Diagnostic and therapeutic potential of exosomes in Alzheimer’s disease

Tânia Soares Martins¹ | Dário Trindade¹ | Margarida Vaz¹ | Inês Campelo² | Martim Almeida² | Guilherme Trigo² | Odete A. B. da Cruz e Silva¹,³ | Ana Gabriela Henriques¹

¹Neurosciences and Signalling Group, Institute of Biomedicine (iBiMED), Department of Medical Sciences, University of Aveiro, Aveiro, Portugal
²Department of Medical Sciences, University of Aveiro, Aveiro, Portugal
³The Discovery CTR, University of Aveiro Campus, Aveiro, Portugal

Abstract

Exosomes are small extracellular vesicles released by almost all cell types in physiological and pathological conditions. The exosomal potential to unravel disease mechanisms, or to be used as a source of biomarkers, is being explored, in particular in the field of neurodegenerative diseases. Alzheimer’s disease (AD) is the most prevalent neurodegenerative disease in the world and exosomes appear to have a relevant role in disease pathogenesis. This review summarizes the current knowledge on exosome contributions to AD as well as their use as disease biomarker resources or therapeutic targets. The most recent findings with respect to both protein and miRNA biomarker candidates for AD, herein described, highlight the state of the art in this field and encourage the use of exosomes derived from biofluids in clinical practice in the near future.

KEYWORDS

Alzheimer’s Disease, biomarker, diagnosis, exosome, therapeutics
INTRODUCTION

1.1 Overview on exosomes biogenesis and secretion

For more than 2 decades exosomes were considered as merely cellular waste disposal systems, but have recently emerged as a novel mechanism capable of mediating cell-to-cell communication. Exosomes belong to the heterogenous family of extracellular vesicles (EVs) which can be distinguish based on their origin, shape, size, composition and functions. Besides exosomes, the other two main subtypes of EVs are microvesicles and apoptotic bodies. Exosomes are the smallest group of EVs (30–150 nm) with an endocytic origin, whereas microvesicles (50–1000 nm) are formed by outward budding from the plasma membrane, and apoptotic bodies (500–4000 nm) are derived from apoptotic cells (Konosheenko, Lekchnov, Vlassov, & Laktionov, 2018; Momen-Heravi, Getting, & Moschos, 2018). The size distribution of these vesicle subtypes can overlap, hindering the isolation of “pure” subpopulations of EVs. Indeed, the precipitation and column-based exosome isolation methods can be most efficient in terms of yield, comparatively to the gold standard ultracentrifugation procedure. However, none are completely free of other vesicle type contaminations, lipoproteins, virus, or protein aggregates that co-purify (He, Zheng, Luo, & Wang, 2018; Li, Kaslan, Lee, Yao, & Gao, 2017; Momen-Heravi et al., 2018). Thus, appropriate characterization of vesicle size (e.g. by Nanoparticle Tracking Analysis, Transmission Electron Microscopy), morphology (Transmission Electron Microscopy, Cryogenic Electron Microscopy) and protein/nucleic acids content is mandatory to better define the nature of the exosomal preparations.

Focusing on exosomes biogenesis, these nanovesicles are formed by inward budding of the plasma membrane and released by fusion of multivesicular bodies (MVBs), an organelle of the endocytic pathway, with the plasma membrane. Indeed, early endosomes mature into late endosomes, forming intraluminal vesicles (ILVs) inside MVBs (Stoorvogel, Strous, Geuze, Oorschot, & Stenmark, 2009), that may have two final destinations: fusion with the lysosome, for ILVs degradation, or fuse with the plasma membrane secreting the exosomes, in a mechanism mediated by several RAB-related proteins. Nonetheless, exosomes can be generated in an ESCRT-independent process that may involve lipids (e.g. as ceramide and sphingomyelin) and tetraspanins. Abbreviations: ESCRT-Endosomal Sorting Complex Required for Transport; ILVs-Intraluminal vesicles; MVBs-Multivesicular bodies; RAB-Ras-related protein

and then interact with the ESCRT-I complex. Consequently, ESCRT-I interacts with ESCRT-II that further recruits ESCRT-III. The budding formation and sequestering of ubiquitinated proteins are processes mediated via the ESCRT-I and II complexes, whereas the ESCRT-III complex is involved in the dissociation and scission of ILVs (Colombo et al., 2013; Schmidt & Teis, 2012) (Figure 1). In turn, it was observed that several small GTPases from Ras-related proteins (RAB) family (Blanc & Vidal, 2018), soluble NSF-attachment protein receptors and RAL-GTPases could be involved in MVBs’ fusion with the plasma membrane (Fader, Sánchez, Mestre, & Colombo, 2009; Hyenne et al., 2015). The first RAB reported to play a crucial role in exosome secretion was RAB11 (Savana, Vidal, & Colombo, 2002). This RAB appears to be involved in MVBs docking with the plasma membrane in an intracellular calcium-dependent mechanism (Savana, Fader, Damiani, & Colombo, 2005). Other RABs are involved in this process, since inhibition of RAB35, RAB27A, RAB27B, RAB9A and RAB2B also resulted in decreased exosome secretion in various cell types (Frihbeis, Fröhli, Kuo, Amphornrat, et al., 2013; Hsu et al., 2010; Ostrowski et al., 2010).

Furthermore, exosome biogenesis can also occur in an ESCRT independent manner, mainly mediated by lipids (e.g. as ceramide and sphingomyelin) and tetraspanins (Guo, Bellingham, & Hill, 2015; van Niel et al., 2011; Perez-Hernandez et al., 2013; Strauss et al., 2010; Stuffers, Sem, Stenmark, & Brech, 2009; Trajkovic et al., 2008).

Of note, a link between exosomes and autophagy (a lysosomal-dependent degradation and recycling pathway) was proposed, since these processes share common molecular machinery. As an example, it was reported that post-translational ubiquitin-like modification (ISGylation) of TSG101, an ESCRT-I complex member, increased TGS101 aggregation and degradation, an event accompanied by...
reduced exosome biogenesis and secretion. Noticeably, inhibition of autophagy could re-establish exosome secretion (Villarroya-Beltri et al., 2016). Furthermore, exosomes and autophagy can act in a coordinated manner to eliminate cellular waste, where both processes compensate each other. The suppression of autophagosome fusion with lysosomes, or autophagosomes maturation, could promote exosome secretion, whereas exosome secretion could be decreased when autophagy was induced by cell starvation (Gudbergsson & Johnsen, 2019; Xu, Camfield, & Gorski, 2018). The interplay between exosome biogenesis, secretion and autophagy can have implications for brain neurodegeneration highlighting the importance of additional studies to clarify this issue.

1.2 | Exosomes in the brain

Although the exact function of exosomes in the brain is not fully understood, it is clear that these small EVs can mediate cell communication at the central nervous system level and play important roles in maintaining normal brain physiology. Neurons—oligodendrocytes communication is such an example of the exosomes—mediated interaction, relevant for myelination and axons survival (Frühbeis, Fröhlich, Kuo, Amphornrat, et al., 2013; Frühbeis, Fröhlich, Kuo, & Krämer-Albers, 2013; Krämer-Albers et al., 2007). The release of glutamate can stimulate exosome secretion from oligodendrocytes, and these nanovesicles can then be endocytosed by neurons. Moreover, addition of oligodendrocytes-derived exosomes to cultured neurons could increase their viability, under stress conditions, exerting a neuroprotective role (Frühbeis, Fröhlich, Kuo, Amphornrat, et al., 2013).

It was also found that glutamatergic activity can regulate exosome secretion from somato-dendritic compartments. Exosomes release could be a possible mechanism for receptor elimination since these nanovesicles could carry AMPA receptors, regulating their number and potentially modulating synaptic transmission and plasticity (Lachenal et al., 2011). It was later reported that exosomes secreted from cortical neurons upon synaptic glutamatergic stimulation were selectively bound and endocytosed by other neurons (Chivet et al., 2014). Upon neurons depolarization a subset of miRNA and proteins were found enriched in exosomes, among them is the microtubule-associated protein 1b (MAP1b), a synaptic plasticity-associated protein, which reinforces the role of exosomes in the synaptic plasticity (Goldie et al., 2014).

Exosomes appear likewise to play a relevant role in synaptic connections elimination, a process known as synaptic pruning and mediated by glial cells, which engulf the neurites that degenerated. It was observed that microglia internalization of exosomes, secreted after PC12 cells depolarization, lead to up-regulated expression of the pro-phagocytic microglial component 3 and stimulated microglia phagocytic activity (Bahrini, Song, Diez, & Hanayama, 2015). Other roles also attributed to exosomes were regulation of neurogenesis and mitigation of inflammation after traumatic injury (Zhang et al., 2015) and involvement in neuronal energy metabolism through the transfer of enzymes that participate in glycolysis and fatty acids synthesis (Drago et al., 2017).

Taken together, the data strengthen the importance of these nanovesicles in the brain and that dysregulation of EVs biogenesis and secretion can impact neurodegeneration, contributing to several neuropathologies, including Alzheimer’s disease (AD).

1.3 | Alzheimer’s disease hallmarks and molecular diagnosis

AD is the most common form of dementia worldwide and it is estimated that the number of individuals affected by this neurodegenerative disease will increase exponentially in the next decades. AD is characterized by memory loss, progressive cognitive decline and hindering of the daily activities until the individuals completely lose their autonomy (DeTure & Dickson, 2019).

The two major disease histopathological hallmarks described are the presence of senile plaques (SPs) and of neurofibrillary tangles (NFTs) in AD brains. Senile plaques consist predominantly of extracellular insoluble deposits of amyloid beta peptide (Aβ) peptides arising from amyloid precursor protein (APP) processing. The two main pathways by which APP can be processed are the non-amyloidogenic and the amyloidogenic pathways, although more recently an additional pathway has been proposed (Willem et al., 2015). In the non-amyloidogenic pathway, APP is cleaved by α-secretases within the Aβ domain generating the large soluble ectodomain sAPPα and the membrane bound carboxy-terminal fragment APP-CTFα that can be further cleaved into the P3 fragment which is less toxic than Aβ peptide, and the APP intracellular domain (AICD) (Dulin et al., 2008). Several members of the ADAM protein family were reported to act as α-secretases such as ADAM10 (Asai et al., 2003; Lammich et al., 1999), ADAM9 (Asai et al., 2003; Koike et al., 1999), ADAM17 (Asai et al., 2003; Buxbaum et al., 1998) and ADAM19 (Tanabe et al., 2007). The former is the main neuronal physiological α-secretase.

