Extracellular vesicles: new horizons in neurodegeneration

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Summary

Extracellular vesicles (EVs) are lipid-enclosed nanovesicles secreted by diverse cell types that orchestrate intercellular communication through cargo delivery. Their pivotal roles span from supporting the development of normal central nervous system (CNS) to contributing to the pathogenesis of neurological diseases. Particularly noteworthy is their involvement in the propagation of pathogenic proteins, such as those involved in neurodegenerative disorders, and nucleic acids, closely linking them to disease onset and progression. Moreover, EVs have emerged as promising diagnostic biomarkers for neurological disorders and as tools for disease staging, owing to their ability to traverse the blood-brain barrier and their specific, stable, and accessible properties. This review comprehensively explores the realm of CNS-derived EVs found in peripheral blood, encompassing their detection methods, transport mechanisms, and diverse roles in various neurodegenerative diseases. Furthermore, we evaluate the potentials and limitations of EVs in clinical applications and highlight prospective research directions in this rapidly evolving field.

Keywords: Extracellular vesicles; Alzheimer's disease; Parkinson's disease; Biomarker

Introduction

Extracellular vesicles (EVs) are lipid bilayer-encapsulated particles released by nearly all cell types, ranging from nanoscale to microscale in size.[1] Traditionally, EVs have been classified into three primary subtypes based on their biogenesis: exosomes, ectosomes, and apoptotic bodies.[2] Exosomes (30–150 nm) originate from the endosomal network and are released via exocytosis from multivesicular bodies (MVBs). Ectosomes (50 nm–1 μm) form through direct outward budding of the plasma membrane. Apoptotic bodies (500 nm–5 μm) are formed during late-stage apoptosis as cells fragment. Due to overlapping size ranges, recent approaches suggest classifying EVs by their biomolecular composition rather than size alone.[1] Despite differences in size and origin, EVs closely reflect the composition of their parent cells, making them valuable biomarkers for assessing the physiological or pathological state of cells. Additionally, EVs carry a mix of lipids, nucleic acids, proteins, and metabolites, enabling them to mediate intercellular communication and regulate various biological processes.[3,4] Indeed, previous research has underscored the pivotal roles of EVs in maintaining brain homoeostasis and fostering neuroprotection.[5]

Neurodegenerative disorders are characterised by progressive neuron degeneration, variable extent of neuroinflammation, and the accumulation of misfolded proteins, such as β-amyloid (Aβ) plaques and neurofibrillary tangles in Alzheimer's disease (AD), and α-synuclein aggregates in Parkinson's disease (PD).[6] Research has proposed a prion-like propagation mechanism for the intercellular transmission of pathological proteins, with EVs serving as potent vehicles in this process.[7,8] In addition to pathogenic proteins, EVs also transport cytokines and miRNAs, contributing to neuroinflammation and accelerating the progression of neurodegenerative diseases.[9] Current diagnosis of these diseases relies on neuroimaging techniques like Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET), along with biomarker analysis in cerebrospinal fluid (CSF), to assess brain structure, cognitive, and motor functions.[10] Despite advancements in neuroimaging, challenges such as high costs and limited early diagnostic capabilities remain. While EVs in CSF are effective for diagnosing neurodegenerative diseases, the invasiveness of the procedure limits repeated sampling, hindering disease monitoring. Peripheral EVs, with their broad availability, high sensitivity, and specificity, hold promise as valuable biomarkers for the early diagnosis and ongoing monitoring of neurodegenerative diseases.[11] Current available treatments for neurodegenerative diseases primarily focus on symptom alleviation and improving quality of life, with limited ability to slow disease progression. Additionally, the blood-brain barrier (BBB) restricts the

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delivery of medications to the brain, reducing their efficacy and often leading to unintended side effects. EVs, capable of crossing the BBB and delivering biomolecules, hold potential as therapeutic tools for neurodegenerative disease.[12]

In this review, we first outline the technologies for detecting and characterising EVs. Next, we delve into the potential transportation mechanism of EVs. We then assess central nervous system (CNS)-derived EVs as key signals in the progression of neurodegenerative diseases and their potential as diagnostic biomarkers. Finally, we discuss the therapeutic applications of EVs for these diseases.

Novel EV isolation and characterisation technologies

The methods of EV isolation

The EV isolation methods include ultracentrifugation, ultrafiltration, precipitation, size exclusion chromatography (SEC), and microfluidics-based technologies. Ultracentrifugation is the most commonly employed method for EV isolation, relying on differences in density, size, and shape.[13] Ultrafiltration, which separates EVs based on molecular size, is a relatively convenient extraction method.[14] Precipitation methods utilise molecules such as polyethylene glycol (PEG) or protamine to encapsulate EVs, promoting their aggregation and subsequent precipitation.[15] SEC separates particles by size using a column filled with porous beads: larger particles elute first as they bypass the pores, while smaller particles take longer as they pass through.[16] Newly developed microfluidics-based technologies can separate EVs from small sample volumes using nano-filters, nanoarrays, or nanowires, while also enabling automated EV characterisation.[17] These methods, though widely used in research, have several limitations (Table 1) and require further improvement.

Ultrafiltration combined with a TiO2-based EV isolation method improves yield and purity by exploiting interactions between metals and phosphate groups on EV membranes. This method is also faster and more efficient for processing large volumes of biofluids than ultracentrifugation.[19] Additionally, tandem or sequentially configured nano-membranes with different molecular weight cut-offs can separate EVs by size into distinct compartments.[14] To avoid chemical contaminants introduced by precipitation, Ludwig et al. incorporated an ultracentrifugation step to wash the PEG precipitates.[20] Pulliam et al. developed a hybrid EV Microfluidic Affinity Purification technique that integrates polymer precipitation with microfluidic chips.[21] Asymmetric flow field-flow fractionation (AF4) relies on a transverse flow that is perpendicular to the parabolic flow to separate biomolecules by size. Wu et al. optimised the AF4 technique for the separation of EVs from human plasma by adjusting key parameters such as the cross-flow gradient, focus timing, sample ultrafiltration conditions, plasma volume, and injection volume. These optimisations reduced lipoprotein contamination, preserved the structural and biological integrity of EVs, and improved the reproducibility of EV purification.[22] More recently, Liu et al. developed an exosome detection method using an ultrafast isolation system (EXODUS), which employs negative pressure oscillation and a double-coupled harmonic oscillator to induce membrane vibration. The dual-frequency transverse waves generated by the oscillators enable EXODUS to outperform other isolation techniques, resulting in improved EV yield and purity.[18]

Taken together, although various methods exist for EV isolation, each has its limitations. Recent studies have focused on optimising EV isolation and improving EV purity and yield by combining different methods such as ultrafiltration or ultracentrifugation with SEC.[23,24] Emerging methods like EXODUS enable ultrafast, high-yield, and high-purity EV isolation. When combined with advanced analysis technologies, these methods are expected to accelerate EV application in life sciences and medicine.

Methods	Yield	Purity	Homogeneity	Integrity	Sample volume	Time-consuming	Contamination	Others	References
Ultracentrifugation or density gradient centrifugation	High	High	High	Low	Large	Large	Lipoprotein; Albumin; EV aggregation	Separate different sizes of EVs	13
Ultrafiltration	High	low	Low	High	Large	Small	Protein; Soluble factors	Easy to operate	14
Precipitation	High	Low	Low	Low	Small	Small	Protein; Chemical substance; EV aggregation	Easy to operate	15
Size-exclusion chromatography	Low	High	High	High	Large	Large	–	High reproducibility; Automated analysis; High costs.	16
Microfluidic technology	–	High	High	High	Small	Small	–	–	17
Exosome detection via the ultrafast-isolation system (EXODUS)	High	High	High	High	Small	Small	–	Small assay capacity; Limited nanopore sizes.	18

Table 1: **Methods for EV isolation.**

The techniques of EV characterisation

EVs display significant heterogeneity, with distinct functions depending on their origin, size, and contents. Traditional isolation methods often lead to co-purification with aggregated proteins, highlighting the need for precise characterisation in research and applications. EV properties are generally divided into physical (size and shape) and biochemical (proteins, lipids, and nucleic acids) categories. Methods for detecting the physical properties of EVs can be categorised into optical and non-optical approaches (Table 2).

