Chapter

Part Two: Extracellular Vesicles as a Risk Factor in Neurodegenerative Diseases

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Abstract

Extracellular vesicles (EVs) involved in the intercellular communication hold cell-specific cargos that contain proteins, various species of RNA and lipids. EVs are emerging as powerful tools for diagnosis and therapy in most diseases but little is known about their role in central nervous system (CNS) physiology or disease. Considering the extraordinary intricated cytoarchitecture of the brain, the implication of EVs in its pathophysiology is difficult to establish. Blood circulating EVs derived from local or distant vascular cells or EVs released from brain into the cerebrospinal fluid (CSF) may influence the brain activity. EVs released in the blood stream from various tissues may influence the brain by passing through the blood-brain barrier (BBB) or through choroid plexus. Since the choroid plexus has also a clearance role, it might be possible that EVs carrying brain abnormal proteins to pass into the blood can be detected. Thus, considering that EVs are specialized cargos bearing combined signals between cells, they might be an interesting therapy target in the future for both regulating neurogenesis and abnormal protein clearance. We present here data gathered about EVs that may influence the CNS functionality and be involved in most common neurodegenerative diseases.

Keywords: extracellular vesicles, exosomes, intercellular communication, brain barriers, neurodegenerative diseases

1. Introduction

Extracellular vesicle (EV) is a term used to define a heterogeneous group of vesicles isolated from biological fluids or tissues. EVs seem to mediate complex cell-to-cell communication over long distances or nearby through various macromolecules: polypeptides, various species of RNAs, and/or lipids.

The classification of EVs is based on their size and mechanism of biogenesis and includes: exosomes, less than 100 nm small vesicles released from multivesicular bodies after endocytosed materials have been sorted in the endolysosomal compartment [1, 2]; ectosomes, up to 500 nm larger vesicles budding from the plasma membrane [2, 3]; and multivesicular cargos, consisting of numerous vesicles, about 150 nm, enclosed in a plasma membrane-derived shield [4]. Although many medical fields experienced real progress with newly discovered diagnostic tools or treatments for several diseases, smaller steps are taken in the field of cerebrovascular and neurodegenerative diseases. Age-related changes, cardiac diseases, and atherosclerosis are known to contribute to the pathogenic mechanism of cerebrovascular and neurodegenerative diseases affecting the elderly.

2. Biological content of extracellular vesicles

An increasing body of evidence proves that EVs are not only involved in the waste disposal system, but, more importantly, they function as membrane-bound carriers for intercellular communication [5, 6]. This type of intercellular communication was proven to modulate cellular functions both in homeostatic and pathological conditions [6, 7]. High concentrations of EVs were detected in culture supernatants and biological fluids [8, 9]. For example, EVs have been isolated from CSF and proved to contain overrepresentation of brain-specific proteins derived from cerebral white mater and choroid plexus [10]. Several studies demonstrated that the level and composition of circulating EVs are altered in disease states, neurodegenerative diseases included [10–12]. Endothelial cells (ECs) and platelets have been most studied as sources for EVs [13, 14], but circulating cells as monocytes or lymphocytes may also be a source for EVs.

Protein composition of the EV-enclosing membrane, mainly different types of integrins, cell adhesion molecules, and tetraspanins, guides the interaction with the recipient cells, the targeting, or recruitment once EVs are released into the extracellular environment [15]. Specific molecules allow EVs either to interact with surface receptors on recipient cells to activate signaling cascades, or to promote their docking and uptake. One potential mechanism is direct membrane fusion with direct transfer of the cargo molecules into the recipient cytosol [16]. Endocytosis, including clathrin-dependent endocytosis, lipid raft-dependent pathways, phagocytosis, and even micropinocytosis, was more frequently considered [15].