In the amyloidogenic pathway, APP can be cleaved at the Aβ amino terminal releasing the sAPPβ ectodomain and leading to the formation of the membrane-bound APP-CTFβ. The later fragment can then be processed by the γ-secretase complex, generating the toxic Aβ peptide, ranging from 39–43 amino acids long and AICD. The β-secretase activity was mainly attributed to β-site APP cleaving enzyme 1 (BACE1), whereas four transmembrane molecular components participate in the γ-secretase complex: presenilin (PS) 1 and presenilin 2 and the three adaptor proteins nicastrin, anterior pharynx-defective 1 (APH1A) and presenilin enhancer protein 2 (Blenno, de Leon, & Zetterberg, 2006; Gandy et al., 2007; Haass, Kaether, Thinakaran, & Sisodia, 2012).

The NFTs are intracellular inclusions, formed by hyperphosphorylated and misfolded Tau proteins. Abnormal phosphorylation is indeed a fundamental process not only related with Tau hyperphosphorylation and NFTs formation (Oliveira, Costa, Almeida, & da Cruz e Silva, Henriques, 2017; Oliveira, Henriques, Martins, Rebelo & da Cruz e Silva, 2015) but also with consequences to APP phosphorylation, processing and Aβ production (da Cruz e Silva et al., 2009; Rebelo et al., 2007; Vieira, Rebelo, Domingues, & Cruz e Silva E. F. & da Cruz e Silva, 2009).
Exosomes secreted from APP transgenic mice brains, exhibited higher levels of hAPP and APP CTFs than wild-type mice, suggesting that AD conditions enhance accumulation of these fragments into exosomes, an event that might contribute to the spreading of amyloidogenic material. Additionally, these exosomes contained some APP cleaving enzymes such as α-secretase (ADAM10), β-secretase (BACE1) and nicastrin, which is part of the γ-secretase complex (Perez-Gonzalez, Gauthier, Kumar, & Levy, 2012). Exosomes isolated from human neural cells expressing mutant PS1 mutation, or from the plasma of two APP/PS1 double-mutant transgenic mice, or from the plasma and CSF of AD patients, presented increased Aβ_42/1-40 ratios. In addition, exosomes isolated from cell media of the former mutant cells and from AD patients CSF, exhibited higher neurotoxic ability, by impairing calcium homeostasis and mitochondrial function (Eitan et al., 2016). The levels of APP, monomeric and oligomeric forms of Aβ, Tau and Tau phosphorylated forms were also found increased in serum-derived exosomes released from a cerebral amyloid angiopathy AD transgenic mouse model, supporting once more the exosome pathological spreading role in AD (Rosas-Hernandez et al., 2019). Exosomes’ spreading was likewise observed in a micro-fluidic model of a neuronal circuit, where these nanovesicles could be easily internalized by neurons; and where exogenous exosomes fuse with endogenous MVBs in order to be re-secreted and exert long distance actions (Polanco et al., 2018).

Regarding human brain studies, exosomes derived from AD brain tissues were enriched in Aβ and Tau (Noglab et al., 2017). Additionally, the exosome marker Alix, which can interact with ESCRT III complex, was present around small SPs and in large diffuse plaques of post-mortem AD brains, supporting that the release of exosomes containing Alix can possibly contribute to plaque formation and disease progression (Rajendran et al., 2006). Microglia surrounding SPs can also play a role in neurodegeneration through the secretion of exosomes containing Aβ species (Joshi et al., 2014). In line with these observations, Aβ oligomer-containing exosomes isolated from post-mortem AD brains could be internalized by neurons, transferred among nearby cells, releasing their cargo and inducing cytotoxicity in the recipient neurons (Sardar Sinha et al., 2018). Furthermore, analysis of post-mortem human brains, from ApoE4 allele carriers, revealed a decrease in the number of exosomes in the extracellular space, when compared with brains from ApoE3 allele carriers. Additionally, brains of ApoE4 mice revealed a decrease in protein and mRNA levels of some exosome pathway regulators, such as TSG101. The data suggest that the decreased exosome number observed in ApoE4 carriers can be explained by reduced exosome biogenesis rather than altered exosome clearance mechanisms. In addition, exosomes isolated from mouse ApoE4 carriers presented a higher content of cholesterol, ceramide and gangliosides (Peng et al., 2019). Of note, ceramide was reported to regulate exosome biogenesis (Guo et al., 2015; Trajkovic et al., 2008). Taken together, data demonstrate that exosomes play an important role in the endosomal and lysosomal dysfunction, an event associated with ApoE4 genotype (Peng et al., 2019). The reduction in exosome biogenesis in ApoE4

2 | EXOSOMES AS CARRIERS OF AD PATHOGENIC CONTENT

Exosomes were reported to carry two of the main molecules involved in AD pathogenesis, Aβ peptide and Tau, thus increasing the interest on these EVs in the disease context. Nonetheless, besides these pathological factors, many other proteins also relevant to AD have been identified into exosomes; these are discussed below.

2.1 | APP and its metabolites in exosomes

Increasing efforts have been made to clarify the exact role of exosomes in AD, in particular regarding their role in APP processing and Aβ production (Lee, Mankhong, & Kang, 2019). It was reported that full-length APP and other APP metabolites can be carried in exosomes secreted by SH-SY5Y cells, expressing wild-type APP, including APP CTFs and AICD (Vingtdeux et al., 2007). Furthermore, exosomes secreted by double-mutant APP Swe/Ind cells, contain full-length APP and APP CTFs, inhibited cell proliferation and promoted apoptosis (Zheng, Pu, Chen, Guo, et al., 2017). Moreover, besides CTFs, Aβ species and sAPPα were found in exosomes secreted by a human cell line expressing wild-type APP. These exosomes also contained BACE, PS1 and PS2 (Sharples et al., 2008). In addition, Aβ_42 peptide was found in MVBs of neurons (Takahashi et al., 2002) and exosomes of HeLa and N2a cells (Rajendran et al., 2006). APP CTFs were also present in exosomes secreted by N2a cells, in conditions of PI3P depletion, which is a regulator of endolysosomal and autophagic functions (Miranda et al., 2018).

MARTINS et al.
carriers (which is a risk factor for AD), and the involvement of exosomes in Aβ clearance, seems to contrast with the data showing that exosomes can be involved in the spreading of amyloidogenic material and in disease progression. This indeed, raises the question, if inhibition of exosomes secretion in AD could have a beneficial or detrimental effect. Nonetheless, it would not be surprising that these EVs play a dual role in AD, which could be triggered or dependent on the surrounding environment; aspects that need further clarification.

2.2 | Exosomes transport of Tau

As mentioned above, like Aβ, Tau can also be found in exosomes. The secretion of Tau-containing exosomes seems to correlate with a relevant disease pathogenic role, since these nanovesicles can carry aggregated and phosphorylated Tau forms, such as p-Tau 181, p-Tau 262, p-Tau 396 and p-Tau 422, acting as seeds of Tau aggregation and misfolding in recipient cells (Ngolab et al., 2017; Polanco, Scicluna, Hill, & Götz, 2016; Rosas-Hernandez et al., 2019; Willén et al., 2017). Exosomes secreted from M1C cells and CSF-derived exosomes, also transported Tau species phosphorylated at several epitopes and exosomes isolated from CSF of AD cases were enriched in p-Tau 181, particularly those from Braak stage III individuals (Saman et al., 2012). Of note, Tau over-expression in 4R0N cells altered the proteome of secreted exosomes, promoting recruitment of known Tau interactors but also of other non-typical exosomal proteins (Saman et al., 2014).

Extracellular Tau truncated at the C-terminus and lacking the microtubule-binding region is the major Tau species released by neurons to the extracellular space, but its secretion into exosomes is only a minor portion of all Tau-truncated species secreted (Kannert et al., 2015). The majority of Tau presenting in neuronal plasma-derived exosomes is free-floating and truncated (Guix et al., 2018). Full-length Tau species were found in low abundance in exosomes, prepared from conditioned media of human induced pluripotent stem cell (iPSC)-derived neuron and also in CSF-derived exosomes (Guix et al., 2018). Particularly noteworthy, exosomes can mediate trans-synaptic Tau transfer between neurons thus contributing to Tau pathology (Wang, Balaji, et al., 2012). Additionally, the spreading of Tau by microglia-derived exosomes could be decreased in in vitro and in vivo models through genetic depletion of BIN1, that is one of the major genetic locus associated with late onset AD (Crotti et al. 2019). Evidence supports that microglia-derived exosomes play a major role in the spread of Tau and thus potentiating new strategies to counteract exosomal Tau spreading can be helpful against AD.

Microglia, neuronal-like models and exosomes can interplay with implications for the inflammatory response. Co-cultured microglial CHME3 cells can internalize SH-SYSY-derived exosomes with consequent release of exosomes carrying the pro-inflammatory marker S100B. Furthermore, the inflammatory-associated miRNA, miR-21, was found in SH-SYSY cells expressing Swedish mutation and CHME3 microglial cells as well as in the exosomes secreted by these two cell lines. This observation suggested that miR-21 can be an exosomal biomarker of microglia activation, enhancing the importance of these vesicles for the neuronal-microglial communication (Fernandes et al., 2018). In addition, in the presence of pro-inflammatory stimulus (lipopolysaccharides, IL-4 and IL-10), Glutaminase C over-expression in primary mouse microglia lead to increased exosome release and alterations in exosome cargo. The released exosomes carried up-regulated pro-inflammatory microRNAs (miRNAs) and down-regulated anti-inflammatory miRNAs, again supporting the role of exosomes in the activation of microglia and in AD inflammatory responses (Gao et al., 2019).

