Published in final edited form as:

Prog Neurobiol. 2019 April ; 175: 96–106. doi:10.1016/j.pneurobio.2019.01.005.

New windows into the brain: Central nervous system-derived extracellular vesicles in blood

Min Shi, PhD^{1,*}, Lifu Sheng, PhD^{1,*}, Tessandra Stewart, PhD¹, Cyrus P. Zabetian, MD, MS^{2,3,4}, and Jing Zhang, MD, PhD^{1,5,†}

¹Department of Pathology, University of Washington School of Medicine, Seattle, WA 98104, USA

²Geriatric Research, Education, and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA

³Parkinson's Disease Research, Education, and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA

⁴Department of Neurology, University of Washington School of Medicine, Seattle, WA 98195, USA

⁵Department of Pathology, Peking University Third Hospital/Institute of Basic Science, Peking University Health Science Center, Beijing 100083, China

Abstract

Extracellular vesicles (EVs), including exosomes and (shedding) microvesicles, are released by nearly all cell types and carry a cargo of proteins and nucleic acids that varies by the cell of origin. They are thought to play critical roles in normal central nervous system (CNS) function and neurological disorders. A recently revealed key characteristic of EVs is that they may travel between the CNS and peripheral circulation. This property has led to intense interest in how EVs might serve as a vehicle for toxic protein clearance and as a readily accessible source of biomarkers for CNS disorders. Furthermore, by bypassing the blood-brain barrier, modified EVs could serve as a unique drug delivery system that targets specific neuronal populations. Further work is necessary to develop and optimize techniques that enable high-yield capture of relevant EV populations, analyze individual EVs and their cargos, and validate preliminary results of EV-derived biomarkers in independent cohorts.

[†]Corresponding Author: Jing Zhang, MD, PhD, Department of Pathology, University of Washington School of Medicine, HMC Box 359635, 325 9th Ave, Seattle, WA 98104, USA. Phone: 1-206-897-5245, Fax: 1-206-897-5452, zhangj@uw.edu.

^{*}These authors contributed equally to this work.

Contributions

J.Z. and M.S. coordinated the writing of the review. M.S. and L.S. did the literature research, wrote the first draft, and prepared tables and figures. All authors contributed to the writing and revisions of the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Competing interests

All authors declare no competing interests.

1. Introduction

Extracellular vesicles (EVs), including exosomes, (shedding) microvesicles, and others, are membrane-bound vesicles released from cells into the extracellular space and capable of transporting cargo (e.g., DNAs, RNAs, and proteins) between cells as a form of intercellular communication (Budnik et al., 2016; Coleman and Hill, 2015; Lee et al., 2011; Yanez-Mo et al., 2015). The first observations of EVs and their relevance occurred in the 1940s – 1970s in various physiological settings, but they were not immediately recognized as a universally shared cell biological mechanism for function and communication (see review by (Yanez-Mo et al., 2015)). In the 1980s, several studies demonstrated that small EVs were released by multivesicular endosomes or elements (now called multivesicular bodies, or MVBs) fusing with the plasma membrane during reticulocyte maturation; these small EVs were believed to be an exocytotic mechanism of clearing transferrin receptors from cells and were named “exosomes” (Harding et al., 1983; Johnstone et al., 1987; Pan and Johnstone, 1983; Pan et al., 1985). One decade later, such EVs/exosomes released from B lymphocytes were found to be antigen-presenting and able to induce T-cell responses (Raposo et al., 1996). Additionally, similar EVs could also be secreted from dendritic cells, and tumor peptide-pulsed dendritic cell-derived EVs could stimulate the immune system to suppress tumor growth in mice (Zitvogel et al., 1998), further suggesting a role of EVs/exosomes in intercellular communication in the immune system. In the 2000s, with the discoveries that EVs carry proteins and RNAs, including microRNAs and mRNAs, and that EVs could transport functional mRNA between cells (Ratajczak et al., 2006; Valadi et al., 2007), their roles as mediators of cell-to-cell communication were further substantiated. Since then, EVs have been reported to be released from different types of cells, including neurons (Faure et al., 2006), astrocytes (Bianco et al., 2009), microglia (Potolicchio et al., 2005), and oligodendrocytes (Kramer-Albers et al., 2007); furthermore, EVs have been detected in blood plasma/serum (Caby et al., 2005; George et al., 1982), cerebrospinal fluid (CSF) (Harrington et al., 2009; Saman et al., 2012; Street et al., 2012), saliva, urine, and other body fluids (see reviews by (Budnik et al., 2016; Yanez-Mo et al., 2015)).

