Extracellular Vesicles in Neurodegenerative Diseases: A Double-Edged Sword

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Abstracts Extracellular vesicles (EVs), a heterogenous group of membrane-bound particles, are virtually secreted by all cells and play important roles in cell–cell communication. Loaded with proteins, mRNAs, non-coding RNAs and membrane lipids from their donor cells, these vesicles participate in normal physiological and pathogenic processes. In addition, these sub-cellular vesicles are implicated in the progression of neurodegenerative disorders. Accumulating evidence suggests that intercellular communication via EVs is responsible for the propagation of key pathogenic proteins involved in the pathogenesis of amyotrophic lateral sclerosis, Parkinson’s diseases, Alzheimer’s diseases and other neurodegenerative disorders. For therapeutic perspective, EVs present advantage over other synthetic drug delivery systems or cell therapy; ability to cross biological barriers including blood brain barrier (BBB), ability to modulate inflammation and immune responses, stability and longer biodistribution with lack of tumorigenicity. In this review, we summarized the current state of EV research in central nervous system in terms of their values in diagnosis, disease pathology and therapeutic applications.

Keywords Extracellular vesicles · Neurodegenerative diseases · Drug delivery system · Blood brain barrier

1 Introduction

Cells in multicellular organisms utilize several modes of communication ranging from direct cell–cell contact to distant paracrine signaling such as growth factors and hormones. Extracellular vesicle (EV), a heterogeneous family of membrane-limited vesicles, is the newest addition to the intercellular communication system. Studies found that these vesicles are loaded with various proteins,

lipids, and nucleic acids, derived from the endosome or plasma membrane [1, 2] and play important roles in normal physiological process as well as in pathological progression [3–5]. EVs have been classically divided into three main categories according to their biogenesis, secretory processes, biophysical properties, size and surface protein markers: (1) exosomes, (2) microvesicles, and (3) apoptotic bodies [6]. In this review, we illustrated the function of EVs and their therapeutic potential in the treatment of neurodegenerative disorders.

Exosomes were discovered over 30 years ago as small vesicles participating in waste disposal [7]. First formed as multivesicular bodies (MVBs) by invagination of the endosomal membrane as intraluminal vesicles (ILV), they may take one of three distinct fates, to form new lysosomes, to fuse with lysosome for degradation or to fuse with plasma membrane [8, 9] thereby releasing exosomes into extracellular space (Fig. 1). Exosomes are typically 40–150 nm in diameter. Biogenesis of exosomes via the formation of MVBs and their secretion appears to be mediated by ESCRT

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Fig. 1 Biogenesis of extracellular vesicles (EVs). Functional EVs are generated via 2 distinct pathways: exosomes and microvesicles. Intraluminal vesicles are formed by invagination of the late endosome generating multivesicular bodies (MVB). The MVB has one of two fates; it will either fuse with the lysosome to degradation of intraluminal content or fuse with the plasma membrane to releases exosome release. The endosomal sorting complex required for transport (ESCRT) contribute to the formation of MVB, protein cargo sorting and lysosomal degradation or exosome secretion. Cargo

content comprises cytoplasmic proteins, membrane proteins and ribonucleic acids (mRNA, miRNA, tRNA and ncRNA). On the contrary, microvesicles (MVs) bud from the plasma membrane. Their cargo contains membrane proteins, cytoplasmic proteins, RNAs and DNAs. Upon interaction with target cells via ligand–receptor interaction, endocytosis, phagocytosis or direct fusion with plasma membrane and membrane of EVs, cargo content can be delivered to recipient cells to regulate the function of recipient cells via intracellular signaling and/or gene regulation

(endosomal sorting complexes required for transport) complexes that recruit ubiquitin-tagged membrane proteins and induce invagination of the endosomal membrane to form ILVs [9–11]. However, ILVs can also be formed even in the absence of ESCRT implying the presence of ESCRT-independent mechanism of ILV formation [12]. Most of exosomes have molecular markers which are involved in their biogenesis process, including CD63, CD9, CD81, tetraspanins, Alix, and TSG101, and have specific proteins that reflect their cellular origin [13, 14] (Table 1).