Moreover, exosomes secreted by astrocytes surrounding SPs could induce apoptosis even in astrocytes not directly exposed to the Aβ peptide. This is presumably because of exosome transfer ability, which can represent a new mechanism for apoptosis induction by Aβ (Wang, Dinkins, et al., 2012). Additional studies support this spreading role of exosomes along considerable distances in the brain. Plasma-derived labelled exosomes were injected into the hippocampus of an AD transgenic mouse model, and these nanovesicles spread in a stable way from the hippocampus to the cortex, accumulating around SPs and taken up by active microglia cells (Zheng, Pu, Chen, Mao, et al., 2017).

Microglia can likewise play a role in Tau spreading. Indeed, depletion of microglia and inhibition of its exosome secretion results in suppression of Tau propagation between neurons in a P301S Tau transgenic mouse model. Furthermore, microglia can contribute to rapid Tau spreading, from entorhinal cortex to the dentate gyrus. The process involves phagocytizing Tau and then secreting it into exosomes, potentially representing a new mechanism for Tau transport that explains non-synaptic propagation (Asai et al., 2015). Additionally, the spreading of Tau by microglia-derived exosomes could be decreased in in vitro and in vivo models through genetic depletion of BIN1, that is one of the major genetic locus associated with late onset AD (Crotti et al. 2019). Evidence supports that microglia-derived exosomes play a major role in the spread of Tau and thus potentiating new strategies to counteract exosomal Tau spreading can be helpful against AD.

In addition, under inflammatory conditions, EVs spreading can promote mitochondrial dysfunction; another pathogenic event that has been implicated in AD pathogenesis. EVs isolated from hippocampal cells, exposed to the pro-inflammatory cytokine TNF-α, could induce mitochondrial dysfunction when internalized by naïve cells. This response is related to increased oxidative phosphorylation, proton leakage and reactive oxygen species production (Russell et al., 2019).
2.4 | Mechanisms of amyloid loading into exosomes

Despite all the above-mentioned evidence on exosomes’ contributions to AD pathogenesis, little is known concerning the mechanisms underlying Aβ binding to these vesicles or how APP and its metabolites are internalized in late endosomes, leading to further exosome-associated release. The growth factor receptor protein-binding protein 2 (Grb2) was reported to be involved in sequestering APP and AICD in the late endosomes of neuro 2A cells (Raychaudhuri & Mukhopadhyay, 2010). APP CTls exosomal secretion and its autophagosomal degradation can be modulated by an active role of Tetraspanin 6, which was found over-expressed in AD brains (Blalock et al., 2004; Bossers et al., 2010; Miller, Woltjer, Goonenbour, Horvath, & Geschwind, 2013). Indeed, it was reported that under tetraspanin 6 over-expressing conditions, APP processing increases and APP CTls can accumulate in the cellular endosomal compartments. This could be explained by a decrease in the fusion between lysosomes and autophagosomes that prevented CTls degradation (Guix et al., 2017).

Furthermore, decreased ubiquitination, particularly because of the lack of APP C-terminal lysine, was related to decreased exosome release of APP CTls. Thus, ubiquitination seems to act as a signal involved in APP endosomal trafficking and metabolism, relocating APP from ILVs to endosomal limiting membrane (Williamson et al., 2017).

Aβ accumulation in MVBs is one of the earliest changes associated with AD onset. The expression of E228Q ATPase-deficient form of VPS4A in a neuroblastoma cell line could increase the intracellular levels of Aβ and p-Tau 396, the number of Aβ oligomers and promote MVBs enlargement. Exosome secretion was not decreased and, instead, an up-regulation of ESCRT-MVB formation mechanism was observed (Willén et al., 2017).

Recently, the development of a highly sensitive analytical platform, termed amplified plasmonic exosome (APEX) offers a new opportunity to assess exosome association to Aβ forms with enhanced sensitivity. APEX demonstrated that the exosomal Aβ1-42 binding is distinct depending on the different exosomal cell origins. While there was a strong association of Aβ1-42 aggregates to neuron, erythrocyte, platelet and epithelial-derived exosomes, the Aβ1-42-binding capacity to glial and endothelial-derived exosomes was comparatively reduced (Lim et al., 2019). Of note, Aβ binding to the exosomal surface can be promoted by glycosphingolipids, particularly glycans (Yuyama et al., 2014).

A recent study revealed that hypoxia can increase the total cellular and exosomal Aβ production not only in vitro but also in vivo, an effect counteracted by the administration of resveratrol, which inhibited CD147 hypoxia-induced expression (Xie et al., 2019). It remains to be elucidated if other external stimuli can modulate APP and Aβ internalization into exosomes or the effects of Aβ per se on exosome secretion. Neurons and glia co-cultures exposed to Aβ1-42 protfibilfs did not lead to variations in exosomes and large vesicles secretion, nonetheless alterations could be found in the protein content, since increased vesicular levels of ApoE were observed after exposure to Aβ1-42 (Nikitidou et al., 2017). The following figure 2 summarizes the exosomal content, in AD-related proteins and cleaved products, found in neurons and other neuronal-derived cells, astrocytes and microglia, also considering the information discussed in subsequent sections.

3 | EXOSOMES PROTECTIVE ROLE AND THERAPEUTIC POTENTIAL IN AD

Despite the harmful effects of exosomes in the spread of amylo- genic molecules some beneficial roles have also been attributed to exosomes in AD. Neuronal exosomes containing Aβ can be uptaken by microglia, thus contributing to peptide clearance from the extracellular space and, in effect, reducing Aβ pathology (Yuyama, Sun, Mitsutake, & Igarashi, 2012; Yuyama et al., 2014).

Furthermore, exosome infusions could sequester Aβ peptides in a process mediated by exosome surface proteins, including the cellular prion protein. These infusions abrogated the synaptic plasticity dysfunction, typically induced by Aβ (An et al., 2013). Additionally, intracerebral infusion with neuronal exosomes or exosomes isolated from hypoxic mesenchymal stromal cells into APP transgenic mice was reported to decrease Aβ accumulation and amyloid deposits (Cui et al., 2017; Yuyama et al., 2015). The administration of these exosomes reduced inflammatory cytokines levels and resulted in improvement of memory and learning capacities in APP/PS1 transgenic mice (Cui et al., 2017).

Exosomes isolated from human umbilical cord mesenchymal stem cells (huc-MSC) and injected into AβPP/PS1 double transgenic mice model for AD, exhibit an interesting therapeutic potential. The injections lead to improved cognitive functions, such as spatial learning and memory function, lower number of SPs in the cortex and hippocampus and up-regulated levels of neprilysin and
Insulin-degrading enzyme (IDE); both are Aβ peptide-degrading enzymes. The injection of huc-MSC exosomes also attenuated neuroinflammation, by decreasing the levels of pro-inflammatory cytokines and up-regulating anti-inflammatory cytokines, nonetheless the mechanism at the basis of this inflammatory response remains unknown (Ding et al., 2018). Interestingly, administration of exosomes secreted from MSC cells to an AD mouse model promoted neurogenesis in the subventricular zone and improved cognitive function (Reza-Zaldivar et al., 2019). Exosomes isolated from adipose-derived stem cells can also play an AD protective role and constitute a novel therapeutic strategy, by reducing Aβ levels, Aβ42/1-40 ratio, as well as apoptosis of neuronal cells mimicking AD, besides increasing neurite outgrowth (Lee, Ban, Yang, Im, & Kim, 2018).

As mentioned, Aβ degrading enzymes like neprilysin and IDE, can be found in exosomes, consistent with the beneficial role of these nanovesicles in AD (Bulloj, Leal, Xu, Castaño, & Morelli, 2010; Ding et al., 2018). Neprilysin is carried by exosomes and its internalization leads to reduction in extracellular and intracellular Aβ levels (Ding et al., 2018; Katsuda, Oki, & Ochiya, 2015; Katsuda et al., 2013). Furthermore, treatment of BV-2 cells with statins enhanced exosome secretion carrying IDE, contributing to Aβ clearance, although the underlying mechanisms remain unclear (Tamboli et al., 2010). Endothelin-converting enzymes 1/2 and metalloproteinases, are also capable of degrading Aβ, and are likewise found into exosomes (Pacheco-Quinto et al., 2018).

Another protein released in association with exosomes which is imbalanced in AD is cystatin C, a cysteine protease inhibitor. Primary cortical neurons, over-expressing the familial AD PS2 mutations, exhibited reduced levels of several exosomal cystatin forms and also of Aβ40 concentrations. Cystatin can potentially exert a trophic role in the brain and thus have a beneficial action against AD, therefore modulation of cystatin levels could be of therapeutic interest (Ghidoni et al., 2011).

The promising therapeutic potential of exosomes in AD is enhanced by the fact that exosomes can cross the blood–brain barrier (Alvarez-Erviti et al., 2011; Haney et al., 2015; Yang et al., 2015). Therefore, exosomes can be a suitable tool for gene therapy because of their capacity to deliver nucleic acids with good specificity (Alvarez-Erviti et al., 2011). For instance, exosome-mediated delivery of short interfering RNA to knock down BACE1 enzyme, which cleaves APP to produce Aβ, is one of the promising approaches in this area. On the other hand, since neuronal exosomes carry Aβ and contribute to amyloidogenesis, it could be likewise a beneficial strategy to reduce exosome secretion or internalization by target cells. Exosome uptake is an active process, regulated by dynamin that can be easily blocked (Sardar Sinha et al., 2018). Furthermore, inhibition of neutral sphingomyelinase 2, the enzyme responsible for ceramide generation, decreased the number of secreted brain- and serum-derived exosomes, SPs formation and neuronal apoptosis in mice (Dinkins, Dasgupta, Wang, Zhu, & Bieberich, 2014; Dinkins et al., 2016). Nonetheless, and as already mentioned, these nanovesicles also appear to have a beneficial role in AD, hence abolish exosomes secretion as a therapeutic approach should be cautiously addressed.

