nerve stem cells loaded with FTY720 promote the recovery after spinal cord injury in rats by PTEN/AKT signal pathway, J. Immunol. Res. 2021 (2021), 8100298.
[114] L.L. Xiong, J. Chen, R.L. Du, J. Liu, Y.J. Chen, M.A. Hawwas, X.F. Zhou, T. H. Wang, S.J. Yang, X. Bai, Brain-derived neurotrophic factor and its related enzymes and receptors play important roles after hypoxic-ischemic brain damage, Neural Regen. Res. 16 (8) (2021) 1453–1459.
[115] F.L. Willekens, J.M. Werre, J.K. Kruijt, B. Roerdinkholder-Stoelwinder, Y. A. Groenen-Döpp, A.G. van den Bos, G.J. Bosman, T.J. van Berkel, Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors, Blood 105 (5) (2005) 2141–2145.
[116] R. van der Meel, M.H. Fens, P. Vader, W.W. van Solinge, O. Eniola-Adefeso, R. M. Schiffelers, Extracellular vesicles as drug delivery systems: lessons from the liposome field, J. Control. Release: Off. J. Control. Release Soc. 195 (2014) 72–85.
[117] L. Deng, F. Zhang, Y. Wu, J. Luo, X. Mao, L. Long, M. Gou, L. Yang, D.Y.B. Deng, RGD-modified nanocarrier-mediated targeted delivery of HIF-1α-AA plasmid DNA to cerebrovascular endothelial cells for ischemic stroke treatment, ACS Biomater. Sci. Eng. 5 (11) (2019) 6254–6264.
[118] S.A.A. Kooijmans, J. Gitz-Francois, R.M. Schiffelers, P. Vader, Recombinant phosphatidylserine-binding nanobodies for targeting of extracellular vesicles to tumor cells: a plug-and-play approach, Nanoscale 10 (5) (2018) 2413–2426.
[119] T. Tian, L. Cao, C. He, Q. Ye, R. Liang, W. You, H. Zhang, J. Wu, J. Ye, B. A. Tannous, et al., Targeted delivery of neural progenitor cell-derived extracellular vesicles for anti-inflammation after cerebral ischemia, Theranostics 11 (13) (2021) 6507–6521.
[120] Y. Xiao, J. Tian, W.C. Wu, Y.H. Gao, Y.X. Guo, S.J. Song, R. Gao, L.B. Wang, X. Y. Wu, Y. Zhang, et al., Targeting central nervous system extracellular vesicles enhanced triiodothyronine remyelination effect on experimental autoimmune encephalomyelitis, Bioact. Mater. 9 (2022) 373–384.
[121] W.C. Wu, J. Tian, D. Xiao, Y.X. Guo, Y. Xiao, X.Y. Wu, G. Casella, J. Rasouli, Y. P. Yan, A. Rostami, et al., Engineered extracellular vesicles encapsulated Bryostatin-1 as therapy for neuroinflammation, Nanoscale 14 (6) (2022) 2393–2410.
[122] X.Y. Wu, B.Y. Liao, D. Xiao, W.C. Wu, Y. Xiao, T. Alexander, S.J. Song, Z.H. Zhao, Y. Zhang, Z.H. Wang, et al., Encapsulation of bryostatin-1 by targeted exosomes enhances remyelination and neuroprotection effects in the cuprizone-induced demyelinating animal model of multiple sclerosis, Biomater. Sci. 10 (3) (2022) 714–727.
[123] J. Tian, G. Casella, Y. Zhang, A. Rostami, X. Li, Potential roles of extracellular vesicles in the pathophysiology, diagnosis, and treatment of autoimmune diseases, Int. J. Biol. Sci. 16 (4) (2020) 620–632.
[124] K.M. Candelario, L. Balaj, T. Zheng, J. Skog, B. Scheffler, X. Breakefield, B. Schüle, D.A. Steindler, Exosome/microvesicle content is altered in leucine-rich repeat kinase 2 mutant induced pluripotent stem cell-derived neural cells, J. Comp. Neurol. 528 (7) (2020) 1203–1215.
[125] S. Lee, S. Mankhong, J.H. Kang, Extracellular vesicle as a source of Alzheimer's biomarkers: opportunities and challenges, Int. J. Mol. Sci. 20 (2019) 7.
[126] D. Kapogiannis, Exosome biomarkers revolutionize preclinical diagnosis of neurodegenerative diseases and assessment of treatment responses in clinical trials, Adv. Exp. Med. Biol. 1195 (2020) 149.