Microvesicles (MVs), also known as shedding vesicles or ectosomes, are a heterogeneous population of membrane vesicles up to 50–1000 nm in diameter. The biogenesis and release of MVs is different from exosome biogenesis and only have been studied in several cell models. In general, they are formed by outward budding and fission of the plasma membrane. Apoptotic signal is also known to promote the release of these particles via the initiation of actin–myosin sliding, resulting in budding and detachment of the membrane from the cytoskeleton [2, 21, 22]. In addition, hypoxia has been shown to promote MV release via HIF-dependent expression of RAB22A [23]. Release of MVs

might be regulated, at least in part, by ESCRT-I and ADP-ribosylation factor 6 (ARF6) [24]. Several studies identified different markers for MVs like CD63, Rab8, HSP90, gp96, CD81 and a high level of phosphatidylserine on the outer surface [18, 25]. However, it is a difficult to experimentally distinguish between exosomes and MVs because of their overlapping biophysical characteristics the lack of discriminating markers, and their biological compositions (mRNA, miRNA, proteins and lipids) [26]. Apoptotic bodies are a heterogeneous population of cell fragments that form during apoptosis. Their diameter is about 500–5000 nm, and they have variable morphology. These vesicles contain the degradation products of subcellular organelles, including organelle fragments, DNA, histones, and cytoplasmic components [21]. It is evident that EVs derived from variety cell types contain functional proteins, mRNA and microRNA (miRNA) and could be delivered by EVs to target cells and translated into the corresponding proteins leading to epigenetic changes of the target cells. These findings have established the novel concept that EVs serve as carriers for intercellular communication and have applied and expanded on various fields including bench to clinic [27–29].

Table 1 Characteristics of extracellular vesicle subtypes

Classes	Biogenesis	Marker	Contents	Refs.
Exosome (40–150 nm)	Invagination of endosomal membrane to form multivesicular bodies (MVBs), exosomes are released from cells upon fusion of MVBs with plasma membrane	CD9, CD63, CD81 Alix, ESCRT and TSG101	Surface and intraluminal proteins including MHC class II, cytokines, adhesion molecules and neurotransmitter receptors, mRNA, non-coding RNA and DNA Enriched in phosphatidylserine, sphingomyelin and cholesterol	[15–17]
Microvesicle (50–1000 nm)	Budding directly from the plasma membrane	No definite markers, Annexin-V, CD63, selectins, integrin, metalloproteinase	Surface and intraluminal proteins including cytokines, adhesion molecules mRNA and non-coding RNA Enriched in phosphatidylserine, sphingomyelin and cholesterol	[18, 19]
Apoptotic bodies (500–5000 nm)	Fragmentation of cells undergoing apoptosis	Annexin-V, DNA, histones	DNA, histones, organelle fragments	[20]

2 Function of extracellular vesicle in the nervous system

In addition to local communication (such as gap junction) and distant communication (soluble factors), EVs are a key mediator in the intercellular communication between cells allowing horizontal transfer of biological signals [30]. Released EVs from parent cell can be internalized via endocytosis or membrane fusion, delivering their cargo molecules to recipient cells. These vesicles not only participate in the regulation of normal physiological processes, such as development, tissue homeostasis, tissue repair and regeneration, immune surveillance, blood coagulation, but also in the disease pathology [4].