Particular noteworthy, exosomes present potential as drug delivery vehicles in AD. To our knowledge, curcumin-primed exosomes was the first attempt to target these nanovesicles as disease therapeutic vehicles. Curcumin-containing exosomes, administered to an AD mouse model treated with okadaic acid (a protein phosphatase inhibitor) abrogated Tau hyperphosphorylation, resulting in beneficial cognitive effects. This Tau phosphorylation inhibitory effect was because of AKT activation and GSK-3β inhibition; a major kinase involved in the formation of paired helical filaments; well-known components of the neurofibrillary tangles (Wang et al., 2019). Although much is still to be done in the area, evidence supports that exosomes hold true AD therapeutic potential.

4 | EXOSOMES AS BIOMARKER RESOURCES IN AD

The diagnostic potential of exosome lies in the fact that these nanovesicles can harbour constituents of their cell of origin like proteins, nucleic acids and lipids; acting as a fingerprint of the releasing cell type and reflecting either the physiological or pathological status of the latter (Colombo, Raposo, & Théry, 2014). In addition, these nanovesicles can be found in various body fluids particularly blood, cerebrospinal fluid, saliva, urine, ascites fluid and breast milk (Armstrong & Wildman, 2018). Hence, these exosomes have emerged as important peripheral biomarker resources in the study of several complex diseases, as is the case for AD. The protein and miRNA contents relevant to AD found in these EVs is discussed bellow.

4.1 | Aβ and Tau as potential exosomal biomarkers

Exosomes are an easily accessible source of biomarkers in AD since these can be found and isolated from distinct body fluids such as serum, plasma and the less accessible CSF (Lee et al., 2019; Pluta, Ulamek-Kozioł, Januszewski, & Czuczwiar, 2018; Soares Martins, Catita, Rosa, da Cruz e Silva, & Henriques, 2018). Thus far several putative protein exosomal biomarker candidates relevant to AD diagnosis have been described and these are summarized in Table 1. Among which are key candidates linked to AD, already used as the gold standards, namely the biomarker triplet in CSF-based neurochemical-diagnosis that includes Aβ42/1-40 ratio, T-Tau and p-Tau 181 (Blennow & Zetterberg, 2009; Welge et al., 2009). These candidates have been mainly tested from distinct exosome sources, the neuronacl derived exosomes (NDEs) and the astrocyte-derived exosomes (ADEs). Despite some inconsistencies in the exosomal Aβ levels in AD among different reports (Table 1), elevated levels of plasma or serum NDEs Aβ1-42 but also of p-Tau 181 and p-Tau 396 were observed in preclinical individuals up to 10 years before AD diagnosis. Levels of these three molecules could predict AD development, before clinical diagnosis, with a sensitivity of 96%. Moreover, Aβ1-42 levels progressively increased from asymptomatic stage to AD diagnosis, supporting its use as a disease progression biomarker.
| Related processes       | Putative biomarker | Change in AD | Exosome enrichment | References                                                                 |
|-------------------------|--------------------|--------------|--------------------|----------------------------------------------------------------------------|
| APP Processing          | Aβ1-42             | ↑            | Neuronal           | Fiandaca et al. (2015); Winston et al. (2016); Goetzl, Mustapic, et al. (2016); Jia et al. (2019) |
|                         |                    | ns           |                    | Kapogiannis et al. (2019)                                                   |
|                         | sAPPβ              | ↓            | Astrocyte          | Goetzl, Mustapic, et al. (2016)                                             |
|                         | sAPPα              | ↑            | Neuronal and astrocyte |                                            |
| BACE-1                  | ns                 |              | Neuronal           | Goetzl, Mustapic, et al. (2016)                                             |
| γ-secretase             | ↑                  |              | Astrocyte          | Goetzl, Mustapic, et al. (2016)                                             |
| Microtubule-associated proteins | T-Tau            | ↑            | Neuronal           | Fiandaca et al., 2015; Jia et al. (2019)                                    |
|                         | p-Tau 396          | ↑            | Neuronal           | Fiandaca et al. (2015); Winston et al. (2016); Goetzl, Mustapic, et al. (2016) |
|                         | p-Tau 181          | ↑            | Neuronal           | Fiandaca et al. (2015); Winston et al. (2016); Goetzl, Mustapic, et al. (2016); Kapogiannis et al. (2019); Jia et al. (2019) |
|                         | p-Tau 231          | ↑            | Astrocyte          | Goetzl, Mustapic, et al. (2016)                                             |
| Synaptic plasticity    | Septin−8           | ns           | Neuronal           | Goetzl, Mustapic, et al. (2016)                                             |
|                         |                    |              | Astrocyte          | Goetzl, Schwartz, et al. (2018); Winston et al. (2019)                      |
| Complement effector proteins | C1q, C3b, C3d, C4b, C5b*, C5b-C9 TCC, Factor B, Factor Bb, Factor D | ↑       | Astrocyte          | Goetzl, Schwartz, et al. (2018); Winston et al. (2019)                      |
| Complement regulatory proteins | CD46, CD59, CR1, DAF | ↓       | Astrocyte          | Goetzl, Schwartz, et al. (2018); Winston et al. (2019)                      |
| Inflammation           | IL−1β, IL−6, TNF−α | ↑            | Astrocyte          | Goetzl, Schwartz, et al. (2018)                                             |
| Synaptic transmission  | Synapsin−1, P-S9, Synapsin−1, Synaptophysin, Synaptotagmin-2 | ↓       | Neuronal           | Goetzl, Kapogiannis, et al. (2016)                                          |
|                         | AMPA4, NLGN1, NPTX2, NRXN2α | ↓       | Neuronal           | Goetzl, Abner, et al. (2018)                                                |
|                         | NRGN               | ↓            |                    | Winston et al. (2016); Goetzl, Kapogiannis, et al. (2016)                   |
|                         | SNAP−25            | ↓            |                    | Agliardi et al. (2019)                                                      |
| Synaptic structure     | Synaptopodin       | ↓            | Neuronal           | Goetzl, Kapogiannis, et al. (2016)                                          |
| Growth Factors         | GAP43              | ↓            | Neuronal           | Goetzl, Kapogiannis, et al. (2016)                                          |
|                         | FGF2, FGF13, HGF, IGF1 | ↓       | CSPG4Es            | Goetzl et al. (2019)                                                        |
|                         | GDNF               | ns           | Neuronal           | Goetzl, Mustapic, et al. (2016)                                             |
|                         |                    |              | Astrocyte          | Goetzl et al. (2015a)                                                       |
| Transcription factors  | HSF1, LRP6         | ↓            | Neuronal           | Goetzl et al. (2015a); Winston et al. (2016)                               |
| Protein Folding        | HSP70              | ↓            | Neuronal           | Goetzl et al. (2015b)                                                       |
| Lysosomal              | Cathepsin D, LAMP−1 | ↑            | Neuronal           | Goetzl et al. (2015b)                                                       |
| Protein Degradation    | Ubiquitin          | ↑            | Neuronal           | Goetzl et al. (2015b)                                                       |

(Continues)
(Fiandaca et al., 2015). Aβ1-42, p-Tau 181 and p-Tau 396 were also found increased in neuronal exosomes derived from plasma of AD patients (Goetzl, Mustapic, et al., 2016) and in neuronal exosomes from individuals with Down Syndrome, which have higher risk of developing AD (Hamlett et al., 2017). The conversion of mild cognitive impairment (MCI) to AD and the discrimination between AD cases and Controls could be achieved with high sensitivity, by combining neuronal exosomal biomarkers exhibited similar diagnostic power to diagnostic power than the individual biomarkers, and the exosomes and in CSF, but it would be relevant to determine the exosomal Aβ1-42/1-40 ratio, as this could provide additional information on the blood-exosomal Aβ rates and potentially constitute a more reliable diagnostic tool.

Another recent study validated blood-neuronally derived exosomes as a source of preclinical AD biomarkers. Longitudinally, the participants that developed AD presented higher exosomal levels of p-Tau 181, p-Tau 231, pSer312-1-42, pY-1-42-1-40 ratio, as this could provide additional information on the blood-exosomal Aβ1-42/1-40 ratio. Since astrocytes contain APP-derived metabolites and some APP processing enzymes, focus has also been given to this ADEs and NDEs cargo. Comparatively to NDEs, ADEs are present at lower levels in plasma, although they can carry higher amounts of the APP processing enzymes (BACE-1, γ-secretase), APP proteolytic fragments such Aβ1-42, sAPPβ and sAPPα, Tau phosphorylated forms (p-Tau 181 and p-Tau 396) and the glial-derived neurotrophic factor (GDNF). In AD, astrocyte-derived exosomal levels of Aβ1-42, sAPPβ, BACE-1, septin-8 and GDNF permitted distinguishing cases from Controls individuals. As referred plasma NDEs presented significantly different levels of Aβ1-42 and Tau phosphorylated forms but also of both sAPPα/β forms. Of note, Aβ1-42 levels exhibited opposite patterns in plasma NDEs versus ADEs (Goetzl, Mustapic, et al., 2016). It was proposed that increased NDEs Aβ levels in AD may reflect the neuronal attempt to eliminate pathogenic material during the neurodegenerative process (Winston et al., 2016). Nonetheless, since data clearly show that different results can arise from different sources of blood-derived exosomes, it would also be relevant the test and compare the data obtained for this triplet in blood-derived exosomes, with no cell-enrichment.

| Related processes | Putative biomarker | Change in AD | Exosome enrichment | References |
|-------------------|-------------------|-------------|-------------------|------------|
| Insulin Signalling | Total IRS, p-pan-Tyr-IRS−1 | ↓ | Neuronal | Kapogiannis et al. (2015) |
|                   | p-Ser312-1-42 | ↑ | | Kapogiannis et al. (2015); Kapogiannis et al. (2019) |
|                   | pY-IRS−1 | ↑ | | Kapogiannis et al. (2019) |

Note: In the results summarized in Table 1, exosomes were isolated using ExoQuick ™ (System Biosciences) and a plasma/serum starting volume of 250 or 500 µl, depending on the study. Most of the studies used ELISA except (Kapogiannis et al., 2019), that employed MesoScale Discovery platforms to quantify p-Tau181, p-Tau231, pSer312-1-42, pY-1-42-1-40 and TSG101 and Single Molecule Array technology to measure Aβ1-42 levels. Only measured in Winston et al., 2019.