A recent quantitative proteomic analysis allowing comparison of different EV subpopulations [17] proved that several classic exosome markers such as flotillin-1, heat-shock 70-kDa proteins, actin, and MHC I and II are present in all EV fractions obtained by successive centrifugation. Moreover, classic exosomal tetraspanins CD9, CD63, and CD81 were unreached in the exosomal fraction but also detected in different amounts in larger EVs. The study suggests a further classification of EV pelleting at high speed into four subcategories: (a) EVs enriched in all tetraspanins and endosome markers (bona fide exosomes); (b) EVs devoid of CD63 and CD81 but enriched in CD9 (associated with plasma membrane and an early endocytic signature); (c) EVs devoid of CD63/CD9/CD81; and (d) EVs enriched in extracellular matrix (ECM) or serum-derived factors. They also propose five categories of proteins with different relative distributions in different EV populations that relate them to their intracellular source [17]. Thus, exosomes contain ECM proteins, receptors, heparin-binding, phospholipid-binding, integrins, immune response,

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and cell adhesion molecules, while ectosomes are enriched in endoplasmic reticulum proteasome and mitochondrial proteins [9].

The amounts of different lipid classes in EVs have been determined in several studies [23, 34], and the enrichment of EV membranes for cholesterol, sphingomyelin, glycosphingolipids, and phosphatidylserine compared with their cellular sources was proved. However, differences in lipid composition were reported between vesicle type and cellular source. Generally, exosomes seem to be enriched in glycolipids, phosphatidylserine, and free fatty acids, while ceramides and sphingomyelins were consistently enriched in ectosomes. Still, phosphatidylcholines were depleted in exosomes but unchanged or enriched in ectosomes [9].

EVs contain not only proteins and lipids, but several classes of RNAs. Most of the recent *in vitro* studies have proved that EVs contain functional RNA molecules that reflect the cellular status and are involved in intercellular crosstalk [18, 19]. Different species of RNA have been reported to be enclosed in EVs derived from various sources—mRNA, rRNA, and tRNA fragments and especially microRNA (miRs) [5]. Several mechanisms for RNA selection, loading into EVs, and their uptake by various target cells have been proposed [5, 20]. Packing into EVs protects the molecules from RNase degradation once released into the extracellular environment. Thus, RNA molecules can be transferred to distant recipient cells, their protein production can be modulated [8, 21], or they may be used as predictive biomarkers for the occurrence of cardiovascular events as demonstrated by the study of EVs containing miR-199a and miR-126 in patients with stable coronary artery disease [22]. In atherosclerotic disease, miR-containing circulating EVs and apoptotic bodies, along with other bioactive molecules, are released by proinflammatory stimulated monocytes and T cells; ECs and activated platelets initiate hyperplasia of vascular smooth muscle cells (VSMCs) which leads to phenotype switching from contractile to synthetic and activates their proliferation and migration [23].

Besides membrane proteins and RNA cargo, EVs may contain cytosolic proteins, such as cytokines, chemokines, growth factors, enzymes or transcription factors, functional organelles, and other bioactive molecules such as lipid mediators, derived from arachidonic acid [5]. Also, some EVs seem to retain the capacity to synthesize eicosanoids using their phospholipid content both by enzymatic and nonenzymatic processes [24].

All neural cells from rodent [25, 26] and human [25, 27], even immortalized human brain microvascular ECs [25, 28], release EVs which contain mRNA and miRs for epigenetic reprogramming of neural cells or post-transcriptional control of specific genes [25]. In vitro studies of brain angiogenesis revealed that EVs deliver proangiogenic protein, mRNAs, and miRs from cultured glioblastoma cells into cerebral ECs [25, 29], especially increased VEGFR-B from immortalized mouse cerebral ECs stimulated with LPS and cytokines into targeted cerebral vascular pericytes [25, 30]. In vitro and in vivo studies showed that neuronal exosomes containing miR-132 could mediate neuronal regulation of brain vascular integrity. Thus, in zebrafish larvae and cultured rodent brain cells, it has been shown that neurons transfer miR-132, a highly conserved and neuron-enriched miR, via secreting exosomes to ECs to maintain brain vascular integrity. Following translocation to ECs through exosome internalization, miR-132 regulates the expression of vascular ECs cadherin (VE-cadherin), an important adherens junction protein, by directly targeting eukaryotic elongation factor 2 kinase [31]. In addition, two proteins found in peripherally circulating plasma EVs, cystatin C and CD14, have been linked to the development of brain atrophy and to cerebral white matter lesions, a small vessel disease within the brain [32]. Because exosomes contain transferrin and insulin receptor [25, 28], which mediate macromolecular passing through the blood-brain