EVs act as a mediator for membrane receptor transfer into target cells thereby conferring their responsiveness to a specific ligand, as in the case of platelet-specific adhesion molecules transfer by platelet-derived EVs to hematopoietic cells [31]. Cytoplasmic molecules, including signaling molecules and transcription factors, can also be transferred by EVs thereby directly activating relevant signaling pathway or inducing target gene expression in neighboring cells leading to changes in the cellular phenotype. Studies have shown that horizontal transfer of RNA by EVs induces epigenetic changes in target cells *in vitro* as well as *in vivo* [15, 32–35]. MicroRNAs (miRNAs) are highly conserved small non-coding RNA molecules (approximately 19–23 nucleotides in length) that involved in the regulation of gene expression by translational repression, mRNA cleavage and de-adenylation [36]. With these molecular cargo, EVs have a significant impact on natural physiological processes including the regulation of cell fate or cell plasticity. However, the ability of EVs to transfer information to other cells can be seen as a two-edged sword; on the one hand, they are beneficial to maintain homeostasis by modulating neighboring cells and

recruiting endogenous stem cells in the local microenvironment while on the other, they can participate in disease propagation through the delivery of genetic material and/or pathogenic proteins as in the cases of neurodegenerative disease [37].

EVs are known to be released by most cell types in the nervous system, including neurons, astrocytes, microglial cells and oligodendrocytes (Fig. 2). Proteomic analysis of EVs extracted from cerebrospinal fluid of healthy subjects revealed the presence of brain-derived proteins of neuron and microglial origins [38] indicating that neurons and glial cells release EVs. Von Bartheld and Altick reported that neuronal MVBs are mainly located in the cell bodies and dendrites than axon of central as well as peripheral nervous systems [39] and Lachenal et al. [40] demonstrated the endosomal origin of exosomes and showed their release from somato-dendritic compartments of cortical neurons in culture by electron microscopy. The function of these EVs in the nervous system is of great importance. In addition to the role of clearance and disposal of metabolites or toxic molecules in local tissues, these particles may participate in the development and remodeling of neural networks, regeneration, protection, immunomodulation and regulation of neural synaptic plasticity [41, 42]. EV-mediated delivery of miRNA is a means to regulate the expression of specific sets of genes that leads to functional and behavioral changes in the target cells. Liu et al. [43] showed that EVs from primary embryonic cortical neuron restrict dendritic growth cell-autonomously during development by miRNAs (let-7c and miR-21) in it. In addition, neuronal EVs mediated synaptic pruning for selective removal of degenerating neurites of inappropriate connections by glial cells implying their roles in development and pathological processes [44]. Fauré et al. [45] reported that EVs from cortical neuron EVs transmit biological information to facilitate neural synaptic function, including passing newly

Fig. 2 EVs (exosomes and microvesicles) in the cell-to-cell communications and their role in nervous system. EVs are produced by neurons, astrocytes, microglial cells and oligodendrocytes and these EVs can transfer biological information in their cargo to other cells via neuron-to-neuron, neuron-to-glia or glia-to-neuron. EVs

contribute to intercellular communication in the brain through their basal release and uptake by surrounding cells. Secreted EVs from host cells get into recipient cell using by fusion with cell membrane and endocytosis. In neurodegenerative disease, EVs are able to play a dual function; neuro-protective and neurotoxic role

synthesized proteins, such as the L1 cell adhesion molecule and the GluR2/3, on to the presynaptic terminals of neighboring neurons and contribute to the local synaptic plasticity. The secretion of EVs in neurons and glial cells appears to be regulated by calcium influx and by glutamatergic synaptic activity [45–47] implying their role in synaptic function. However, the relationship between exosome release and exocytosis of synaptic vesicle during neurotransmission is unclear. In addition to their protein cargo, EVs deliver functionally active RNA molecules in neuronal synaptic terminals [48]. Immunomodulatory role of EVs was demonstrated by Cossetti et al. [49] that EVs from neural stem/precursor cells (NPCs) treated with proinflammatory cytokines induced interferon gamma (IFN- γ) pathway in target cells via activating STAT1 implying that grafted stem cells may utilize their EVs in communication with host immune system (Fig. 3).