Abbreviations: AD—Alzheimer’s disease; AMPA4—GluA4-Containing glutamate receptors; APP—Amyloid precursor protein; Aβ—Amyloid β; BACE-1—β-site APP cleaving enzyme 1; C5b-C9 TCC—C5b-C9 terminal complement complex; CR1—Complement receptor type 1; CSGP4Es—Chondroitin sulphate Proteoglycan (CSPG) 4 Type Neural precursor cells-derived exosomes; DAF—Complement decay-accelerating factor; FGF—fibroblast growth factor; GAP43—Growth-Associated Protein 43; GDNF—Glial-derived neurotrophic factor; HGF—hepatocyte growth factor; HSF1—Heat-Shock Factor-1; HSP70—Heat-Shock Protein 70; IGF1—Insulin-like growth factor 1; IL—Interleukin; IRS—Insulin Receptor Substrate; LAMP-1—lysosome-associated membrane protein 1; LRP6—Low-Density Lipoprotein Receptor-Related Protein 6; NLGN1—Neuroligin 1; NPTX2—Neuronal pentraxin 2; NRGN—Neurogranin; NRXN2α—Neurexin 2α; p-pan-Tyr IRS−1—Phospho-Tyrosine Type 1 Insulin Receptor Substrate; P-S9-Synapsin-1—phosphorylated synapsin-1; p-Ser312 IRS−1—Phospho-Serine 312 Type 1 Insulin Receptor Substrate; p-Tau—phosphorylated Tau; pY-1-42—phosphorylated tyrosine residues in IRS-1; REST—Repressor Element 1-Silencing Transcription Factor; sAPP—Soluble amyloid precursor protein; SNAP-25—Synaptosomal nerve-associated protein 25; TNF-α—Tumour Necrosis Factor α; T-Tau—Total Tau. ns—No significant change.
The combination of this non-specific exosomal enrichment with Fourier-Transformed Infrared Spectroscopy analysis of Controls versus ADs spectra, was recently shown to have potential disease discriminatory value (Soares Martins et al., 2020).

Furthermore, complementary biomarker studies should also consider novel analytical EVs platforms that can measure exosomes-bound and unbound Aβ1-42 levels as well as their potential contribution to AD prediction. As already mentioned, recent findings using APEX showed that plasma exosomes bind preferentially to Aβ pre-fibrillar forms and that Aβ1-42 is strongly co-localized with several neuronal markers, supporting that the major source of exosomes in human plasma is of neuronal origin. The exosome-bound Aβ1-42 strongly correlated with brain plaque loads, even better than PET brain scan imaging (Lim et al., 2019). The highest correlation was achieved between the exosome Aβ binding and the brain plaque load at the cingulate region where Aβ is deposited in early AD stages. In addition, the levels of Aβ1-42 bound to exosomes had differential diagnostic relevance, by distinguishing AD cases from MCI, no cognitive impairment, vascular dementia, vascular mild cognitive impairment and acute stoke (Lim et al., 2019).

4.2 | Other AD relevant exosomal protein biomarker candidates

As referred above, chronic inflammation is a key event in AD (Domingues et al., 2017). Exosomes spreading may deliver severe inflammatory mediators to various central nervous system cells and contribute to the neuronal damage, typical of the disease late inflammatory phase (Goetzl, Schwartz, Abner, Jicha, & Kapogiannis, 2018). High levels of several complement effector proteins, and inflammatory mediators, were detected in astrocyte-derived exosomes of AD patients versus Controls (Goetzl, Schwartz, et al., 2018) and in mild cognitively impaired individuals that converted to dementia within 3 years (MCIC) versus mild cognitive cases without conversion (MCIS), whereas complement regulatory proteins were decreased in MCIC versus MCIS (Winston, Goetzl, Schwartz, Elahi, & Rissman, 2019).

Other promising exosomal biomarker candidates for AD are synaptic proteins. In AD brains there is a decrease in several synaptic proteins that significantly correlates with progressive cognitive decline. Interestingly, besides NRGN, the levels of synapsin 1, synaptophysin, synaptotagmin-2, synaptotodin and growth-associated protein 43 (GAP43) were all significantly reduced in plasma NDE from the AD cases (Goetzl, Kapogiannis, et al., 2016). In addition, the pyramidal neuron excitability is altered in AD, which is reflected in decreased levels of neuronal pentraxin 2 complexes (NPTX2) and GluA4-containing glutamate (AMPA4) receptors, not only in brain tissues but also in NDEs. Post-synaptic adhesion protein neurelin 1 (NRG1) and pre-synaptic neurexin 2α (NRXN2α), two synaptic proteins that interact with each other to provide structural stability to excitatory synapses, were also diminished in plasma NDE of AD cases when compared with Controls. These two synaptic proteins and AMPA4, involved in synaptic transmission, exhibited decreased NDE levels at the AD preclinical stage. In this longitudinal study, the levels of NLGN1, NRXN2α, AMPA4 and NPTX2 were likewise significantly decreased in mild to moderate stages, reflecting the typical AD cognitive loss (Goetzl, Abner, Jicha, Kapogiannis, & Schwartz, 2018).

Neuronal Pentraxin 1 (NPTX1) is another pre-synaptic protein mainly expressed in excitatory neurons and whose levels are elevated in the brains of APP/PS1 mice, sporadic late-onset AD individuals and, more recently, reported to be altered in plasma of MCI individuals and early stage AD, particularly in ApoE4 carriers. The levels of NPTX1 increase in plasma and plasma-derived exosomes of E4FAD mice (ApoE4+/−/FAD+/−), reinforcing the role of exosomes as a vehicle to transport NPTX1 from the brain to the plasma. Considering NPTX1 nature, it may represent a putative biomarker of synaptic excitatory response in AD (Ma et al., 2018). Furthermore, the serum NDE levels of the pre-synaptic protein SNAP-25 also show discriminatory power between AD and healthy Controls. The exosomal levels of SNAP-25 were significantly decreased in the AD group, with a sensitivity and specificity over 70%, supporting its value as a relevant synaptic degeneration biomarker (Agliardi et al., 2019).

In addition to NDE, it is expected that exosomes derived from other central nervous system cells exhibit distinct neuronal signatures. In fact, exosomes isolated from plasma of AD individuals and derived from CSPG4 cells, which are neural precursor cells, carry lower levels of four neurotropic growth factors when compared with exosomes derived from Control individuals: FGF2, FGF13, HGF and IGF1. These four growth factors remain decreased in AD preclinical stages when compared with Controls while no differences were found in the following 3–8 years. Changes in the levels of growth factors involved in neuronal repair enhance the importance of developing new therapeutic strategies aimed at compensating these changes (Goetzl et al., 2019).

AD neurotoxicity is typically associated with deficiency of survival factors in diseased brains of the affected individuals. Consistently, the levels of low-density lipoprotein receptor-related protein 6 (LRP6), heat-shock factor-1 (HSF1) and repressor element 1-silencing transcription factor (REST) were decreased in neuronal-plasma exosomes when compared with Control individuals (Goetzl et al., 2015a). LRP6 can modulate the Wnt signalling, a relevant pathway in AD neuropathology (da Cruz e Silva, Henriques, Domingues, & da Cruz e Silva, 2010).

Lysosomal dysfunction is another AD feature related to APP impaired metabolism that probably precedes AD pathogenesis (Nixon, 2017). Changes in lysosomal proteins were detected in plasma-derived exosomes, including high levels of cathepsin D, lysosome-associated membrane protein 1 (LAMP-1) and ubiquitinylated proteins; whereas lower levels were detected for the heat-shock protein 70 (Hsp70). These distinct protein alterations were observed 10 years before AD diagnosis and all, except Hsp70, presented discriminatory power to distinguish AD from frontotemporal dementia individuals (Goetzl et al., 2015b).

The neurovascular dysfunction in AD can also be reflected in EVs. Comparison of the protein content from hypoperfused mice
brain-derived EVs versus human brain-derived EVs from post-mortem individuals with early AD and other dementias, revealed an overlap of proteins implicated in angiogenesis, hypoxia, protein quality control and vesicle sorting (Gallart–Palau et al., 2019). Future studies may strengthen the use of EVs as peripheral tools to monitor the neurovascular dysfunction associated with dementia.

NDEs from AD patients also presented distinct phosphorylation patterns of the adaptor insulin receptor substrate 1 (IRS-1), even though lower IRS levels were reported. Specifically, higher levels of pSer312-IRS-1, lower p-panTyr-IRS-1 and a higher ratio of pSer312-IRS-1 to p-panTyr-IRS-1 were detected in cases, supporting their potential as AD exosomal phosphospecific biomarkers (Kapogiannis et al., 2015). This is not surprising since protein phosphorylation is a key event in AD and even Aβ can lead to alterations in the neuronal phosphoproteome (Henriques et al., 2016). Brain tissues of AD individuals typically exhibit altered phosphorylation patterns of IRS-1 and IRS-2, mimicking insulin resistance (Kapogiannis et al., 2015) and, more importantly, exosomal pSer312-IRS-1 and p-panTyr-IRS-1 blood levels correlate not only with brain molecules composition but also with regional atrophy in AD brains (Mullins, Mustapic, Goetzl, & Kapogiannis, 2017). AD has been closely linked to type 2 Diabetes (Chatterjee & Mudher, 2018). Of note, the phosphorylation patterns of pY-IRS-1 and pSer312-IRS-1 in neuronal-derived plasma EVs were correlated with changes in cognitive performance supporting the usefulness of EVs in monitoring therapeutic responses (Mustapic, Tran, Craft, & Kapogiannis, 2019).