Optical methods include nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), flow cytometry, Raman spectroscopy (RS), single particle interferometric reflectance imaging sensing (SP-IRIS), stochastic optical reconstruction microscopy (STORM), and direct-STORM (dSTORM). NTA and DLS are widely employed to measure the quantity and size of EVs by tracking their Brownian motion.[25,26] Flow cytometry measures particle size through light scatter and detects EV components when combined with fluorescence.[28,48] RS provides information on the purity and quantity of EVs through inelastic photon scattering, revealing their chemical composition without requiring specific labelling or targeting.[27] SP-IRIS enhances the contrast of scattering signals from nanoparticles using a layered silicon substrate, enabling precise measurements of EV quantity and size.[29] The recently developed ExoView platform integrates SP-IRIS technology with a chip-based system that captures EVs based on their surface marker expression on antibody-coated spots. Beyond detecting surface proteins, this platform can also identify internal markers through specific permeabilization protocols.[30] STORM utilises the photoswitchable properties of specific fluorescent probes to achieve high-precision localisation of molecular events on the EV membrane. While traditional STORM primarily relies on immunoaffinity for detection, dSTORM employs lipophilic dyes that are highly compatible with the lipid membrane structure of EVs, thus, allowing dSTORM to provide high-resolution visualisation of EV membrane structures without solely depending on surface protein markers.[31]

Non-optical methods for physical characterisation include electron microscopy (EM), atomic force microscopy (AFM), and resistive pulse sensing (RPS). EM techniques, including transmission electron microscopy (TEM), scanning electron microscopy (SEM), and cryo-electron microscopy (Cryo-EM), provide high-resolution images of EV morphology and enable precise measurement of EV diameters.[32] AFM utilises a microscopic physical probe to scan the surface of

	Techniques	Information provided	References
Physical properties			
Optical detection	NTA	Concentration, size distribution	25
	DLS	Size distribution, zeta potential of EVs in monodisperse fluids	26
	RS	Concentration, components on the EVs	27
	Nano-flowcytometry	Concentration, size, components on the EVs but may overestimate particle size due to the Swarm effect	28
	SP-IRIS, ExoView	Concentration, size, components on the EVs	29,30
	STORM, dSTORM	Size, high-resolution of components on the EVs	31
Non-optical detection	EM	High-resolution of EV morphology (cup-shaped for TEM and SEM, near-spherical for Cryo EM), size, components on the EVs	32
	AFM	Three-dimensional morphology, size, components on the EVs	33
	RPS	Concentration, size distribution	34
Biochemical properties			
Protein	Bradford assay, bicinchoninic acid assay	Total protein content	35
	ELISA, nano-flowcytometry, Simoa, nPES, Tango	Specifically targeted protein	36–40
	Proteomics	Protein profile	41
Glycan	EVLET	Glycan profile	42
Lipids	Sulfo-phospho-vanillin assay	Total lipid content	43
	Lipidomics and metabolomics	Lipid profile and other metabolites	44
Nucleic acid	High-throughput sequencing	mRNA, lncRNA, circRNA profiles	45
	RT-qPCR	Specific RNA sequence	46
	Nano-flowcytometry	DNA on a single EV	47

Abbreviations: Nanoparticle tracking analysis: NTA; dynamic light scattering: DLS; Raman spectroscopy: RS; single particle interferometric reflectance imaging sensing: SP-IRIS; stochastic optical reconstruction microscopy: STORM; direct-STORM: dSTORM; electron microscopy: EM; atomic force microscopy: AFM; resistive pulse sensing: RPS; single molecule array: Simoa; nanoplasmon-enhanced scattering: nPES; thermophoretic AND gate operation assay: Tango; lectin-based thermophoretic assay: EVLET; reverse transcriptase quantitative polymerase chain reaction: RT-qPCR.

Table 2: **Methods for EV characterisation.**

specimens, providing detailed information about their three-dimensional morphology.[33] Combining immunoaffinity techniques with EM or AFM enables the acquisition of protein information on the EVs.[49] RPS relies on the Coulter effect to detect the concentration and size distribution of EVs, where particles passing through a nanopore induce a temporary change in resistance.[34]

The detection of biochemical properties of EVs primarily involves the analysis of their contents, including proteins, nucleic acids, lipids, and metabolites. Bradford assay and bicinchoninic acid assay are widely used to measure the total protein content of EVs,[35] while immunoaffinity-based assays like ELISA and flow cytometry target specific proteins within EVs. The single molecule array (Simoa) platform is an advanced ultrasensitive technology that measures proteins using ELISA-based assays in a fully automated system. Wei et al. were the first to apply this technology for EV detection, targeting EV markers like CD9 and CD63, along with tumour markers such as epithelial cell adhesion molecule (EpCAM), to profile tumour-derived EVs directly from plasma samples.[36] Similarly, Liang et al. developed a nano-plasmon-enhanced scattering (nPES) assay, which utilises antibody-conjugated gold nanospheres and nanorods to bind EVs, subsequently captured by a sensor chip coated with EV-specific antibodies.[37] A recent study introduced the Tango assay, a one-step thermophoretic AND (Aptamer, Connector, Detection) gate method that enhances EV detection and diagnostic precision. This assay uses a mix of EVs, an Aptamer, a Connector, and PEG in a microchamber heated with an infrared laser to create gradients that concentrate EVs. Probes in the assay include an aptamer targeting specific EV membrane proteins (PSMA or EpCAM), the complementary sequences for the Connector and fluorophores (Cy3 or Cy5). When both proteins are present, the Connector aligns the probes, generating a Förster resonance energy transfer (FRET) signal. Tango assay achieved 91% accuracy in distinguishing prostate cancer from benign prostatic hyperplasia in cases with borderline PSA levels, demonstrating high sensitivity and clinical potential.[38] Finally, in addition to measurements of targeted proteins, proteomics technologies are widely used to profile novel proteins in secreted EVs.

For metabolites, Sun et al. developed a lectin-based thermophoretic assay (EVLET) to profile glycans on the EV membrane by combining vibrating membrane filtration (VMF) with thermophoretic amplification. Specifically, EVs were first incubated with fluorophore-conjugated lectins to specifically label glycans associated with triple-negative breast cancer (TNBC) on the EV surface. The circulating proteins and unbound lectins were subsequently effectively removed by the customised VMF system, which consists of a nanoporous anodic aluminium oxide (AAO) membrane and a high-frequency oscillator. These purified EVs were then subjected to thermophoretic analysis, where a radial temperature gradient in a laser-irradiated microchamber amplified the fluorescence signal of lectin-bound EVs, enabling quantitative analysis of EV glycans.[42]

Like proteomics, lipidomics can be used to profile lipids within various EVs.[50] Besides, the total lipid content can be measured using a sulfo-phospho-vanillin assay, a two-step quantitative colourimetric method. Specifically, the concentrated sulphuric acid initially reacts with unsaturated lipids at high temperatures to form alkenyl cations. These lipid cations then react with vanillin in an acidic solvent, producing a pink-coloured complex.[43]

As discussed earlier, EVs also contain nucleic acids. Traditionally, nucleic acid detection relied on high-throughput sequencing, validated by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).[46] Liu et al. developed a single-EV DNA detection method using nano-flow cytometry. EVs were stained with SYTO16, a membrane-permeable nucleic acid dye, and analysed by flow cytometry. The fluorescence peaks corresponded to different DNA fragment sizes, enabling sensitive and quantitative DNA analysis in single EVs, particularly when the total fragment length exceeds approximately 200 bp.[47]

It is important to note that no single method currently captures the full spectrum of biological information from EVs. Therefore, combining multiple techniques for both quantitative and qualitative analysis is more effective in achieving a comprehensive understanding of their physiological and pathological roles. Newly developed approaches, such as Tango and EVLET, provide detailed insights into EVs using minimal biological samples, positioning them as promising tools for clinical translation.