Although EVs may serve as messengers for reciprocal communication between neuron and glial cells, EVs from cortical neuron can only bind and be endocytosed by neurons, but not glial cells, implying that there is a cell-selectivity as well as cell-specific function [46]. For example, proteomic analysis of EVs from oligodendrocytes revealed that they contain major myelin proteolipid protein

and stress-protective proteins implying their roles in balanced production of myelin proteins and lipids [50] and trophic support of axons. Bidirectional action of EVs from neurons and glial cells for neuronal integrity was demonstrated by Frühbeis et al. [41, 47]. They showed that activity-dependent release of glutamate induced EV release from oligodendrocytes and the released EVs, containing superoxide dismutase and catalase, were internalized by neurons leading to improved cell viability under stressed condition. EVs from Schwann cells also exhibit a neuro-protective role in the periphery by increasing neurite growth and enhanced axonal regeneration *in vitro* and *in vivo* [51]. Wang et al. [52] reported that EVs from astrocytes promoted neurite outgrowth and neuronal survival under conditions of oxidative stress or high neuronal activity via synapsin I in the coculture experiment. Microglial cells, the resident immune cells in the brain, play essential roles in regulating inflammatory responses during infection and injury. Functional communication of microglial cells with neuron via EVs was demonstrated by coculturing microglial cells with serotonergic neuron *in vitro* where serotonin induces the release of EVs from microglial cells [53]. Contribution of EVs in microglial-to-neuron communication was demonstrated by injection of

Fig. 3 Hypothetical mechanisms of cell-to-cell transmission of cytosolic protein aggregates in neurodegenerative diseases. Misfolded protein seeds (oligomers and fibrils) are formed in the cytoplasm of affected neuron (left) where normal protein monomers are recruited to the growing aggregates. More seeds are generated by fragmentation of aggregates that serve as a secondary nucleation in a self-perpetuating process. Pathogenic protein aggregates can be released

into extracellular space as the monomer, oligomer or via EVs (exosome or microvesicle). Seeds may enter by ① direct penetration to the plasma membrane, ② endocytosis, ③ receptor-mediated endocytosis, or ④ membrane fusion with the neighboring cells (right). Internalized seeds then serve as templates to drive fibrillation of pathogenic aggregates in the cytoplasm, thereby spreading pathological proteins to interconnected neurons and glial cells

microglial MVs into rat visual cortex showing that stimulation of synaptic activity by microglia-derived EVs led to enhanced sphingolipid metabolism [54]. These studies provided evidence that the interactive exchanges of EVs among neurons and glial cells are essential for synapse function and plasticity.

3 The role of EVs in the pathology of neurodegenerative diseases

Neurodegenerative diseases are a group of heterogeneous disorders that can be defined as a progressive loss of structure and function of central or peripheral nervous system. Although the cause of diseases appears to be different, neurodegenerative diseases share similarities in molecular mechanisms underlying their pathogenesis including the involvement of misfolding and aggregation

of specific proteins (known as proteinopathies) and cell-to-cell propagation of these proteins [55]. The aggregates of misfolded proteins are highly resistant to degradative pathway [56]. Normally, misfolded proteins generated by genetic mutations or errors during protein synthesis or protein assembly, are subjected to refolding, degradation or sequestration and chaperone proteins are the key protein components in sensing and determining the fate of these misfolded proteins [57]. Once these misfolded proteins escape the clearance due to either functional defects in proteolytic system or mutations in the affected proteins and begin to accumulate into non-native multimeric fibers. A hallmark of the neurodegenerative proteinopathies is the formation and propagation of misfolded protein aggregates that cause neurotoxicity via prion-like manner from neurons to other neurons/glial cells by recruiting normal cellular proteins of their own kind or other proteins to take pathogenic conformation (Table 2).