In addition to all these proteins, some astrocyte and neuronal-related exosomal markers, were also found changed in AD cases. The levels of glial fibrillary acidic protein (GFAP), neurofilament light chain and neuron-specific enolase were significantly increased in neuronal-derived plasma EVs compared with Controls, whereas CD81 was found decreased. However, other neuronal-derived exosomes (NDEs) from AD cases comparatively show different miRNA profiles in AD and the detailed information is summarized in Table 2. Noticeably, most of the serum-derived exosomal miRNAs increase in AD, whereas miRNAs isolated from plasma-derived exosomes from disease patients are mainly decreased. Of note, the majority of the miRNAs found altered in AD, target genes related to APP, APP-related processing proteins, and even to Tau processing or phosphorylation, like the kinase GSK3β (Table 2). Besides targeting APP-, Aβ- and Tau-related genes, several miRNAs can also target genes related to oxidative phosphorylation and mitochondrial dysfunction. Nonetheless, the relation between these exosomal miRNAs and their gene targets in AD is poorly explored.

Overlap of the data obtained from distinct body fluids reveals two common exosomal miRNA: the miR-193b and the miR-125b-5p (Figure 3).

It would be expected that these two microRNAs constitute putative peripheral biomarker candidates worthy of further studies. Nonetheless, one must register that the miR-125b-5p presents inconsistent expression patterns across distinct biofluids (Barbagallo et al., 2019; Lugli et al., 2015; McKeever et al., 2018). While miR-125b-5p is increased in serum- and CSF-derived exosomes of AD cases, from distinct biofluids, including serum, plasma and CSF, were collected and the common candidates identified. Data clearly show different miRNA profiles in AD and the detailed information is summarized in Table 2. Noticeably, most of the serum-derived exosomal miRNAs increase in AD, whereas miRNAs isolated from plasma-derived exosomes from disease patients are mainly decreased. Of note, the majority of the miRNAs found altered in AD, target genes related to APP, APP-related processing proteins, and even to Tau processing or phosphorylation, like the kinase GSK3β (Table 2). Besides targeting APP-, Aβ- and Tau-related genes, several miRNAs can also target genes related to oxidative phosphorylation and mitochondrial dysfunction. Nonetheless, the relation between these exosomal miRNAs and their gene targets in AD is poorly explored.

Overlap of the data obtained from distinct body fluids reveals two common exosomal miRNA: the miR-193b and the miR-125b-5p (Figure 3).

It would be expected that these two microRNAs constitute putative peripheral biomarker candidates worthy of further studies. Nonetheless, one must register that the miR-125b-5p presents inconsistent expression patterns across distinct biofluids (Barbagallo et al., 2019; Lugli et al., 2015; McKeever et al., 2018). While miR-125b-5p is increased in serum- and CSF-derived exosomes of AD cases.

**FIGURE 3** miRNAs differentially expressed in exosomes isolated from serum, plasma and CSF of AD cases. The miR-193b is indicated in bold and underlined as it is found in the exosomes of the three biofluids with similar expression patterns. The miR-342-3p is common to exosomes from the serum and plasma and exhibited in both biofluids similar expression patterns. The miR-125b-5p was found in exosomes from both plasma and CSF although it has different expression patterns; it is increased in CSF-derived exosomes and decreased in plasma-derived exosomes. Green – Increased expression; Red – Decreased expression; # – Opposite expression patterns in biofluids; Bold and italics – exosomal miRNA common to the three biofluids with similar expression patterns; Bold and underlined – exosomal miRNA common to the three biofluids with similar expression patterns. Abbreviations: CSF – Cerebrospinal fluid.
| Biofluid | Change in AD | miRNA     | APP-related | Tau-related | References       |
|----------|--------------|-----------|-------------|-------------|-----------------|
| Serum    | ↑            | miR-15a−5p| ADAM10; APH1A; APP; BACE1; MME | CDK5R1      | Cheng et al. (2015) |
|          | ↑            | miR-18b−5p| APH1B; GAPDH | –           | Cheng et al. (2015) |
|          | ↑            | miR-20a−5p| APP         | –           | Cheng et al. (2015) |
|          | ↑            | miR-22−5p (miR-22) | ADAM10; LPL | CDK5R1; CASP3 | Barbagallo et al. (2019) |
|          | ↑            | miR-23a−3p (miR-23a) | ADAM10; PSEN1; NCSTN; APH1A | CASP3; CASP7 | Barbagallo et al. (2019) |
|          | ↑            | miR-29a−3p (miR-29a) | APH1A; GAPDH; BACE1; BACE2; LPL | GSK3β; CASP7 | Barbagallo et al. (2019) |
|          | ↑            | miR-30a−5p | APP; NAE1   | CASP3       | Cheng et al. (2015) |
|          | ↑            | miR-93−5p | APP; GAPDH; LRP1 | GSK3β       | Cheng et al. (2015) |
|          | ↑            | miR-101−3p | ADAM10; APP; PSEN1 | CAPN2; CASP3; GSK3β | Cheng et al. (2015) |
|          | ↑            | miR-106a−5p | APP; ADAM17 | CAPN2       | Cheng et al. (2015) |
|          | ↑            | miR-106b−5p | APP         | CASP7       | Cheng et al. (2015) |
|          | ↑            | miR-125b−5p (miR-125b) | ADAM10; APH1A; GAPDH; LRP1 | –           | Barbagallo et al. (2019) |
|          | ↑            | miR-135a  | nf          | nf          | Yang, Liu, Gao, Zhang, and Wang (2018) |
|          | ↑            | miR-143−3p | ADAM10; LRP1 | –           | Cheng et al. (2015) |
|          | ↑            | miR-335−5p | APBB1; LRP1 | CASP7       | Cheng et al. (2015) |
|          | ↑            | miR-361−5p | ADAM10; APH1B; LRP1 | –           | Cheng et al. (2015) |
|          | ↑            | miR-384   | nf          | nf          | Yang et al. (2018) |
|          | ↑            | miR-424−5p | APBB1; APH1A; APP; LRP1 | CDK5R1; GSK3β | Cheng et al. (2015) |
|          | ↑            | miR-582−5p | IDE         | –           | Cheng et al. (2015) |
| Plasma   | ↓            | miR-15b−3p | nf          | nf          | Cheng et al. (2015) |
| Plasma   | ↓            | miR-193b  | nf          | nf          | Yang et al. (2018); Liu et al. (2014) |
| Plasma   | ↓            | miR-223   | nf          | nf          | Wei et al. (2018) |
| Plasma   | ↓            | miR-342−3p | APH1B; MME; LPL | GSK3β      | Cheng et al. (2015) |
| Plasma   | ↓            | miR-1306−5p | nf          | nf          | Cheng et al. (2015) |
| Plasma   | ↓            | miR-548at−5p | nf          | nf          | Lugli et al. (2015) |
| Plasma   | ↓            | miR-21−5p | GAPDH; NCSTN; APH1A | CAPN2       | Gáméz-Valero et al. (2019) |
| Plasma   | ↓            | miR-23a−3p (miR-23a) | ADAM10; PSEN1; NCSTN; APH1A | CASP3; CASP7 | Gáméz-Valero et al. (2019) |
| Plasma   | ↓            | miR-23b−3p | ADAM10; APH1A; GAPDH; NCSTN; PSEN1 | CASP3; CASP7 | Lugli et al. (2015) |
| Plasma   | ↓            | miR-24−3p | APH1A; NCSTN | CAPN1; GSK3β | Lugli et al. (2015) |
| Plasma   | ↓            | miR-29b−3p | BACE1; GAPDH; LPL | CASP7; GSK3β | Lugli et al. (2015) |
| Plasma   | ↓            | miR-125b−5p (miR-125b) | APP         | –           | Lugli et al. (2015) |
| Plasma   | ↓            | miR-126−3p | –           | CAPN1       | Gáméz-Valero et al. (2019) |
| Plasma   | ↓            | miR-132−3p | APH1A       | CAPN2; CASP7; GSK3β | Cha et al. (2019) |
| Plasma   | ↓            | miR-139−5p | nf          | nf          | Lugli et al. (2015) |
| Plasma   | ↓            | miR-141−3p | APH1B; GAPDH; PSEN1 | –           | Lugli et al. (2015) |

(Continues)
cases, it is decreased in plasma-derived exosomes (Table 2 and Figure 3). The inconsistency of miRNA pattern between biofluids is evident, but the distinct patterns are hard to explain. Pre-analytical and analytical differences cannot be ruled out as possible sources of variation (Saliminejad, Khorram, Ghaffari, 2019). These may arise because of distinct sample collection and storage procedures, different methodologies for exosome isolation and the employ of distinct RNA extraction and miRNA expression approaches. In addition, free circulating miRNA in blood stream can be originated from platelets and blood cells (Wang, Yuan, et al., 2012) and constitute important contaminants in exosomal
preparations. The lack of standardized protocols to miRNA analysis is an important limitation, particularly in the case of CSF that render in lower RNA yields than blood (Kopkova et al., 2018).

Consistently, however, were the observations found for miR-193b. This miRNA was previously reported to be decreased in the hippocampus of APP/PS-1 transgenic mice and in the CSF and serum of the same mice (Liu, Song, Zhang, & Wang, 2014). Noticeably, the exosomal levels of miR-193b and the levels of Aβ1-42 were found negatively correlated in the CSF of AD cases. In the same study, a bioinformatic analysis was carried out and revealed that miR-193b can target the 3′-untranslated region (UTR) of APP (Liu et al., 2014). As such, since miR-193b can regulate APP levels, its decrease in AD may correlate with increased APP expression and augmented Aβ production. Hence, this miRNA is highly relevant not only as a diagnostic biomarker but also as a potential therapeutic avenue.

Another miRNA potentially interesting as an AD target or blood-derived diagnostic tool is the miR-342-3p. This miRNA was significantly decreased in both serum and plasma-derived exosomes from AD cases (Cheng et al., 2015; Lugli et al., 2015) and its serum levels also correlated with Mini-Mental State Examination score (Tan et al., 2014). Furthermore, miR-342-3p is highly expressed in the brain of AD patients and its inhibition improved learning, memory capacities, probably by reducing Aβ species production through the modulation of the APP processing in an transgenic AD mouse model (Fu et al., 2019). Additionally, this miRNA directly targets 3′-UTR of PP2A, modulating Tau phosphorylation (Wang, Min, et al., 2017). The miR-342-3p can also target membrane metalloendopeptidase and lipoprotein lipase genes that encode proteins related to Aβ degradation and aggregation respectively. It would be relevant to determine the levels of miR-342-3p in CSF and CSF-derived exosomes of AD patients and non-demented Controls, to check if the profile is similar to the blood-derived biofluids, serum and plasma.