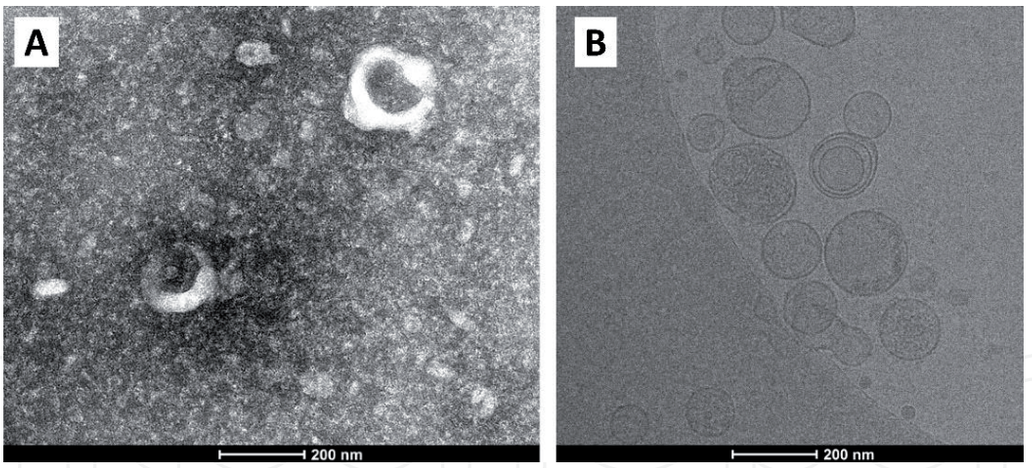

Figure 1.
Transmission electron microscopy of the isolated extracellular vesicles: (A) negative staining and (B) cryo-electron microscopy.

barrier (BBB), peripherally infused modified exosomes containing specific RNA were used to knockdown a specific gene in mouse brain [32–34]. Considering the extraordinary intricate cytoarchitecture of the brain, the presence of EVs in the adult brain is hard to be documented. Fetal brain and neurospheres contain cells which seem to release vesicles into the extracellular space (**Figure 1**). EVs are easier to be seen near ependymal cells floating in the ventricles from where they can be isolated [11].

3. Methods of isolation and analysis of extracellular vesicles

Minimal experimental requirements for definition of EVs and their functions have been proposed [35], and isolated EVs should be characterized, and the morphology, protein composition, and functionality should be tested before any new enquiries are pursued. Whether the chosen isolation technique for the experiment consists in ultracentrifugation or any other technique, after collecting the sample it is necessary to perform two low-speed centrifugations as soon as possible after the sample collection [36]. The first low-speed centrifugation (300–800×g) removes cells, lipid droplets, and macromolecules from the sample, while the second low-speed centrifugation (2.500×g) removes platelets and apoptotic bodies. Cell removal is an important step; otherwise, the following high-speed centrifugations break the cells, leading to EV release and sample contamination [37].

Differential ultracentrifugation is the most widely used method for EV isolation [36], which involves multiple centrifugations at increasingly higher speeds obtaining a particle separation based on the sedimentation coefficient [38]. Establishing the appropriate speed and duration of centrifugation is a very important step in EV isolation [36]. Several inconveniences can occur using this technique such as the loss of certain subpopulations of EVs or simultaneous isolation of lipo/protein aggregates [39, 40].