Table 2 Overview of misfolded proteins associated with EVs in the neurodegenerative diseases

Disease	Aggregating proteins	Refs.
Alzheimer's disease (AD)	Amyloid- β peptide, tau protein	[58–60]
Parkinson's disease (PD)	α -synuclein (α -syn)	[61, 62]
Huntington's disease (HD)	Polyglutamine-expanded (polyQ) proteins	[63]
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase 1, TDP-43, FUS, C9ORF72, ubiquitin-2	[64–66]
Spongiform encephalopathies	Prion proteins	[67, 68]

Alzheimer's disease (AD), the most common form of dementia, is a neurodegenerative disorder in which the death of neuronal cells in brain causes gradual memory loss and cognitive decline. Two toxic protein derivatives, amyloid- β and microtubule-associated protein tau, are the key pathogenic molecules for Alzheimer's disease [69]. The pathogenesis of the disease is characterized microscopically by the presence of two highly insoluble, densely packed structures in the brain; extracellular plaques, deposits of amyloid- β , and intraneuronal neurofibrillary tangles, twisted fibers of tau. While amyloid- β and tau are the independent seeds (building blocks) for plaques and tangles, respectively, studies have shown that misfolded amyloid- β requires tau for disease pathogenesis [70, 71]. The spreading of toxic, misfolded amyloid- β and tau during disease progression resembles to the pathogenesis of prion protein (PrP).

Parkinson's disease (PD) is a progressive neurodegenerative disorder. Dopaminergic neurons in the substantia nigra are the primarily affected cell types resulting in gradual loss of control in movement and coordination [72]. Although the cause of the disease is unknown, it involves the aberrant soluble oligomeric aggregates of α -synuclein (α -syn) known as Lewy bodies in the neurons [73]. The physiological function of α -syn is not clearly defined, but studies proposed a role in maintaining a supply of synaptic vesicles in the presynaptic terminals by clustering synaptic vesicles [74, 75] and in regulating dopamine release [76]. While α -helical conformation is typical for α -syn, this protein adopts β -sheet-rich conformation under certain circumstance leading to polymerization of α -syn proteins. As of prion, aggregated fibrils of α -syn are highly toxic to neurons and secreted misfolded α -syn is known to serve as a seed for subsequent aggregation of α -syn in unaffected neurons, thus contributing to disease propagation [77]. Indeed, a single intracerebral injection of the misfolded α -syn mimicked the early stages of Parkinson's disease in mice including cell death of dopaminergic neuron [78].

Huntington's disease (HD) is an inherited neurodegenerative disorder that causes the progressive neural cell loss in the caudate, putamen and the cerebral cortex resulting in movement, cognitive and emotional disorders. It is caused by dominant mutation in the *IT-15* gene that expands the number of CAG repeats within an existing poly-glutamine (polyQ) repeat sequence of a gene leading to the translation of huntingtin protein with polyQ repeats [79]. The ubiquitously expressed huntingtin protein is known to interact with over 100 other proteins [80] implying their pleiotrophic functions. Although the gene and mutations that cause Huntington's disease is unknown, the hallmark of the disease pathology is the formation of intraneuronal fibrillar aggregates of mutant huntingtin protein forming intracellular inclusion bodies. Similar to other amyloid-related

neurodegenerative diseases, the overabundance of a polar glutamine makes huntingtin proteins prone to aggregate by converting them into β -sheet-rich conformation [81, 82] and mutant huntingtin protein recruits wild type proteins to the aggregates [83]. Although studies have shown that mutated huntingtin protein-containing inclusion bodies are not correlated with neurotoxicity [84], but may be protective [85], the accumulation of protein aggregates may interfere the function of neurons [86] and the loss of normal huntingtin protein itself may also contribute to the disease pathology [87].