Likewise, the miR-451a was found decreased in plasma- and CSF-derived exosomes of AD cases when compared to Controls (Gámez-Valero et al., 2019; McKeever et al., 2018). This miRNA levels were decreased in the hippocampus, temporal cortex and CSF of AD individuals (Cogswell et al., 2008; McKeever et al., 2018; Takousis et al., 2019; Villela et al., 2016). However, perhaps because of the presence of small amounts of this miRNA in neuronal exosomes, the significant differences between disease and Control groups were only observed when using non-neuronal enriched plasma exosomes (Cha et al., 2019; Gámez-Valero et al., 2019). The levels of miR-451a in plasma-derived exosomes had a discriminatory value to distinguish AD from Controls and AD from Lewy bodies dementia (Gámez-Valero et al., 2019). In our perspective the above-mentioned three miRNAs can constitute interesting targets in AD deserving further investigation.

5 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Exosomes are involved in many physiological and pathological processes gaining increased relevance in neurodegenerative diseases. In AD, exosomes can contribute to disease progression through spreading and formation of SP and NFTs, as well as to other pathogenic events, like neuroinflammation. Nevertheless, exosomes can likewise exert beneficial effects in the context of AD. This is supported by exosomes ability to sequester Aβ and promote its clearance. Hence, these nanovesicles seem to have dual actions in AD, reinforcing the need for complementary studies to better clarify the underlying molecular mechanisms and exosomes exact roles in disease pathogenesis.

On the other hand, exosomes have gained increased attention in the biomarker discovery field. Preference would be given to methodologies less time-consuming and that render in higher exosomes yields, as the case of precipitation-based approaches, which represent an advantage in clinical settings. Optimization and standardization of these procedures would be fundamental to increase data reproducibility.

Several putative biomarker candidates, from changes in peptides and proteins directly linked to AD, to altered miRNA profiles, have arisen. Three distinct miRNAs found in peripheral exosomes are proposed as potential disease biomarker candidates. Although much still has to be done to validate the candidates, including multicentre studies, it is undeniable that exosomes hold promising potential not only in the diagnostic but also in the therapeutic field for AD and dementia.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Alzheimer’s Association (2019-AARG-644347), and also by PTDC/DTPPIC/5587/2014 and POCI-01-0145-FEDER-016904 and Instituto de Biomedicina (IBIMED)-UIDB/04501/2020, the Fundação para a Ciência e Tecnologia (FCT) of the Ministério da Educação e Ciência, COMPETE program, the QREN and the European Union (Fundo Europeu de Desenvolvimento Regional) and by the Integrated Programme of SR&TD “PAG” (CENTRO 2020 - CENTRO-01-0145-FEDER-000003), co-funded by Centro 2020 program, Portugal 2020, European Union, through the European Regional Development Fund. AGH is supported by the FCT stimulus of scientific employment, individual support (CEECIND/01803/2017). TSM is supported by the FCT through the individual PhD grant (SFRH/BD/145979/2019).

Figures 1, 3 and graphical abstract were produced using Servier Medical Art (http://smart.servier.com/). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Tânia Soares Martins https://orcid.org/0000-0003-3017-2092
Dário Trindade https://orcid.org/0000-0002-8536-4609
Margarida Vaz https://orcid.org/0000-0001-5425-1495
Odete A. B. da Cruz e Silva https://orcid.org/0000-0003-3718-9874
Ana Gabriela Henriques https://orcid.org/0000-0003-0851-6979
REFERENCES

Agliardi, C., Guerini, F. R., Zanottore, M., Bianchi, A., Nenmi, R., & Clerici, M. (2019). SNAP-25 in serum is carried by exosomes of neuronal origin and is a potential biomarker of Alzheimer’s disease. *Molecular Neurobiology*, 56, 5792–5798. https://doi.org/10.1007/s12035-019-1501-x.

Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., & Wood, M. J. A. (2011). Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnology*, 29, 341–345. https://doi.org/10.1038/nbt.1807.

An, K., Klyubin, I., Kim, Y., Jung, H. J., Mably, A. J., O’Dowd, S. T., ... Kim, J.-H. (2013). Exosomes neutralize synaptic plasticity-disrupting activity of Aβ assemblies in vivo. *Molecular Brain*, 6, 47. https://doi.org/10.1186/1756-6606-6-47.

Armstrong, D., & Wildman, D. E. (2018). Extracellular vesicles and activity of Aβ. *Frontiers in Neuroscience*, 12, 383. https://doi.org/10.3389/fnins.2018.00383.

Cheng, L., Doecke, J. D., Sharple, R. A., Villemagne, V. L., Fowler, C. J., Rembach, A., ... Hill, A. F. (2015). Prognostic serum miRNA biomarkers associated with Alzheimer’s disease shows concordance with neuropsychological and neuroimaging assessment. *Molecular Psychiatry*, 20, 1188–1196. https://doi.org/10.1038/mp.2014.127.

Chivet, M., Javale, C., Lauragnier, K., Blot, B., Hemming, F. J., & Sadoul, R. (2014). Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. *Journal of Extracellular Vesicles*, 3, 1. https://doi.org/10.3402/jev.v3.24722.

Cogswell, J. P., Ward, J., Taylor, I. A., Waters, M., Shi, Y., Cannon, B., ... Richards, C. A. (2008). Identification of miRNA changes in Alzheimer’s disease brain and CSF yields putative biomarkers and insights into disease pathways. *Journal of Alzheimer’s Disease*, 14, 27–41. https://doi.org/10.3233/JAD-2008-14103.

Colombo, M., Moita, C., van Niel, G., Kowal, J., Vigneron, J., Benaroch, P., ... Raposo, G. (2013). Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *Journal of Cell Science*, 126, 5553–5565. https://doi.org/10.1242/jcs.128868.

Colombo, M., Raposo, G., & Théry, C. (2014). Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annual Review of Cell and Developmental Biology*, 30, 255–289. https://doi.org/10.1146/annurev-cellbio-101512-122326.

Crotti, A., Sait, H. R., McAvoy, K. M., Estrada, K., Ergun, A., Szak, S., ... Ranshoff, R. M. (2019). BIN1 favors the spreading of Tau via extracellular vesicles. *Scientific Reports*, 9, 9477. https://doi.org/10.1038/s41598-019-45676-0.

Cui, G.-H., Wu, J., Mou, F.-F., Xie, W.-H., Wang, F.-B., Wang, Q.-L., ... Fang, J. (2017). Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice. *The FASEB Journal*, 32, 654–668. https://doi.org/10.1096/fj.201700600R.

da Cruz e Silva O. A. B., Henriques, A. G., Domingues, S. C., da Cruz e Silva, E. F. (2010). Wnt signalling is a relevant pathway contributing to amyloid beta-peptide-mediated neuropathology in Alzheimers disease. *CNS & Neurological Disorders – Drug Targets*, 9, 720–726.

da Cruz e Silva, O. A. B., Rebelo, S., Vieira, S. I., Gandy, S., da Cruz e Silva, F. E., & Greengard, P. (2009). Enhanced generation of Alzheimer’s amyloid-β following chronic exposure to phorbol ester correlates with differential effects on alpha and epsilon isoforms of protein kinase C. *Journal of Neurochemistry*, 108, 319–330. https://doi.org/10.1111/j.1471-4159.2008.05770.x.

da DeTure, M. A., & Dickson, D. W. (2019). The neuropathological diagnosis of Alzheimer’s disease. *Molecular Neurodegeneration*, 14, 32. https://doi.org/10.1186/s13024-019-0333-5.

Ding, M., Shen, Y., Wang, P., Xie, Z., Xu, S., Zhu, Z. Y., ... Yang, H. (2018). Exosomes isolated from human umbilical cord mesenchymal stem cells alleviate neuroinflammation and reduce amyloid-beta deposition by modulating microglial activation in alzheimer’s disease. *Neurochemical Research*, 43, 2165–2177. https://doi.org/10.1007/s11064-018-2641-5.

Dinkins, M. B., Dasgupta, S., Wang, G., Zhu, G., & Biberich, E. (2014). Exosome reduction in vivo is associated with lower amyloid plaque load in the 5xFAD mouse model of Alzheimer’s disease. *Neurobiology of Aging*, 35, 1792–1800. https://doi.org/10.1016/j.neurobiaging.2014.02.012.

Dinkins, M. B., Enasko, J., Hernandez, C., Wang, G., Kong, J., Helwa, I., ... Biberich, E. (2016). Neutral sphingomyelinase-2 deficiency in neurally derived plasma exosomes of Alzheimer’s patients. *Frontiers in Neuroscience*, 13, 1208. https://doi.org/10.3389/fnins.2019.01208.

Chatterjee, S., & Mudher, A. (2018). Alzheimer’s disease and Type 2 diabetes: A critical assessment of the shared pathological traits. *Frontiers in Neuroscience*, 12, 383. https://doi.org/10.3389/fnins.2018.00383.
stem cells secrete functional neuropilin-bound exosomes. *Scientific Reports*, 3, 1197.https://doi.org/10.1038/srep01197.

Koike, H., Tomioka, S., Sorimachi, H., Saito, T. C., Maruyama, K., Okuyama, A., ... Ishiura, S. (1999). Membrane-associated metallo-protease MDC9 has an alpha-secretase activity responsible for processing the amyloid precursor protein. *The Biochemical Journal*, 343(Pt 2), 371–375.

Konoshenko, M. Y., Lekchnov, E. A., Vlassov, A. V., & Laktionov, P. P. (2018). Isolation of extracellular vesicles: general methodologies and latest trends. *BioMed Research International*, 2018, 1–27. https://doi.org/10.1155/2018/8545347.

Kopkova, A., Sana, J., Fadrus, P., Machackova, T., Vecera, M., Vybihal, V., ... Slaby, O. (2018). MicroRNA isolation and quantification in cerebrospinal fluid: A comparative methodical study. *PloS One*, 13, e0208580. https://doi.org/10.1371/journal.pone.0208580.