Density gradient ultracentrifugation is a method that isolates the particles by size and mass density [41], and its usefulness lies in the fact that it can isolate several subpopulations of EVs which are lost using differential ultracentrifugation; therefore, it increases the purity of the isolated EVs [42].

Size exclusion chromatography is an easy handling method by which a size-based separation is achieved [43]. It allows the electron microscopy to be performed immediately after isolation and the proteins and lipoproteins are removed

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from the sample without losing any subpopulation, thus achieving a high-yield separation [44].

Precipitation kits gained a lot of attention during the last years involving a concentration method based on the use of polyethylene glycol polymers. The price for the kits is low, the protocol is easy to perform, it is compatible with both low and high volumes of samples, and the method can be applied on large scale [45]. It must be taken into account that the precipitation kits are not the best method for EV isolation, having a low-yield purification because of the co-precipitations [37]. Anyway, for validating the results, it is recommended to use one of the ultracentrifugation methods in parallel with this method.

After the isolation step, a **general description of the protein composition** should be made even though there are no specific EV markers discovered yet. EVs are enriched in certain proteins such as tetraspanins (CD63, CD81, and CD9) and TSG 101 for exosomes, annexin V for ectosomes, etc. [46]. To exclude a contamination, the presence or absence of proteins that are not expected to be found in EVs, intracellular proteins like calnexin, cytochrome C, histones, GRP94, and Argonaute complex must be detected [35]. Western blot, flow cytometry, or mass spectroscopy can be used for this protein characterization step.

The next step consists in **individual characterization** of the EVs using at least two methods: electron microscopy [35, 45], atomic force microscopy [47], or nanoparticle tracking analysis [48]. **Transmission electron microscopy** (TEM) is the most used method for visualization of EVs considering their nanoscale size (**Figures 1** and **2**). Negative staining [49], TEM on thin section from plastic