Amyotrophic lateral sclerosis (ALS) is the most common fatal adult-onset disorder with progressive degeneration of motor neurons in the brain and spinal cord. More than 90% of ALS cases are sporadic with the remaining 5–10% being familial. A number of ALS-associated gene defects have been identified including *SOD1* (encoding copper zinc superoxide dismutase 1), *TARDBP* (encoding TAR DNA-binding protein 43), *hnRNPA2B1/A1*, *SETXN*, *PFN1*, *TAF15*, *ATXN2*, *C9ORF72*, *UBQLN2*, *OPTN*, *VCP*, and *FUS* genes [88]. As of other neurodegenerative disorders, the hallmark of the disease pathology is formation of protein inclusion that contains SOD1 (a ubiquitous mitochondrial enzyme scavenging reactive oxygen species), TDP-43 (a ubiquitous RNA transporting protein), FUS (fused in sarcoma, a RNA binding protein), FUS, p62, UBQLN2, and C9ORF72 dipeptides protein [89]. *In vitro* and *in vivo* studies provided evidence of insoluble fibrillar aggregate formation by these proteins that act as seed for subsequent aggregation of normal proteins [90, 91]. In addition to serve as seed for aggregate formation, misfolding of mutated SOD1 leads to the mitochondrial dysfunction [92]. Since two structurally and functionally similar RNA/DNA binding proteins, TDP-43 and FUS, are participating in multiple steps of RNA processing, the consequences of mutations in these proteins is both gain of toxic function and loss of normal function effects [93]. Aggregates of some of these pathogenic proteins are also observed in other neurodegenerative disorders, such as frontotemporal lobar degeneration (FTLD), Alzheimer's disease and Huntington's disease [94].

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a rare group of fatal progressive neurodegenerative disease with characteristic spongiform changes associated with neuronal loss in the brain cortex. PrP or prion protein (PrP^C for the native form, PrP^{Sc} for the misfolded form), the causative agent for the disease, are known to play multiple cellular functions including ion channel activity and neuronal excitability, misfolded PrP^{Sc} leads to the formation of pleiomorphic aggregates that can be transmitted from cell to cell or host to host by transforming normal protein (PrP^C) into pathogenic conformation resulting in neuronal degeneration. Although an

autosomal dominant mutation in the prion protein gene (*PRNP*) were found in 10–15% of the patients [95], there is no disease-specific modification identified in the majority of patients.

Although controversial, the involvement of extracellular vesicles in the inter-neuronal spread of misfolded proteins has been proposed [37, 96]. Accumulating evidence suggests that EVs have the capacity to deliver the misfolded and mutant proteins related to neurodegenerative diseases (Table 2).

EV-mediated transmission of amyloid- β in Alzheimer's disease was first reported by Rajendran et al. [58], their physiological role is controversial. While some considered the EV compartment as a neuroprotective process to secrete toxic oligomeric amyloid- β [97], others see this process for the propagation of neurotoxic oligomer [98]. Detection of EVs carrying enzymes that degrade amyloid- β [99] and carrying miRNAs that activate microglial cell function [44] supports the notion of pathogenic amyloid β clearance by EVs. *In vitro* and *in vivo* studies have shown that secreted EVs participate in the transmission of pathogenic tau protein [100, 101]. In addition, inhibition of exosome biogenesis by neural sphingomyelinase-2 either by siRNA or chemical drug (GW4869) reduced the propagation of tau *in vitro* and *in vivo* [102]. Yet, the relative contribution of EV-mediated transmission and direct cell-to-cell transmission in the pathological spreading of misfolded protein aggregates in Alzheimer's disease is undetermined and additional studies are necessary.

Although EVs' role in the pathogenesis of Parkinson's disease is a matter of debate, Lee et al. [103] first discovered the EV-mediated secretion of α -syn. They found that a fraction of newly synthesized α -syn, localized in the intraluminal vesicles, are more prone to aggregation and are secreted from cells. In the study, they also reported that α -syn secretion is elevated by proteosomal and mitochondrial dysfunction, the defects typically associated with various neurodegenerative disorders including Parkinson's disease [104]. Since then, a number of studies have shown that EVs participate in the α -syn spreading [61, 105–107]. Although the underlying mechanism of α -syn incorporation to EVs is not clear, its involvement in synaptic vesicular trafficking [108] during may provide a clue for the process.