Krämer-Albers, E. M., Bretz, N., Tenzer, S., Winterstein, C., Möbius, W., Berger, H., ... Trotter, J. (2007). Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? *Proteomics - Clin. Appl.*, 1, 1446–1461. https://doi.org/10.1002/prca.200700522.

Lachenal, G., Pernet-Gallay, K., Chivet, M., Hemming, F. J., Kelly, A., Bodon, G., ... Sadoul, R. (2011). Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. *Molecular and Cellular Neurosciences*, 46, 409–418. https://doi.org/10.1016/j.mcn.2010.11.004.

Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasonowski, M., ... Fahrenholz, F. (1999). Constitutive and regulated alpha-secretase cleavage of Alzheimer’s amyloid precursor protein by a disintegrin metalloprotease. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 3922–3927. https://doi.org/10.1073/pnas.96.7.3922.

Lee, M., Ban, J.-H., Yang, S., Im, W., & Kim, M. (2018). The exosome of adipose-derived stem cells reduces β-amyloid pathology and apoptosis of neuronal cells derived from the transgenic mouse model of Alzheimer’s disease. *Brain Research*, 1691, 87–93. https://doi.org/10.1016/j.brainres.2018.03.034.

Lee, S., Mankhong, S., & Kang, J.-H. (2019). Extracellular vesicle as a source of Alzheimer’s biomarkers: opportunities and challenges. *International Journal of Molecular Sciences*, 20, 1728.https://doi.org/10.3390/ijms20071728.

Li, P., Kaslan, M., Lee, S. H., Yao, J., & Gao, Z. (2017). Progress in exosome isolation techniques. *Theranostics*, 7, 789–804. https://doi.org/10.7150/thno.18133.

Lim, C. Z. J., Zhang, Y., Chen, Y., Zhao, H., Stephenson, M. C., Ho, N. R. Y., ... Shao, H. (2019). Subtyping of circulating exosome-bound amyloid β reflects brain plaque deposition. *Nature Communications*, 10, 1144.https://doi.org/10.1038/s41467-019-09030-2.

Liu, C.-G., Song, J., Zhang, Y.-Q., & Wang, P.-C. (2014). MicroRNA-193b is a regulator of amyloid precursor protein in the blood and cerebrospinal fluid derived exosomal microRNA-193b is a biomarker of Alzheimer’s disease. *Molecular Medicine Reports*, 10, 2395–2400. https://doi.org/10.3892/mmr.2014.2484.

Lugli, G., Cohen, A. M., Bennett, D. A., Shah, R. C., Fields, C. J., Hernandez, A. G., & Smalheiser, N. R. (2015). Plasma exosomal miRNAs in Persons with and without Alzheimer Disease: Altered expression and prospects for biomarkers. *PloS One*, 10, e0139233. https://doi.org/10.1371/journal.pone.0139233.

Ma, Q.-L., Teng, E., Zuo, X., Jones, M., Teter, B., Zhao, E. Y., ... Zhu, C. (2018). Neuronal pentraxin 1: A synaptic-derived plasma biomarker in Alzheimer’s disease. *Neurobiology of Disease*, 114, 120–128. https://doi.org/10.1016/j.nbd.2018.02.014.

McKeever, P. M., Schneider, R., Taghdidi, F., Weichert, A., Multani, N., Brown, R. A., ... Tartaglia, M. C. (2018). MicroRNA expression levels are altered in the cerebrospinal fluid of patients with young-onset Alzheimer’s disease. *Molecular Neurobiology*, 55, 8826–8841. https://doi.org/10.1007/s12035-018-1032-x.
Mullins, R. J., Mustapic, M., Goetzl, E. J., & Kapogiannis, D. (2017). Exosomal biomarkers of brain insulin resistance associated with regional atrophy in Alzheimer’s disease. Human Brain Mapping, 38, 1933–1940. https://doi.org/10.1002/hbm.23494

Mustapic, M., Tran, J., Craft, S., & Kapogiannis, D. (2019). Extracellular vesicle biomarkers track cognitive changes following intranasal insulin in Alzheimer’s disease. Journal of Alzheimer’s Disease, 69, 489–498. https://doi.org/10.3233/JAD-180578

Nicolae, J., Trinh, L., Rockenstein, E., Mante, M., Flurion, J., Trejo, M., ... Risman, R. A. (2017). Brain-derived exosomes from demen-
sity compromises brain exosome production. Current Alzheimer Research, 20, 275–292.

Rebelo, S., Vieira, S. I., Esselman, H., Wiltfang, J., da Cruz e Silva, E. F., & da Cruz e Silva, O. A. B. (2007). Tyr687 dependent APP endocytosis and Abeta production. Journal of Molecular Neuroscience, 32, 1–8. https://doi.org/10.1007/s12031-007-0001-z.

Reza-Zaldivar, E., Hernández-Sapiens, M., Gutiérrez-Mercado, Y., Sandoval-Avila, S., Gomez-Pinedo, U., Márquez-Aguirre, A., ... Canales-Aguirre, A. (2019). Mesenchymal stem cell-derived exosomes promote neurogenesis and cognitive function recovery in a mouse model of Alzheimer’s disease. Neurological Research, 41, 1626–1634.

Rosas-Hernández, H., Cuevas, E., Raymick, J. B., Robinson, B. L., Ali, S. F., Hanig, J., & Sarkar, S. (2019). Characterization of serum exosomes from a transgenic mouse model of Alzheimer’s disease. Current Alzheimer Research, 16, 388–395. https://doi.org/10.2174/1567205016666190321155422

Russell, A. E., Jun, S., Sarkar, S., Geldenhuys, W. J., Lewis, S. E., Rellick, S. L., ... and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. Journal of Biological Chemistry, 278, 3842–3849.

Saman, S., Lee, N. C. Y., Ino, Y., Jin, J., Li, Z., Doyle, T., ... Hall, G. F. (2014). Proteins recruited to exosomes by tau overexpression implicate novel cellular mechanisms linking tau secretion with Alzheimer’s disease. Journal of Alzheimer’s Disease, 40(Suppl 1), S47–70. https://doi.org/10.3233/JAD-132135

Sardar, S. M., Ansell-Schultz, A., Civitelli, L., Hildesjö, C., Larsson, M., Lannfelt, L., ... Hallbeck, M. (2018). Alzheimer’s disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. Acta Neuropathologica, 136, 41–56. https://doi.org/10.1007/s00476-018-1868-1

Savina, A., Fader, C. M., Damiani, M. T., & Colombo, M. I. (2005). Rab11 promotes docking and fusion of multivesicular bodies in a calcium-dependent manner. Traffic, 6, 131–143. https://doi.org/10.1111/j.1600-0854.2004.00257.x

Savina, A., Vidal, M., & Colombo, M. I. (2002). The exosome pathway in K562 cells is regulated by Rab11. Journal of Cell Science, 115, 2505–2515.

Schmidt, O., & Teis, D. (2012). The ESCRT machinery. Current Biology, 22, R116–R120. https://doi.org/10.1016/j.cub.2012.01.028
from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimer’s Dement*, 3, 63–72. https://doi.org/10.1016/j.dadm.2016.04.001.

Winston, C. N., Goetzl, E. J., Schwartz, J. B., Elahi, F. M., & Rissman, R. A. (2019). Complement protein levels in plasma astrocyte-derived exosomes are abnormal in conversion from mild cognitive impairment to Alzheimer’s disease dementia. *Alzheimer’s Dement* (amsterdam, Netherlands), 11, 61–66. https://doi.org/10.1016/j.dadm.2018.11.002.

Xie, J., Li, X., Zhou, Y., Wu, J., Tan, Y., Ma, X., ... Zhao, Y. (2019). Resveratrol abrogates hypoxia-induced up-regulation of exosomal amyloid-β partially by inhibiting CD147. *Neurochemical Research*, 44, 1113–1126. https://doi.org/10.1007/s11064-019-02742-3.

Yang, T. T., Liu, C. G., Gao, S. C., Zhang, Y., & Wang, P. C. (2018). The serum exosome derived microRNA-135a, -193b, and -384 were potential Alzheimer’s disease biomarkers. *Biomedical and Environmental Sciences*, 31, 87–96.

Yuyama, K., Sun, H., Mitsutake, S., & Igarashi, Y. (2012). Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia. *Journal of Biological Chemistry*, 287, 10977–10989.

Yuyama, K., Sun, H., Sakai, S., Mitsutake, S., Okada, M., Tahara, H., ... Igarashi, Y. (2014). Decreased amyloid-β pathologies by intracerebral loading of glycosphingolipid-enriched exosomes in Alzheimer model mice. *Journal of Biological Chemistry*, 289, 24488–24498.

Zetterberg, H. (2018). Blood-based biomarkers for Alzheimer’s disease—An update. *Journal of Neuroscience Methods*, 319, 2–6. https://doi.org/10.1016/j.jneumeth.2018.10.025.

Zhang, Y., Chopp, M., Meng, Y., Katakowski, M., Xin, H., Mahmood, A., & Xiong, Y. (2015). Effect of exosomes derived from multipuripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. *Journal of Neurosurgery*, 122, 856–867. https://doi.org/10.3171/2014.11.JNS14770.

Zheng, T., Pu, J., Chen, Y., Guo, Z., Pan, H., Zhang, L., ... Zhang, B. (2017). Exosomes secreted from HEK293-APP Swe/Ind cells impair the hippocampal neurogenesis. *Neurotoxicity Research*, 32, 82–93. https://doi.org/10.1007/s12640-017-9713-1.

Zheng, T., Pu, J., Chen, Y., Mao, Y., Guo, Z., Pan, H., ... Zhang, B. (2017). Plasma exosomes spread and cluster around β-amyloid plaques in an animal model of Alzheimer’s disease. *Frontiers in Aging Neuroscience*, 9, 12. https://doi.org/10.3389/fnagi.2017.00012.

How to cite this article: S. Martins T, Trindade D, Vaz M, et al. Diagnostic and therapeutic potential of exosomes in Alzheimer’s disease. *J. Neurochem*. 2021;156:162–181. https://doi.org/10.1111/jnc.15112.