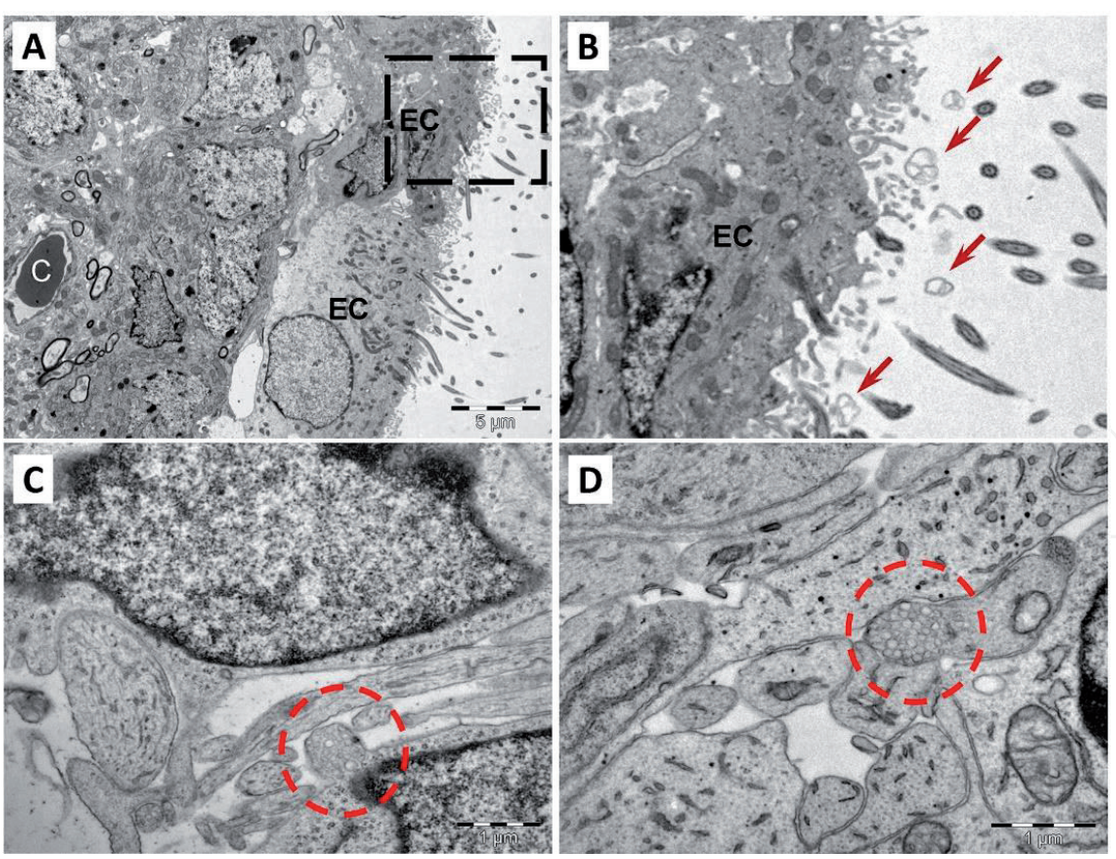

Figure 2.
Transmission electron microscopy: (A) ependymal cells (EC) facing the ventricular system of the mouse brain control the communication between cerebrospinal fluid and brain parenchyma; C—capillary. (B) Higher magnification of the square-marked area in (A) shows extracellular vesicles (arrows) close to cilia and microvilli of the ependymal cell (EC). Volume transmission may be mediated by multivesicular cargoes (encircled) which could be seen released from neurons in fetal mouse brain (C) or neurons from neurospheres generated from stem cells (D).

embedded EVs [49], can also be used to confirm the presence of the lipid bilayer of the isolated EV membrane and an election method to investigate EVs in tissue. **Cryo-TEM** [50] is a high resolution method to visualize the lipid bilayer. Electron tomography [4, 51] allows 3D reconstruction of the EVs with a very good imaging of the different types. For surface protein recognition, immuno-EM [52, 53] allows imaging of EV-specific markers.

Flow cytometry is a method based on measuring the signal of the light scattering from a structure passing through a laser beam. The smaller the particles, the less the light scattering, and therefore, conventional cytometry has a detection limit of approximately 200 nm [54]. Fluorescence-based detection is used to overcome this inconvenience [45, 55], the background noise being higher than the EV signal.

Nanoparticle tracking analysis involves the passing of a laser beam through a suspension that contains EVs and the scattering light is captured by a camera [48]. The Brownian movement of the EVs in the suspension is recorded, and according to their movement, the size can be measured [48].

The last step to fulfill the minimal experimental requirements involves the **functional assays** [35]. Multiple *in vitro* models can be designed for this step; co-incubating EVs with different culture cells and migration, proliferation, coagulation, and fibrinolysis may be quantified depending on the chosen model [45].

There is no gold-standard method yet, but researchers are encouraged to explore and to standardize their methods of isolation with the hope that someday, easy EV isolation will be possible.

4. Extracellular vesicles and cardiovascular risk for brain pathology

All cells are capable of secreting specific arrays of EVs. Furthermore, the same cells may produce EVs by different biogenesis pathways, with different intracellular origins, of various sizes, with diverse composition and consequently, different functional properties [17]. Crucial feature for the normal functionality of the CNS and brain microenvironment homeostasis is sustained by vascular integrity which is highly dependent on the systemic status.

Several studies of patients with **stable coronary artery disease** have reported increased levels of circulating EVs which may influence the BBB integrity. Specific EV subpopulations, especially those of ECs origin, characterized by $CD144^+$, $CD131^+$/annexin $A5^+$, or EVs containing miR-199a and miR-126, are currently researched as interesting biomarkers for cardiovascular risk and mortality in these patients [22, 56, 57].