Neurons secreting EVs loaded with mutant SOD1 and TDP-43 to the extracellular spaces and the transmission of these misfolded proteins were demonstrated *in vitro* [65, 109] implying their roles in amyotrophic lateral sclerosis pathogenesis. Thus, EVs function as the shuttling vehicles for amyloids in plaque formation that can be decreased by inhibitor of exosome secretion in the mouse model of ALS [97, 110]. Basso et al. showed that EV-

loaded with mutant SOD1 from astrocytes is neurotoxic to motor neurons [64] indicating the pathogenic nature of EV-loaded mutant SOD1. In addition, *in vivo* study showed that EVs containing TDP-43, the target of protein aggregation containing prion-like domain in ALS, participate in the disease progression by axonal transportation in brain and spinal cord in a prion-like manner [111]. More recently, dipeptide repeat proteins (DPRs), produced by one of the most common mutated gene, C9orf72, is also associated with exosome [112] further strengthening the role of EVs in the ALS pathogenesis.

Involvement of EVs in Huntington disease transmission was elegantly demonstrated *in vitro* and *in vivo* of inter-species model, where human exosome carrying mutated huntingtin protein induced Huntington disease-like symptoms and pathology in newborn mice [113] indicating that mutant huntingtin protein via EVs can infect and induce the pathogenic protein aggregation. In prion diseases, PrP is one of the most common proteins identified in EVs [114] implying their role in the transmission of pathological protein. More recently, Guo et al. [115] showed that stimulation of exosome release by ionophore, monensin increased the intercellular infectivity of prion, whereas inhibition of exosome biogenesis by GW4869 decreased the prion transmission highlighting their pivotal role in the prion transmission. Collectively, EVs may serve as a double-edge sword in nervous system; the protective pathogenic roles.

4 Neuroprotective role and therapeutic potentiation of EVs in neurodegenerative disease

A number of studies have showed that therapeutic effects of mesenchymal stem cells (MSCs) and other adult stem cells are mostly attributed to paracrine action rather than direct action of transplanted cells [116–119]. Paracrine factors from transplanted cells promote proliferation of tissue cells, induce revascularization [120], modulate immune response and protect apoptosis via various pathways [121, 122]. Interestingly, purified EVs from MSCs exerted most, if not all, of these protective effects suggesting that EVs are the major mechanism of MSC-mediated tissue repair and regeneration [123–125]. As of other tissues [126, 127], EVs represent a typical mode of inter-cellular communication by between neurons and glial cells that is essential for the maintenance of functional and structural integrity of the central nervous system [128].

A number of studies provided evidence that EVs contribute to neural restoration in experimental models of brain injury or neurodegenerative diseases via neuroprotection, angiogenesis and neurogenesis. Intracerebrally administration of neuroblastoma-derived exosomes

reduced deposition of amyloid- β and induced its clearance by microglial cells in amyloid- β precursor protein transgenic mice [129]. Systemic administration of brain extract-treated MSCs derived EVs reduced inflammation on ischemic area and increased the number of neuroblasts in subventricular zone (SVZ) and hippocampus in rat ischemic stroke model [130]. They suggested that miR-133b in MSC-derived EVs modulate gene expression in astrocytes and neurons, subsequently leading to neurite remodeling, axonal plasticity and functional recovery after stroke [131]. Lee et al. [132] found that MSCs delivered exogenous pre-miRNAs and miRNA (miR-124 and miR-145) to human neuronal precursor cells (NPCs) and astrocytes leading to alterations in the expression of target mRNA and increased the neuronal differentiation of the NPCs. Also, MSC-derived EVs improve functional recovery by increasing endogenous angiogenesis and neurogenesis and by diminishing inflammation in rat model with traumatic brain injury (TBI) and ischemic brain injury [133]. In addition, EVs are known to stimulate angiogenesis in damaged brain. BM-MSC derived EVs demonstrated a significant increase in the percentage of vWF-positive cells and α -SMA-positive cells in ischemic zone after injected with EVs in stroke rat [131, 134]. Dental pulp stem cell derived EVs suppressed 6-OHDA-induced apoptosis in dopaminergic neurons *in vitro* [135]. Katsuda et al. [136] recently proposed a new therapeutic possibility for Alzheimer's disease using MSC-derived EVs that contain neprilysin, the amyloid- β degrading enzyme, in the brain. Hence, EVs from MSCs emerge as a novel cell-free therapy for brain injury and other CNS diseases.