More recently, EVs have been implicated in neurological disorders, particularly in the transportation of key proteins [e.g., amyloid beta (A β), tau, and α -synuclein involved in Alzheimer disease (AD) and Parkinson disease (PD)] between different cells in the central nervous system (CNS) or between different brain regions (Budnik et al., 2016; Coleman and Hill, 2015; Thompson et al., 2016) (see more details in section 2 below). Trafficking of these EVs between brain and the peripheral systems is much less appreciated. In 2014, we demonstrated that α -synuclein, a protein that plays a central role in the pathophysiology of PD and related neurological disorders, could be transported from the brain into the peripheral circulation via EVs, and that CNS-derived EV-contained α -synuclein in plasma was diagnostically and prognostically relevant to PD (Shi et al., 2014). Since then, bloodborne CNS- or neuron-derived EVs, and the proteins and RNAs they carry, have shown substantial potential as “windows into the brain,” points of transparency allowing examination of peripheral blood to reveal CNS disorder-associated changes in brain biochemistry and intercellular communication (Fiandaca et al., 2015; Goetzl et al., 2015a; Goetzl et al., 2015b; Goetzl et al., 2016b; Goetzl et al., 2018b; Hamlett et al., 2017;

Kapogiannis et al., 2015; Mustapic et al., 2017; Shi et al., 2016; Winston et al., 2016). This review will briefly highlight current understanding on the roles of EVs in CNS disorders, and then focus on the translational potential of CNS-derived EVs in developing novel clinical technologies. Particular emphasis will be placed on the necessity of improving existing protocols and the importance of developing novel tools for isolation, purification, and quantification of CNS-derived EVs, to enable accurate analysis of EV protein and RNA constituents.

2. Extracellular vesicles and their roles in neurological disorders

EVs have generally been divided into two different classes by size and origin. The smaller “exosome” class is relatively homogeneous in size (30–40 to ~150 nm) and is defined by origin within the endosomal network as intraluminal vesicles, formed by inward budding of the limiting membrane of late endosomes or MVBs, which subsequently fuse with the plasma membrane and release exosomes into the extracellular space (Coleman and Hill, 2015; Xu et al., 2016; Yanez-Mo et al., 2015). In contrast, the larger “shedding microvesicle” (“ectosome”) class is heterogeneous in size (~100 to ~1,000 nm) and such vesicles bud directly from the plasma membrane. Other EV types, such as apoptotic bodies that are generally even larger and are released by cells undergoing apoptosis, will not be covered in this review. Currently, there are no consensus criteria to define different types of EVs (Yanez-Mo et al., 2015), and they often overlap in size and share some surface markers (e.g., tetraspanins CD9, CD63, CD81) (Mora et al., 2015; Xu et al., 2016; Yanez-Mo et al., 2015). Given the current lack of clarity on the definition of EVs and that many of the studies to date analyzed crude EV preparations largely due to the limitations of current technologies, guidelines published by the International Society of EVs (ISEV) propose (Gould and Raposo, 2013; Yanez-Mo et al., 2015) the use of the term EVs instead of exosomes and (shedding) microvesicles. We will thus use EVs from this point forward in this review.

As indicated in section 1, EVs can be secreted by most cell types *in vitro*, including neurons and astrocytes, and are detectable in different body fluids *in vivo* (Budnik et al., 2016; Yanez-Mo et al., 2015). The functions of EVs remain to be fully defined, but they have been recognized as an important cell-to-cell communication and signaling pathway in the body in both normal and disease settings (Budnik et al., 2016; Lee et al., 2011; Yanez-Mo et al., 2015). The many functions of EVs in the nervous system, including intercellular communication, maintenance of myelination, synaptic plasticity, antigen presentation, and trophic support of neurons, have been reviewed elsewhere (Budnik et al., 2016; Coleman and Hill, 2015; Thompson et al., 2016), and the current review focuses on the aspects that are relevant to CNS disorders, especially development of novel biomarkers.