Calcifications present in the atherosclerotic plaque [58] have a destabilizing effect in early lesions, favoring the rupture, but gain a potential protective effect in advanced lesions with heavy calcium deposits [59]. As several studies have shown, atherosclerotic plaque calcifications are associated with EVs of ECs, VSMCs, and macrophage origin. VSMCs can release EVs with low levels of calcification-inhibiting enzymes and annexin A6/phosphatidylserine nucleation complexes. Exposure of VSMCs to proinflammatory cytokines can stimulate the release of EVs that can mineralize when inhibitors of calcification are missing [60]. Also, ECs exposed to proinflammatory stimuli can release EVs rich in bone morphogenetic protein 2, promoting calcification in VSMs [61]. Alterations in local homeostasis of calcium and phosphate lead to the formation of macrophage-derived exosomes which stimulate mineralization, through an annexin-dependent mechanism [62].

Recent studies have also noted that in humans, advanced atherosclerotic plaques have a high content of procoagulant EVs, originating form leukocytes, erythrocytes, and VSMCs [63, 64]. In opposition to ectosomes, exosomes have shown

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antithrombotic effects. In animal studies, platelet-derived exosomes suppressed platelet aggregation and occlusive thrombosis by inhibiting platelet CD36 and decreasing CD36-dependent oxidized LDL binding and macrophage cholesterol loading [65]. EVs influence the different mechanisms that lead to plaque destabilization and rupture. Intraplaque hemorrhages are produced by neovascularization originating from adventitial tissue, stimulated by plaque EVs, such as CD40+ vesicles of macrophage origin. Hemorrhages are also favored by leukocyte and ECs-EVs with fibrinolytic activity [66, 67]. Fibrous cap weakening is associated with VSMC apoptosis, induced by the presence of EVs, released in some pathological conditions [100]. Moreover, EVs can influence breakdown of matrix structural proteins through metalloproteinase interactions [68].

Circulating levels of procoagulant EVs are higher in patients with **acute coronary syndromes** compared to healthy controls or patients with stable coronary artery disease [57, 69]. Circulating EVs alter endothelial-dependent NO mediated vasodilation, and endothelial EVs increase ECs thrombogenicity [70, 71]. Circulating EVs have also been investigated as prognostic markers in secondary prevention, in order to identify patients at high cardiovascular risk [56, 57]. Increased levels of $CD11b^+$/$CD66^+$ leukocyte-derived EVs could be useful in identifying asymptomatic patients at high risk for plaque rupture [72], while $CD3^+$/$CD45^+$ EVs could identify individuals who will develop a major cardiovascular event [73].

All these circulating EVs associated with coronary disease [74] may affect the vascular bed of the brain and disrupt the functionality of BBB. Aging and cardiovascular-associated disease are associated with BBB alteration [75] and blood circulating EVs may mediate early dysfunctions or progression of cerebral associated pathology. EVs associated with hypercholesterolemia [76] and atherosclerosis [77, 78] may have an impact on BBB function, and reducing the proinflammatory cytokines enrolling in EVs [79] may be beneficial.

Different neuronal cell types and molecules concur to regulate the improvement of brain vasculature [31, 80–82]. Each cell, including neurons and astrocytes [32, 83], is able to produce EVs enriched in specific proteins, lipids, and RNAs. EVs can stimulate targeted neural cells and surrounding neural tissues, which are important elements of vascular integrity preservation. The brain pathological condition changes the EV content profile of proteins and miRs [84]. A number of studies have shown that EVs regulate arterial stiffness [32, 85] which is linked to small vessel [32, 86–88], and platelet-derived EVs seem to be important players in the formation of cerebral microthrombi which lead to brain atrophy and consequent cognitive degeneration. It is believed that the prothrombotic nature of an elevated number of platelet-derived EVs reported in the acute phase of cerebral infarction may conduct to infarct progression [32, 84, 89, 90]. EV proteins cystatin C and CD14 have been shown to be related to cerebral white matter lesions and the progression of brain atrophy in patients with manifest vascular disease, suggesting a role for EVs in the etiology of structural brain changes [32].