Mechanisms of EVs transport across blood-brain barrier

Advancements in technology have greatly enhanced the detection and characterisation of EVs, allowing for more precise tracing of their cellular origins. This progress has led to the remarkable discovery of CNS-derived EVs in peripheral fluids, such as plasma and saliva.[51,52] These findings open promising avenues for non-invasive diagnostics and monitoring of neurological disorders through peripheral samples. Under normal conditions, the BBB limits the transfer of large molecules between the CNS and peripheral circulation.[53] However, the detection of CNS-derived EVs in peripheral fluids suggests that EVs can indeed cross the BBB. Conversely, Ridder et al. utilised a transgenic mouse model expressing Cre mRNA in haematopoietic cells under the *Vav1* promoter to demonstrate that haematopoietic cell-derived EVs can enter the brain from peripheral circulation.[54] Later, using the same model, their group

revealed that neurons and microglia show increased uptake of haematopoietic cell-derived EVs, especially under inflammatory conditions.[55] These findings highlight the continuous EV-mediated communication between the peripheral and CNS under both pathological and physiological conditions.

Although the mechanisms underlying CNS-peripheral crosstalk require further investigation, it is hypothesised that EVs potentially cross the BBB through mechanisms such as transcytosis or by passing through a compromised BBB, which becomes more permeable under certain pathological conditions (Fig. 1).[56] The transcytosis of EVs involves the processes of adhesion to endothelial cells, endocytosis, and subsequent release. Current research indicates that the adhesion capability of EVs is regulated by surface molecular markers such as integrins and heparan sulphate proteoglycans[57,58] and is influenced by microenvironmental factors, including hypoxia and inflammatory responses.[59,60] In 2021, Joshi et al., using an *in vitro* assay, demonstrated that the ability of neural stem cell-derived EVs to adhere to the endothelium depends on heparan sulphate proteoglycans.[58] Besides, Banks et al. utilised EVs derived from both human and murine cell lines and identified CD46 as a potential regulator in the process of EV's adhesion to endothelial cells. Furthermore, they observed that inflammation increases the influx of peripheral-derived EVs into the brain, even without compromising the integrity of the BBB.[60]

EV transcytosis is typically an active process, as EVs rarely cross an intact BBB at low temperatures.[61] There are four main mechanisms of transcytosis: clathrin-mediated endocytosis, caveolae-mediated endocytosis, adsorptive-mediated transcytosis, and micropinocytosis.[56] In 2020, Cheng et al. used an *in vitro* assay to demonstrate that neural stem cell-derived EVs can cross the BBB. This process was effectively blocked by dynasore, an inhibitor of clathrin-mediated endocytosis that prevents the detachment of clathrin-coated invagination pits from the plasma membrane.[62] Recently, Lin et al. found that EVs derived from glioma cells overexpressing cavin1 contained more cavin1 and exhibited a greater ability to cross the BBB compared to EVs derived from normal glioma cells. This enhanced

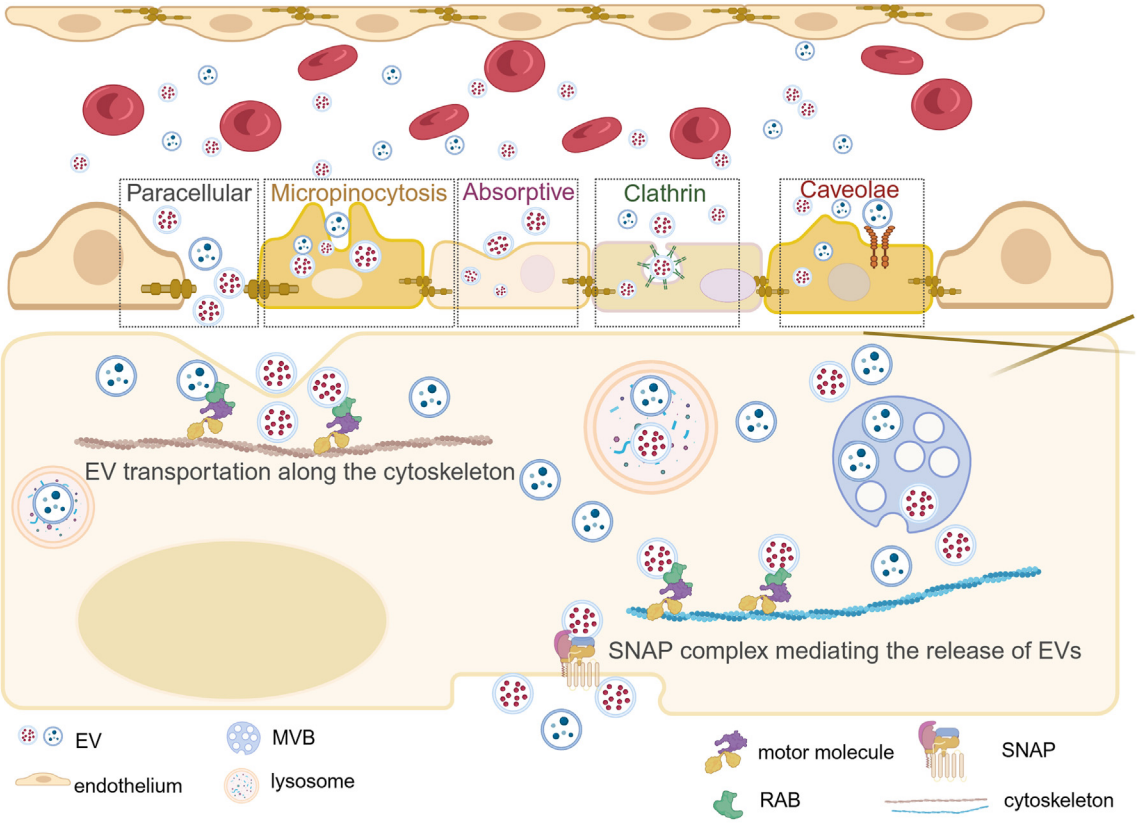

***Fig. 1:* Mechanisms of EVs traversing the BBB.** EVs can traverse the BBB through the paracellular pathway or transcytosis, which involves four key mechanisms, clathrin-mediated endocytosis, caveolae-mediated endocytosis, adsorptive-mediated transcytosis, and micropinocytosis. Once internalised into the endothelium, the RAB protein family potentially bind to the EV membrane, directing them either to lysosomes for degradation or to the plasma membrane via the cytoskeleton, with the SNAP complex facilitating their release into the extracellular space.