Although significant advances in understanding the molecular mechanisms underlying the pathogenesis of neurodegenerative diseases has been made, numerous attempts to targeting the initiation and propagation of these devastating diseases with chemical drugs, proteins, gene therapy and cell therapy have met with little success in patients with advanced stages so far. With their ability to cross BBB, EVs are gaining increasing attention as an ideal drug delivery system over conventional synthetic drug delivery vehicles [137] in a number of animal models or *in vitro* models with neuronal disease. EVs can be engineered to deliver molecule therapeutics, including small chemicals, nucleic acids and/or proteins by chemical, biological or physical means. For example, catalase-loaded exosome exhibited significant neuroprotective function in *in vitro* and *in vivo* models of PD [138]. Exosomes from dendritic cells stimulated with low level of IFN γ promoted remyelination *in vitro* as well as *in vivo* by reducing oxidative tolerance [139] implying their therapeutic potential in multiple sclerosis and demyelinating diseases. In another study, neuronal EVs containing exogenously transfected miR-124a led to

increase GLT1 protein, an important glutamate transporter selectively lost in animal model of ALS, *in vitro* as well as *in vivo* implying their therapeutic potential for ALS [140].

Alternatively, siRNA loaded EVs can selectively silence the expression of pathogenic genes. Systemic administration of dendritic cell-derived EVs loaded with exogenous siRNA against BACE1, a therapeutic target in AD, led to significant reduction of BACE1 mRNA and protein in mouse brain [141]. Systemic administration of engineered EVs with exogenous siRNA to α -syn significantly decrease the level of endogenous mouse α -syn, and a pro-aggregating human form of α -syn in a transgenic PD mouse model [142]. Taken together, these evidences suggest that the EVs can be potent therapeutics for neurodegenerative disease and other CNS diseases.

5 Conclusion and future perspectives

In the present review, we have illustrated the EVs' therapeutic potential and their involvement in the pathogenesis of neurodegenerative diseases. As evidenced by a number of studies, the role of EVs are continuously expanding from maintenance of tissue homeostasis, disease diagnosis, disease progression and therapeutics for neurodegenerative diseases. While they play a key role in cell-to-cell communication under normal physiological conditions, EVs from neurodegenerative diseases contain pathogenic protein aggregates and participate in the transmission of disease pathology. Thus, proteomic and genetic profiling may provide a panel of biomarkers as well as therapeutic targets of neurodegenerative disorders.

With their natural ability of transferring biological information, EVs became a highly attractive candidate for therapeutic delivery vehicle in neurodegenerative diseases. They possess the characteristics of ideal drug delivery vehicles; ability to cross the blood–brain barrier, stability in circulation due to their natural, non-synthetic, origin, and little or low immunogenicity in the host. These vesicles can also be engineered to deliver therapeutic agents efficiently to affected tissues in brain. However, several critical issues remain to be addressed for the clinical translation of EVs as molecular therapeutics. They include the identification of specific EV class, selection of donor cells, purification protocols for large-scale production, the definition of therapeutic potency and the delineation of their underlying mechanism of actions in the treatment of neurodegenerative diseases. Despite preliminary experiments in animals that demonstrated the safety of EVs, additional experiments are essential to investigate long-term safety, bio-distribution and persistency of the biologic effects. With the advances of molecular techniques, the

engineering of the EVs with therapeutic molecules to enhance their target specificity may be anticipated.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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

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