The role of cardiovascular disease risk factors in the occurrence and progression of cognitive impairment is widely accepted. There is a link between elevated levels of cholesterol and amyloid deposition in the brain, and the relationship between atherosclerotic injury and sporadic Alzheimer's disease is investigated.

5. Extracellular vesicles in neurodegenerative diseases

The pathology of Alzheimer's disease (AD) consists in the extracellular amyloid plaques formed by aggregated amyloid beta peptides and in the intraneuronal neurofibrillary tangles made of hyperphosphorylated tau proteins [91]. The

accumulation of the proteins induces an apoptotic response with neuronal loss and occurs especially in the cerebral cortex [92].

It has been observed that the pathological findings in AD have a typical spatial distribution suggesting a neuron-to-neuron spread of the amyloid and hyperphosphorylated tau proteins, which promote aggregation, acting as "seeds" [93]. Therefore AD is considered to have a prion-like model of propagation [94]. The immediate question that rises is: what is the mechanism of the propagation?

Amyloid beta is mainly formed extracellularly from the cleavage of APP by beta and gamma secretases, which are found at the level of the plasma membrane [95]. But in some degree, the secretases are present in endoplasmic reticulum and Golgi apparatus, and there is a certain intracellular production of amyloid beta [96]. It is removed from the cell via exosomes embedded in a multivesicular body as an alternative pathway to lysosomal degradation [97]. A new study suggests that exosomes containing amyloid beta are present in higher concentrations in the AD brain compared to the healthy brain [98]. Moreover, the study shows that exosomes are the carriers of the toxic amyloid beta from one neuron to another [98]. Also, it is thought that exosomes mediate the intercellular transfer of hyperphosphorylated tau protein, and the exosome-mediated tau protein induces the formation of neurofibrillary tangles [99].

The evolution of AD is insidious with an asymptomatic stage that lasts several years [100]. Although asymptomatic, the pathological changes in the brain are present in this stage [93]. These findings suggest the value of discovering biomarkers that can anticipate the onset of the clinical symptoms or can facilitate a window for the possibility of a future therapy that could stop the progression. Amyloid beta and hyperphosphorylated tau proteins are of great value as biomarkers when dosed from the cerebrospinal fluid [101]. Nevertheless, performing lumbar puncture in a wide population is almost impossible. Thereby, the discovery of new biomarkers is a valuable research theme.

Several types of miRs isolated from cerebrospinal fluid are differentially expressed in AD, such as miR-100, miR-146, miR-505, and miR-1274a [102]. The presence of several types of exosomal miRs isolated from serum (miR-361-5p, miR-93-5p, miR-335-5p, and miR-30e-5p) correlates with the neuropsychological evaluation and brain imaging [103]. It is to be mentioned that exosome-containing proteins like tau, apolipoprotein E, cystatin E, and HSP-90 alpha were isolated in the cerebrospinal fluid and were present in patients with AD [104]. This evidence proposes both miRs and protein-containing exosomes as a promising source of biomarkers for AD.

Parkinson's disease (PD) is a neurodegenerative disease that consists in the loss of dopaminergic neurons localized in the substantia nigra [105]. The pathology of the disease implies the deposition of Lewy bodies in the neurons which are mostly made of misfolded and aggregated alpha-synuclein protein [105]. The Braak staging explains the spatio-temporal dissemination of Lewy bodies into the neurons from caudal to rostral, starting in the medulla oblongata and spreading to the level of the cerebral cortex, damaging various structures on this way [106]. The starting point is thought to be either the enteric nervous system or the olfactory bulb [106]. Thereby, it is suggested that PD has a prion-like propagation [94]. Are exosomes responsible for carrying alpha-synuclein from neuron to neuron? The mechanism could be similar to the one described in AD, but there are fewer studies to draw certain conclusions regarding the involvement of exosomes in the pathogenesis of PD. Excessive intracellular alpha-synuclein is thought to be transported out of the cell via multivesicular body containing exosomes in a similar manner as in AD in an attempt to clean the intracellular space [107].