transcytosis is likely due to improved caveolae-mediated transport, as cavin1, in combination with caveolin1, facilitates the formation of caveolae by shaping the plasma membrane.[63] Adsorptive-mediated transcytosis is initiated by the binding of polycationic substances to negatively charged components on the plasma membrane, thereby accelerating endocytosis.[64] Research on erythrocyte-derived EVs revealed that these EVs could traverse the BBB and accumulate in microglia, likely via adsorptive-mediated transcytosis.[65] Under inflammatory conditions, macromolecular entities can cross the BBB via micropinocytosis, a process in which irregular spherical or tubular vesicles protrude from the plasma membrane to encapsulate macromolecules.[66] A study on Enterovirus-71 (EV71)-induced CNS infection demonstrated that EV71-infected cells release small EVs could across the brain endothelium through micropinocytosis during the early stages of infection.[67]

Once internalised into endothelial cells, these vesicles may avoid lysosomal degradation and be released into the extracellular space. However, the precise mechanisms governing this process remain largely unexplored.[68] Studies on MVBs have shown that RAB family proteins can dock onto the MVB membrane and recruit motor molecules such as kinesins, dyneins, and myosins to bind with cytoskeletal structures like actin or microtubules, directing vesicle transport to the plasma membrane or lysosome, where SNAP receptor proteins on the target membrane interact with those on the vesicle membrane, facilitating vesicle fusion and subsequent release.[69] The intracellular trafficking of exogenous EVs within recipient cells may follow a similar pathway. Morad et al. revealed that internalised breast cancer-derived EVs could be transferred into EEA1-marked early endosomes and sorted into rab11-labelled recycling endosomes, which then fuse with the plasma membrane with the assistance of the SNAP complex.[61]

Under various conditions, including ageing, inflammation, tumours, and neurodegenerative diseases, the integrity of BBB can be compromised, allowing EVs to cross the BBB via paracellular pathways.[70,71] In a mouse model of periodontitis, Elashiry et al. demonstrated that increased BBB permeability enabled gingiva-derived exosomes to traverse the BBB. The influx of these exosomes further compromised BBB integrity, creating a vicious positive feedback loop.[72] CNS infection could also facilitate the crossing of EVs from EV71-infected cells by disrupting the tight junctions between brain microvascular endothelial cells.[67]

It is crucial to note that most studies on EVs and the BBB focus on the transport of peripheral EVs into the CNS. The primary mechanisms involved may differ for the transport of CNS-derived EVs across the BBB to the periphery, highlighting a critical area in need of further research. In 2016, Yoshimura et al. developed a transgenic rat model expressing human CD63-GFP under the CAG promoter, enabling the *in vivo* tracking of EVs.[73] To specifically trace neural stem cell-derived EVs, they later constructed a model expressing the CD63-GFP gene under the Sox2 promoter.[74] To provide a more convenient method for tracing EVs from various cell types, Rufino-Ramos et al. developed the NoMi platform (Nanoluciferase outside, mCherry inside), a lentiviral-based system that tags CD63 with both bioluminescent and fluorescent markers. This system selectively detects EVs originating from pre-labelled cells.[75] Taken together, these innovative tools have significantly advanced the study of EV dynamics, opening new horizons for research on how CNS-derived EVs cross the BBB to the periphery. Elucidating the mechanisms by which EVs traverse the BBB bidirectionally offers significant potential for advancing our understanding of EV-mediated peripheral-CNS communication. Such insights could not only shed light on critical aspects of intercellular signalling but also pave the way for the development of biomarkers and EV-based drug delivery systems, enabling the targeted transport of therapeutics from the bloodstream to the brain.

The implication of CNS-derived EVs

CNS-derived EVs can serve as intercellular signalling molecules in the onset and progression of diseases

EVs containing proteins like β-amyloid (Aβ), Tau, and α-synuclein have been identified as significant contributors to the progression of AD and PD (Fig. 2).[76] Sardar et al. found that EVs extracted from the brains of AD patients contained increased levels of Aβ oligomers, which could be internalised by neurons and transferred to other neurons, mimicking the spread of Aβ and leading to cell death.[77] The co-localisation of Aβ oligomers and EVs suggests that EVs may play a dual role in both facilitating the propagation of Aβ and aiding in its sorting and removal from neurons. EVs also appear to contribute to the propagation of tau proteins. In two mouse models of tau pathology, the AAV-P301L-tau model, involving adeno-associated virus (AAV)-mediated neuron-specific expression of human P301L tau, and the PS19 mouse model, administration of GW4869, an EV biogenesis inhibitor, effectively suppressed tau propagation.[78] This study, led by Ikezu and colleagues, further demonstrated that tau-containing EVs are predominantly released by microglia in a sphingomyelinase-2-dependent manner. Depleting microglia *in vivo* inhibited the release of tau-containing EVs and their subsequent transmission to neurons.[78] Using high-resolution imaging and immuno-gold labelling, the researchers confirmed that EVs containing the microglial-specific mEmerald-CD9 (mE-CD9) fusion protein co-localised with phosphorylated tau.[79] Moreover, inhibiting the P2X purinoceptor 7 (P2X7R), an ATP-gated cation channel highly expressed in microglia, with GSK1482160 significantly reduced the release of

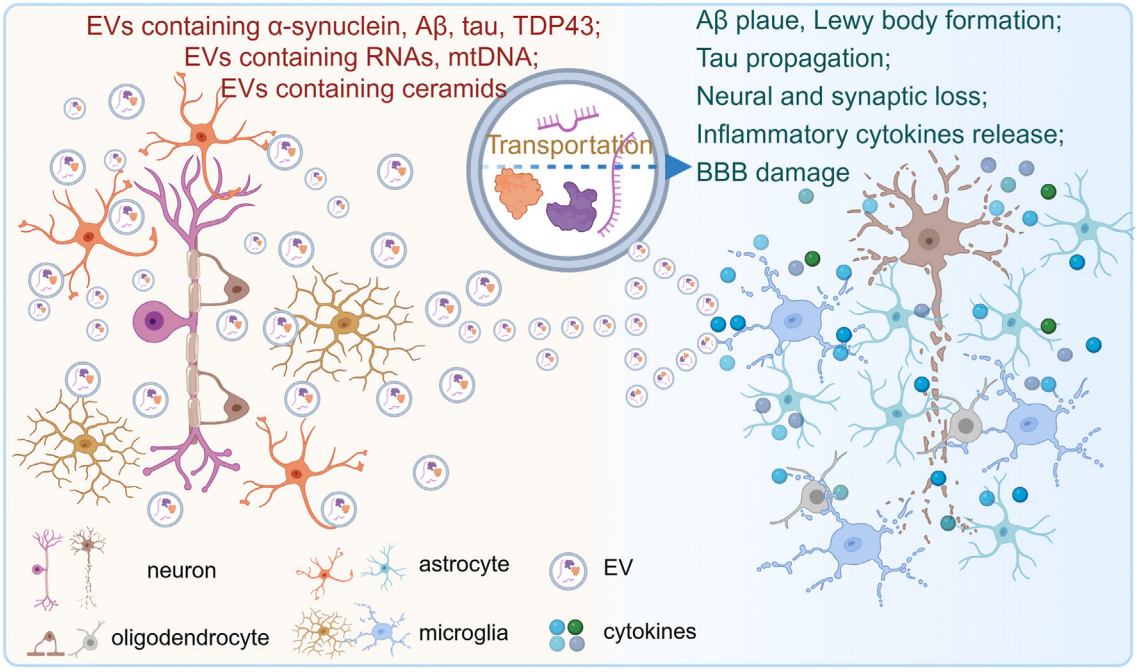

***Fig. 2:* Pathological roles of EVs in neurodegenerative diseases.** CNS-derived EVs containing pathological proteins or nucleic acids, once internalised by recipient cells, can induce abnormal protein aggregation, activate microglia and astrocytes, and trigger neuroinflammation, ultimately leading to neural loss and BBB damage.

microglial EVs and alleviated disease phenotypes in P301S mice.[80]

Similar to Aβ and tau, the pathological protein associated with PD, α-synuclein, has also been widely detected in EVs. Katerina et al. demonstrated in an α-synuclein preformed fibril (PFF) mouse model that α-synuclein can propagate via brain-derived EVs, but these EVs only develop Lewy body-like pathological features in the presence of endogenous α-synuclein.[81] It should also be noted that EV-mediated intercellular protein propagation extends beyond AD and PD, highlighting its broader relevance in neurodegenerative disorders. For example, recent research has revealed that CNS-derived EVs are also enriched with TDP-43[82] and misfolded superoxide dismutase 1 (SOD1).[83] These EVs can accelerate the spread of pathological proteins and drive the progression of amyotrophic lateral sclerosis (ALS)[82] or potentially TDP-43 related frontal temporal dementia (FTD).[84]

In addition to transporting pathological proteins, EVs also transfer nucleic acids, regulating disease onset and progression. In AD, with long RNA profiling, Dan et al. identify distinct RNA profiles in EVs from AD brain tissue compared to healthy controls. Their findings revealed that lncRNAs in AD-derived EVs were associated with processes such as metal ion transport, calcium ion homoeostasis, neuron spines, and neuronal cell bodies. These associations mirrored those of differential mRNAs, suggesting that these lncRNAs may act as competing endogenous RNAs (ceRNAs).[85] Whether these lncRNAs in EVs regulate the biological functions of recipient cells through their ceRNA role remains to be explored. In PD, miRNA sequencing of plasma neuron-derived EVs identified elevated levels of miR-4443, which inhibits heparan sulphate expression, thereby reducing EV release and leading to intracellular α-synuclein accumulation, which exacerbated PD pathology.[86] Similar mechanisms were observed in ALS, where astrocytes overexpressing the ALS-linked mutant Cu/Zn SOD1 released EVs enriched with miR-155-5p. When taken up by motor neurons, these EVs induced cell death and reduced neurite length.[87] Mitochondrial dysfunction is a common hallmark of neurodegenerative diseases. In a sporadic PD model with dementia, Emilie et al. found that neurons lacking IFNβ/IFNAR signalling promoted the release of EVs containing damaged mitochondrial DNA (mtDNA). These mtDNA-containing EVs, when internalised by recipient neurons, triggered mitochondrial oxidative stress, and neuronal cell death both *in vitro* and *in vivo*.[88] Another example of the involvement of EV-related nucleic acids is Huntington's disease (HD), a disorder caused by an expanded CAG repeat mutation in the first exon of the huntingtin gene (HTT). A study demonstrated that striatal cells with mutant HTT preferentially release expanded CAG repeat mRNA into EVs.[89] Moreover,

defects in the mitochondria-lysosomal axis in fibroblasts and neurons of HD patients led to increased release of mtDNA-containing EVs.[90]

Abnormalities within metabolites in EVs have likewise drawn considerable attention in neurodegenerative disease research, as disruptions in brain metabolism are often believed precede the onset of symptoms. For example, in presymptomatic HD mouse models, a recent study revealed that the ascorbic acid flux from astrocytes to neurons was impaired. Astrocytes expressing the mutant HTT gene showed reduced release of ascorbic acid-containing EVs, therefore, contributing to increased reactive oxygen species (ROS) stress and redox imbalance in neurons.[91] Similarly, stressful stimuli, such as exposure to Aβ oligomers, increased ceramide levels in microglia via acid sphingomyelinase activation, leading to the release of proinflammatory factors. These factors could subsequently activate astrocytes, prompting the release of ceramide-enriched EVs.[92] When microglia took up these EVs, the inflammatory response was amplified, creating a self-perpetuating cycle of inflammation that accelerated disease progression. In parallel, Ahmed et al., using lipid-mediated affinity chromatography analysis, demonstrated that astrocyte-derived EVs was enriched in ceramide and Aβ. These EVs can be internalised by neurons and transported to mitochondria, where ceramide facilitated the formation of a voltage-dependent anion channel 1 (VDAC1)-Aβ complex, ultimately inducing apoptosis.[93] Furthermore, EVs isolated from the brains of patients with Lewy body disorders were found to contain high levels of ceramides. This enrichment is thought to be associated with impaired lysosomal degradation, potentially contributing to the pathological aggregation of α-synuclein.[94] However, the underlying reasons for the dysregulated metabolism observed in neurodegenerative disease patients and the specific ways in which these metabolic abnormalities influence disease progression remain to be fully elucidated.

In addition to facilitating intercellular communication within the brain, CNS-derived EVs also play a vital role in the interaction between CNS and peripheral system. An *in vitro* experiment found that EVs derived from astrocytes of 3xTG-AD mice can damage tight junctions and impair the integrity of BBB.[95] Immune profiling has revealed a distinct immune signature on the surface of plasma EVs in PD patients, which also be linked to maintaining BBB integrity.[96] Once brain-derived EVs traverse the BBB, they may contribute to pathological alterations in peripheral organs such as heart and gut. For example, brain-derived EVs can influence gut permeability, leading to heightened inflammation and an increased risk of inflammatory bowel disease (IBD).[97] In addition, under pathological conditions, brain-derived EVs can induce cardiomyocyte hypertrophy and fibrosis.[98] In PD, plasma α-synuclein-containing EVs, some of which may originate from the CNS, have been shown to hyperactivate monocytes, further contributing to peripheral immune activation and inflammation.[99] Multiple sclerosis (MS) is a chronic autoimmune disease of the CNS characterised by myelin destruction, loss of oligodendrocytes, and axonal damage. While previous studies have primarily focused on the dysregulation of peripheral immune cells like T and B cells,[100,101] recent research has highlighted oligodendrocyte-derived EVs enriched with P2X purinoceptor 7 (P2X7R).[102] P2X7R are known to regulate inflammation and immune responses.[103] Further investigation is essential to determine whether EVs containing P2X7 receptors mediate communication between peripheral and CNS immune responses, offering potential therapeutic targets for immune-related diseases.

In summary, as growing research highlights the critical roles of CNS-derived EVs in mediating the pathological processes of various neurodegenerative diseases[104] -both within the CNS and in interactions with peripheral organ systems- further exploration of their functions could unveil new therapeutic strategies (Table 3).

Blood based CNS-derived EVs as biomarkers in disease diagnosis and prognosis

Definitive diagnosis of most neurodegenerative diseases typically requires invasive histopathological procedures, such as brain biopsies or post-mortem autopsies. EVs, which retain the characteristics of their source cells, offer valuable potential for early diagnosis and prognosis (Fig. 3). Previous studies have highlighted the potential of plasma bulk EVs as biomarkers to distinguish AD and PD from the healthy population.[105] However, since most plasma EVs are derived from adipose tissue and muscles, with only a small portion originating from the CNS,[106] specifically detecting CNS-derived EVs can reduce peripheral noise and increase sensitivity to brain pathology.