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Studies of neuronal cell cultures expressing alpha-synuclein noticed that the protein is released by the cell free in the extracellular space but also incorporated in the exosomes [108]. Furthermore, neighboring cells take up the exosomes, and the transferred alpha-synuclein acts as seed for the formation of the aggregate [109]. Evidence to prove that in vivo alpha-synuclein is transferred by exosomes is not available yet, but it can be speculated that exosomes play an important role as in vitro.

The use of EVs as a biomarker tool in PD is a promising field too [110]. Several miRs carried by exosomes were increased in the CSF of PD patients, such as miR-10a-5p, miR-153, and miR-409-3p, while some miRs such as miR-19b-3p and miR-1 were significantly reduced [111]. Exosomes containing alpha-synuclein and LRRK proteins were also isolated in the CSF of PD patients [109, 112].

6. Extracellular vesicles' perspective use in brain pathology

The discovery of the EV involvement in several biological processes gave hope that some questions regarding neurodegenerative diseases will be answered. First of all, it is important to clarify which are the cellular mechanisms involved in the progression of the disease and if exosomes play any role. Based on the spatio-temporal spreading of the pathological proteins in the brain, an appealing theory is the prion-like propagation theory [113]. It is presumed that exosomes play an important role by facilitating the interneuronal transport of the proteins [114]. As well, there is a critical need in finding accessible biomarkers that can diagnose a neurodegenerative disease in the asymptomatic stage [115]. Dosing certain free proteins in biofluids can be an option, but several problems are experienced because of their low concentrations [115]. Therefore, a new approach is being attempted consisting in finding the proteins encapsulated in extracellular vesicles [116]. Micro-RNAs and different proteins carried by exosomes are attractive options for finding new biomarkers in several diseases as well as in neurodegenerative diseases [117].

Recently, it was discovered that an important player in the field of neural diseases is EV derived from stem cells [118], mainly in the case of stroke [118–120]. In stroke pathophysiology, inflammation plays a significant role, circulating EV-activating immune cells. Neurons quickly depolarize and die, being next phagocytosed by infiltrating circulating macrophages and microglia [84, 121].

Because of their β1 and α2b integrin-enriched content, human neural stem cell-derived EV administration recovers both tissue and sensorimotor function and may protect the BBB integrity, in the preclinical mouse thromboembolic model of stroke [122, 123]. Similarly, multipotent mesenchymal stromal cells (MSCs), through their capacity to secrete soluble factors, play an important role in brain repair. It was demonstrated that MSC cargos modulate cell signaling in ischemic stroke by PI3K/Akt pathway activation [123, 124] and EVs facilitated secretion of miRs sustaining MSC neuroprotective effects in ischemic stroke as well. Previous research demonstrated that intravenous administration of bone marrow-MSCs containing exosomes transferred miR-133b to astrocytes and neurons into the ischemic boundary zone [120, 123], and MSCs cultured in the presence of extracts from rat ischemic brain induced increased expression of exosomal miR133b [123, 125]. Also, EVs released by human MSCs seem to have an anti-inflammatory effect on mast cells, by increased prostaglandin E2 (PGE2) synthesis and up-regulation of EP4 receptor which might prevent the rupture of intracranial aneurysms [126]. All these data suggest that EVs from various sources may contribute to the neurogenesis and angiogenesis during brain repair processes in cerebral diseases.

7. Conclusion

The EVs emerge as a powerful tool for early diagnosis and subsequent prevention of pathologies with high risk for the brain dysfunction. Still, EVs investigation as biomarkers and therapeutic agents is in its infancy. There is increasing evidence that EVs have an important role in brain pathophysiology. Thus, their potential application as prognostic and diagnostic biomarkers and targeted therapeutic tools relies on their subsequent isolation for molecular and functional characterization that can relate them with the cellular source and cellular mechanisms in both health and disease.

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Conflict of interest

The authors declare that the research was conducted in the absence of any either commercial or financial relationships that could be construed as a potential conflict of interest.

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