L1CAM, also known as CD171, is a well-recognised neural cell marker.[107] As early as 2014, it was discovered that α-synuclein-containing plasma-based CNS-derived L1CAM$^+$ EVs could differentiate PD patients from healthy individuals and assess disease severity, with higher levels of α-synuclein in L1CAM$^+$ EVs from PD patient plasma.[108] Recently, a cross-sectional study with 576 participants found that increased serum α-synuclein in L1CAM$^+$ EVs could distinguish individuals with isolated rapid eye movement sleep behaviour disorder from controls and accurately identified 80% of those who later developed PD and related dementia, highlighting the potential of α-synuclein-containing serum-based CNS-derived EVs for screening individuals at risk of PD and related dementia.[107] Joseph et al. demonstrated that the levels of α-synuclein, pTau181, and pY-IRS-1 in plasma L1CAM$^+$ EVs could effectively differentiate PD patients from control

Disease	Source of EVs	Contents of EVs	Effects of EVs	References
AD	Brain	Aβ oligomer	Spread the Aβ proteinopathy and induce neuronal toxicity	77
AD	Microglia	Tau	Spread the tau proteinopathy	78–80
AD	Brain	lncRNAs	Regulate processes such as metal ion transport, calcium ion homoeostasis, neuron spines, and neuronal cell bodies	85
AD	Astrocyte	Ceramides	Promote neuroinflammation and neural apoptosis	92,93
PD	Brain	α-synuclein	Promote the synucleinopathy	81
PD	Neuron	miR-4443	Promote α-synuclein accumulation and neural degeneration	86
PD	Neuron	mt-DNA	Induce neural oxidative stress	88
LBD	Brain	Ceramides, α-synuclein	Induce synucleinopathy	94
ALS	Neuron	TDP-43	Promote TDP-43 proteinopathy	82
ALS	Astrocyte, Neuron	Misfolded SOD1	Prion-like spreading of misfolded proteins	83
ALS	Astrocyte	miRNA-155-5p	Reduce motor neuron survival and shorten neurite length	87
HD	Striatal cells	Expanded CAG repeat mRNA	Potential toxicity to recipient cells	89
HD	Fibroblast, Neuron	mtDNA	Counteraction of defective mitochondrial quality control system	90
HD	Astrocyte	Ascorbic acid	Reduce reactive oxygen species	91
MS	Oligodendrocyte	P2X7R	–	102

Abbreviations: Alzheimer's disease: AD; Parkinson's disease: PD; Lewy body disorder: LBD; Amyotrophic lateral sclerosis: ALS; Huntington's disease: HD; Multiple sclerosis: MS; β-amyloid: Aβ; Mitochondrial DNA: mtDNA; P2X purinoceptor 7: P2X7R.

Table 3: **Pathological roles of CNS-derived EVs in neurodegenerative diseases.**

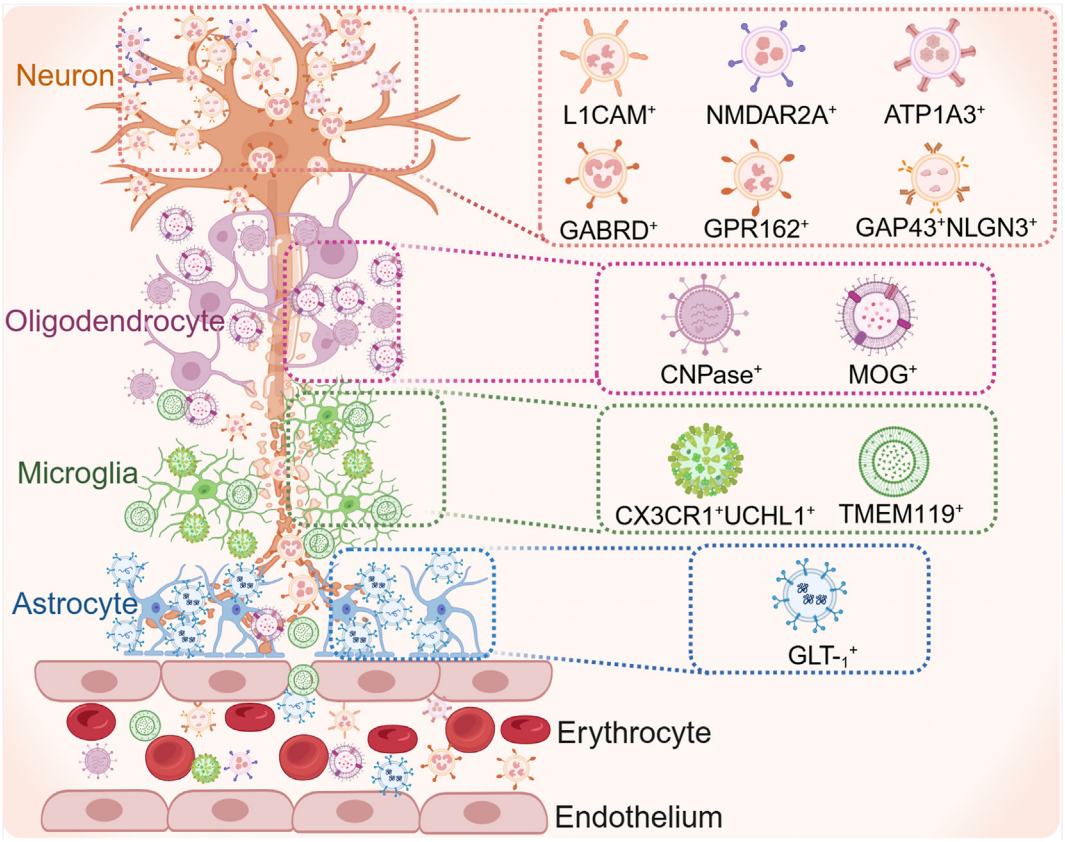

Fig. 3: **CNS-derived EVs in peripheral system.** CNS-derived EVs carrying parental cell information can cross the BBB and be detected in plasma, serving as valuable biomarkers for disease diagnosis.

subjects.[109] They also found that PD patients with cognitive impairment exhibited decreased α-synuclein and pY-IRS-1 levels and increased pTau181 levels in L1CAM$^+$ EVs compared to non-cognitively impaired PD patients.[109] Researchers enriched L1CAM$^+$ EVs from plasma and revealed that AD patients had higher Aβ42 in these EVs, which correlated with worsening cognitive function and increased amyloid deposition.[110] A recent study suggests that L1CAM may not be associated with the membrane of plasma EVs, as significantly more soluble L1CAM was detected in the plasma.[111] However, Carlos et al. used high-resolution immunofluorescent confocal microscopy and immuno-TEM technology to visually confirm the colocalisation of L1CAM and EVs.[112] Additionally, nanoflow technology, which requires a specific particle size for detection, effectively excluded interference from soluble proteins and verified that plasma L1CAM$^+$ EVs contained neuronal proteins.[112]

In addition to L1CAM, a recent study identified ATPase Na^+/K^+ transporting subunit alpha 3 (ATP1A3) as a newly developed neuronal marker in EVs. Using dSTORM, they revealed a higher ratio of ATP1A3$^+$ EVs compared to L1CAM$^+$ EVs in brain tissue, neuron-derived culture medium, CSF, and plasma. Proteomic analysis indicated that ATP1A3$^+$ EVs showed a greater enrichment of neuron-specific proteins and AD-related molecules compared to L1CAM$^+$ EVs. Moreover, researchers observed increased levels of Aβ$^+$ATP1A3$^+$ EVs in AD patients compared to those with mild cognitive impairment (MCI) or healthy controls. Beyond distinguishing AD patients from healthy individuals, plasma Aβ$^+$ATP1A3$^+$ EVs positively correlated with tau pathology and cognitive impairment, underscoring their potential as both a diagnostic biomarker and staging biomarker for AD.[113]

While the discovery of general neuron-specific represents significant progress, identifying brain region-specific EVs in blood samples could more accurately reflect pathological changes in the brain, thus improving diagnostic precision. Using mass spectrometry, Guo et al. identified GABRD and GPR162 as potential markers for cortical and hippocampal neurons, revealing a decreased ratio of GABRD$^+$pTau217$^+$ or GPR162$^+$pTau217$^+$ EVs in the plasma of AD patients compared to healthy individuals.[39] This ratio was slightly higher in non-AD dementia patients, effectively distinguishing between AD and non-AD dementia populations. Tian et al. focused on plasma EVs containing NMDAR2A, a protein associated with synaptic function, as a potential brain marker in the peripheral system, discovering that AD patients exhibited a lower ratio of EVs carrying NMDAR2A compared to control subjects.[40] Similarly, Eitan et al. recently developed a novel immunoaffinity-based method to isolate plasma neuron-derived EVs by targeting the axonal marker growth-associated protein 43 (GAP43) and the synaptic protein neuroligin 3 (NLGN3). This approach demonstrated enhanced neuron specificity, and the EVs enriched for GAP43 and NLGN3 exhibited elevated levels of pTau181 and Aβ42 in AD patients, suggesting their utility as neuron-derived biomarkers for distinguishing AD cases.[114] As synaptic and axonal degeneration typically precedes neuronal loss, future research should focus on synaptic and axonal markers in EVs for diagnostic purposes or as indicators of disease progression. These markers may offer earlier and more precise insights into neurodegenerative disease stages, aiding both diagnosis and therapeutic intervention strategies.

Neurodegenerative diseases are characterised not only by synaptic and axonal damage but also by glial cell dysfunction. Detecting glial cell-derived EVs in plasma may offer valuable insights into brain function. For instance, astrocytes have been found to exhibit α-synuclein deposition. A recent study found a significant increase in astrocyte-derived glutamate transporter 1 (GLT-1) enriched EVs (GLT-1^+EV) in the plasma of PD patients compared to healthy controls. Specifically, astrocyte-derived EVs containing α-synuclein (GLT-1^+/SYN_{211}^+) or aggregated α-synuclein (GLT-1^+/$MJFR_{14}^+$) were able to differentiate PD from healthy controls and multiple system atrophy (MSA), with higher ratios of GLT-1^+/SYN_{211}^+ or GLT-1^+/$MJFR_{14}^+$ EVs in PD patients. An integrated analysis combining GLT-1^+/SYN_{211}^+ and GLT-1^+/$MJFR_{14}^+$ EVs demonstrated strong diagnostic capability in distinguishing PD from healthy controls and MSA patients.[115]

Detecting microglia-derived EVs in plasma poses challenges due to overlapping molecular markers with monocytes and macrophages, which are abundant in the peripheral system. TMEM119 has been widely regarded as a microglial marker, suggesting its potential utility for identifying microglia-derived EVs. A study found that TMEM119-enriched EVs were more abundant in patients with MCI compared to healthy controls or AD patients. Using Canevelli's Frailty Index (FI), frail MCI patients showed elevated levels of TMEM119-enriched EVs compared to non-frail healthy controls.[116] However, research on ischaemic stroke raised concerns, showing TMEM119 could not reliably distinguish resident microglia from infiltrating macrophages during the acute phase.[117] Therefore, the specificity of TMEM119 as a marker for microglia-derived EVs in plasma requires further validation. To enhance specificity, combining multiple cell-specific markers may improve the identification of EV origins. Recently, Duan et al. to identified UCHL1 as a protein exclusively expressed in EVs derived from BV2 microglial cells but absent in EVs from Raw264.7 macrophages using mass spectrometry. By combining UCHL1 with CX3CR1, a myeloid cell marker, they identified CX3CR1$^+$UCHL1$^+$ EVs as microglia-specific. Their findings revealed elevated levels of these microglia-derived EVs in multiple sclerosis (MS) patients compared to healthy controls.[118] These combined markers could advance our understanding of microglial

activity and improve diagnostic approaches in neurodegenerative diseases.

The abnormal accumulation of α-synuclein in oligodendrocytes is the main characterisation of MSA. Yu et al. explored whether plasma oligodendrocyte-derived EVs could assist in diagnosing MSA. They first identified CNPase$^+$ EVs as a reliable plasma marker for oligodendrocytes. Further research showed that CNPase$^+$ EVs were reduced in the plasma of MSA patients compared to healthy controls and PD patients. This decrease may contribute to α-synuclein accumulation, leading to impaired SNARE complex-mediated EV release in oligodendrocytes.[119] Following this research, Dutta et al. measured α-synuclein levels in plasma oligodendrocyte-derived EVs using myelin oligodendrocyte glycoprotein (MOG) as a specific marker for oligodendrocytes. They discovered elevated concentrations of α-synuclein in these MOG$^+$ EVs, which could effectively distinguish MSA patients from healthy controls. Additionally, the ratio of α-synuclein concentration in oligodendrocyte-derived EVs versus neuronal EVs was identified as a sensitive biomarker for differentiating PD from MSA, offering potential for more precise clinical diagnostics.[120]

In conclusion, with CNS-specific EVs in plasma, convenient peripheral fluids can serve as effective vehicles for real-time monitoring of brain pathology. Combining multiple brain cell-specific markers with pathology-related proteins can enhance the ability to monitor cellular status. However, current technologies are limited in their capacity to perform triple or quadruple staining of EVs, which should be improved in the future to increase accuracy. Additionally, existing biomarkers, which primarily focus on pathological proteins, often face challenges in differentiating preclinical patients from healthy controls or individuals at high risk. Identifying differentially expressed novel proteins and RNAs in EVs from neurological disorders offers valuable insights into the development of new biomarkers for early disease diagnosis.[121,122] The combined detection of proteins and RNAs in EVs could provide another strategy for detecting the earliest stages of neurodegeneration. Finally, more research is needed to explore the heterogeneity in EVs across various diseases, which could improve the specificity and sensitivity of diagnostic efficiency for different neurodegenerative diseases.

Therapeutic applications of EVs

EVs have significant therapeutic potential for neurodegenerative diseases due to their ability to cross the BBB, protect cargo from degradation, and target specific tissues through surface biomolecules.

Mesenchymal stem cell (MSC)-derived EVs have been extensively studied as efficient therapeutic approaches in mouse models of neurodegenerative disease. Morris et al. intranasally administered bone marrow MSC-derived EVs to 3xTg AD mice, observing inhibition of microglial activation and increased dendritic spine density.[123] In another study, intranasal delivery of 3D-cultured bone marrow MSC-derived EVs significantly improved cognitive function in 5XFAD mice, while also reducing astrogliosis and Aβ deposition.[124] Like those from bone marrow MSCs, adipose-derived MSC EVs containing proteins with neuroprotective and neurogenic properties have also shown protective effects in AD mice.[125] When intranasally administered to APP/PS1 mice, they primarily increased the expression of genes related to neurogenesis and neurite growth, and reduced Aβ1-42 oligomer or glutamate-induced neuronal toxicity. In PD, Eun et al. demonstrated that neural stem cell-derived EVs reduced 6-OHDA-induced intracellular reactive oxygen species (ROS) accumulation, decreased pro-inflammatory factor levels, and mitigated dopaminergic neuronal loss *in vivo*.[126] Another study revealed human tooth stem cell-derived EV could effectively reversed 6-OHDA-induced spatial learning and memory impairments, with effects lasting up to 6 days post-treatment. Remarkably, the reversal of 6-OHDA-induced gait disturbances lasted even longer, with benefits observed up to ten days after treatment concluded.[127] However, further research is needed to fully understand their mechanisms.

While stem cell-derived EVs exhibit neuroprotective effects, their inherently complex cargo can sometimes produce unpredictable outcomes. Selectively packaging therapeutic molecules into EVs holds the potential to enhance their efficacy. Among such molecules, miRNAs stand out due to their ability to regulate entire cellular pathways by interacting with a broad range of target genes. Their small size and high potency make miRNAs particularly attractive for EV-based therapies.[128] For instance, Marta et al. loaded miR-124-3p, a miRNA previously shown to exhibit neuroprotective effects in PD models, into EVs extracted from human umbilical cord blood-derived mononuclear cells (hUCB-MNCs), designating these EVs as miR-124-3p sEVs. *In vitro* experiments demonstrated that miR-124-3p sEVs facilitated the differentiation of neural stem cells into neurons. Although the treatment did not increase neuronal numbers in the striatum of PD model mice, it protected dopaminergic neurons from 6-OHDA-induced damage and significantly alleviated motor impairments.[129] Engineered EVs have also been used to encapsulate proteins for therapeutic purposes. Fang et al. engineered MSCs to overexpress tyrosine phosphatase-2 (SHP2) via lentiviral transduction, creating SHP2-enriched EVs (MSC-EVs-SHP2). These EVs promoted mitophagy and reduced neuronal death caused by Aβ42 stimulation. *In vitro*, MSC-EVs-SHP2 suppressed NLRP3 inflammasome-mediated microglial activation. *In vivo*, intravenous administration of MSC-EVs-SHP2 alleviated

oxidative stress, decreased Aβ deposition, and improved cognitive function.[130]

Despite the advancements in encapsulating therapeutic molecules into EVs, significant challenges persist, particularly when addressing neurodegenerative diseases. First, the relatively low concentration of therapeutic molecules that EVs can carry may reduce their efficacy in treating. This challenge becomes especially pronounced in patients with genetic mutations, such as *SNCA* in PD or *TARDBP* in ALS, where the pathology is driven by underlying genetic abnormalities.[131] A promising strategy to overcome this limitation is combining EV-based therapy with the CRISPR-Cas9 system for gene editing.[132] This approach could enable EVs not only to deliver therapeutic molecules but also to achieve precise genetic modifications in recipient cells, targeting the root cause of the disease. By editing pathogenic mutations, this combination therapy could provide more efficient and sustainable treatment options for genetic neurodegenerative diseases.

Second, enhancing the brain-targeting capabilities of EVs is critical for minimising side effects in neurodegenerative diseases. Various strategies have been explored to improve targeting specificity. For example, EVs modified with rabies viral glycoprotein (RVG) enhanced BBB penetration by binding to nicotinic acetylcholine receptors on endothelial cells, while transferrin receptor antibodies exploited the transferrin receptor to facilitate EV transport across the BBB.[133] Beyond crossing the BBB, some modifications have been developed to target specific brain cell populations. For instance, EVs engineered with platelet-derived growth factor A (PDGFA) can selectively target oligodendrocyte progenitor cells (OPCs), which express PDGFRα.[134] Similarly, mannose-coated EVs were taken up by microglia via mannose receptors (CD206).[135] However, targeting microglia specifically poses challenges due to the presence of mannose receptors on peripheral macrophages, which can reduce targeting efficiency. One study used dendritic cell-derived EVs coated with cationized mannan (polymers of mannose) to saturate liver macrophages by reducing their "eat me" signals, minimising peripheral clearance. In a subsequent step, CD47-enriched EVs were coated with a PEG-nanocarrier functionalized with c(RGDm7) peptide (which binds to αvβ3 integrins on tumour vascular cells), reducing macrophage uptake and prolonging circulation.[136] This highlights the need for CNS-specific targeting strategies that distinguish microglia from peripheral macrophages, potentially through a combination of markers or advanced engineering approaches, to ensure precise delivery and enhanced therapeutic outcomes.

Third, maintaining the stability of surface-modified EVs is a significant challenge, as they are prone to degradation in both *in vivo* and *in vitro* environments. Engineered EVs, particularly those modified with peptides, face vulnerability to protease degradation in the extracellular matrix, which reduces their targeting efficiency. Strategies such as incorporating D-isomers or glycosylation motifs into peptides have shown promise in enhancing resistance to proteolysis.[137] Additionally, proper storage practices, like maintaining low temperatures and minimising freeze-thaw cycles, are essential to preserve EV structural integrity and prevent *in vitro* degradation.

Fourth, producing EVs on a large scale remains a substantial hurdle. Techniques to boost EV production, such as overexpressing EV-associated proteins (e.g., CD63, CD81) or exposing cells to stimuli like mechanical stress, chemical agents, starvation, or hypoxia, can increase yield.[138] However, these methods may simultaneously alter the parent cells, increasing EV heterogeneity and complicating downstream applications. Balancing production efficiency and maintaining consistency in EV composition requires careful optimisation.

Fifth, the significant heterogeneity of EVs complicates assessments of their immunogenicity. Depending on their origin and cargo, EVs can exhibit immunostimulatory or immunosuppressive effects. While they generally have lower immunogenicity than their parent cells, the unpredictability of immune responses presents challenges for their clinical application. Developing methods to standardise EV subpopulations with defined immunological properties is essential for therapeutic development.

Lastly, there are no established standardised guidelines for determining optimal EV dosages. This gap extends to critical pharmacokinetic and pharmacodynamic considerations, such as biodistribution, clearance rates, and dose-response relationships. Addressing these challenges through improved isolation methods, robust characterisation tools, and comprehensive clinical studies will be pivotal in advancing the clinical translation of EV-based therapies.

Search strategy and selection criteria

Data for this review were identified through Google Scholar searches and by examining references from relevant articles. The search terms included: "extracellular vesicles" or "vesicle isolation" or "neurodegenerative diseases" or "Alzheimer's disease" or "Parkinson's disease" or "biomarker" or "intercellular communication" or "blood-brain barrier" or "extracellular vesicle therapy" and "engineered extracellular vesicles". Articles published between 2014 and 2024 were screened and selected from the Google Scholar database for inclusion in this review.

Conclusion

EVs, as powerful mediators of intercellular and inter-organ communication, exhibit immense potential in

driving the onset and progression of neurodegenerative diseases. The properties of CNS derived EVs, inherited from parental cells, combined with their accessibility in peripheral fluids, make them invaluable for clinical applications, particularly as biomarkers for disease diagnosis, staging, and prognosis. Moreover, EVs can function as carriers for therapeutic molecules or serve as treatment agents themselves for neurodegenerative disorders. In this review, we provide a comprehensive overview of EV isolation and characterisation methods, focussing on recent advancements such as ExoView, Simoa, and EVLET. These innovative technologies have improved the sensitivity and specificity of EV detection and characterisation, particularly for their application as biomarkers in clinical settings. Additionally, we delve into the mechanisms enabling EVs to traverse the BBB, underscoring their therapeutic potential in neurodegenerative diseases. Looking ahead, engineering EVs with optimised brain-targeting properties, coupled with precise therapeutic cargos, offers a promising pathway to enhance EV-based therapies. Such advancements hold the potential to significantly improve the precision and efficacy of treatments for neurodegenerative disorders, representing a critical step forward in translating EV research into clinical applications.

Outstanding questions

1. A more detailed classification of EVs is needed to reduce their heterogeneity and better understand their potential roles in regulating disease progression.
2. Standardised methods for EV extraction, storage, and transport could enhance their potential as diagnostic biomarkers and therapeutic vehicles in clinical applications.
3. New biomarkers capable of diagnosing diseases at preclinical stages are urgently needed.

Contributors

JZ contributed to designing the outline and revising the manuscript; JC drafted the manuscript and prepared the tables and figures; CT, YY, and XX assisted with revising the draft. All authors have read and approved the final version of the manuscript.

Declaration of interests

All of authors declare no competing interests.

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