EVs are thought to contribute to the pathogenesis of neurodegenerative diseases, including AD and PD. In these disorders, protein oligomers and aggregates (e.g., A β and tau in AD and tauopathies, α -synuclein in PD and other synucleinopathies) are widely considered to have a central role in neurodegeneration, and the role of EVs in toxic protein disposal is believed to be especially pertinent (Coleman and Hill, 2015; Thompson et al., 2016). Evidence suggests that up-regulation of EV secretion from neuronal cells enhances A β uptake into microglia and significantly decreases extracellular levels of A β (Yuyama et al.,

2012), and injecting EVs into the brains of AD transgenic mice resulted in a significant reduction in the brain A β load (Yuyama et al., 2014). Secretion, particularly via EVs, has also been proposed as a toxicity attenuation mechanism by which excess tau is eliminated (Medina and Avila, 2014; Simon et al., 2012).

On the other hand, mounting evidence also suggests EVs have a role in spreading of pathological proteins in these CNS disorders (Coleman and Hill, 2015; Howitt and Hill, 2016; Thompson et al., 2016), though the evidence is far from conclusive in conditions other than the classic prion diseases (e.g., Creutzfeldt–Jakob disease) (Fevrier et al., 2004; Guo et al., 2016; Vella et al., 2008; Vella et al., 2007). In AD models, some cleavage of amyloid precursor protein (APP) to A β peptides was found to occur on early endosomes, which subsequently resulted in the trafficking of A β to MVBs, and a minute fraction of A β was secreted into the extracellular space bound to EVs (Rajendran et al., 2006). Since this discovery, the full-length APP protein and many of its metabolites as well as tau have been found to be secreted into EVs (see reviews by (Howitt and Hill, 2016; Thompson et al., 2016)). Associations between EVs and amyloid plaque formation (Dinkins et al., 2014) or tau aggregate propagation (Polanco et al., 2016) have also been recently reported *in vivo*. In addition to AD and tauopathies, EVs are also reported to be associated with spreading neuropathological proteins in other neurological disorders, including α -synuclein in PD and dementia with Lewy bodies (Emmanouilidou et al., 2010; Grey et al., 2015; Ngolab et al., 2017; Stuendl et al., 2016), and transactive response DNA-binding protein 43 kDa (TDP-43) and superoxide dismutase 1 (SOD1) protein in amyotrophic lateral sclerosis (Feiler et al., 2015; Gomes et al., 2007; Grad et al., 2014).

3. Transportation of extracellular vesicles between the brain and peripheral blood

Although the evidence is still limited, studies have suggested that EVs may move from the brain to the systemic circulation. Earlier studies suggested that EVs may cross multiple layers of the blood-brain barrier (BBB) (Alvarez-Erviti et al., 2011; Matsumoto et al., 2017; Shi et al., 2014), possibly by moving from cell to cell via the MVB compartment and endocytosis (Record et al., 2011). A more recent study confirmed that EVs derived from orthotopic xenotransplant of human glioma-cancer stem cells in mouse brain could cross intact BBB into the bloodstream (Garcia-Romero et al., 2017). This is in line with observations that EVs containing glioma-specific mRNA mutant/variants, microRNAs, and DNAs could be detected in peripheral serum of glioblastoma patients (Chen et al., 2013; Garcia-Romero et al., 2017; Noerholm et al., 2012; Skog et al., 2008). To isolate brain-derived EVs in peripheral blood for more general purposes, we recently introduced a method that targets L1 cell adhesion molecule (L1CAM), the founding member of a subfamily of cell adhesion molecules primarily expressed in the nervous system that is thought to be a surface marker of CNS-derived EVs (Faure et al., 2006; Kenwrick et al., 2000; Lachenal et al., 2011; Simpson et al., 2012). Moreover, radiolabeled α -synuclein or tau injected intracerebroventricularly in mouse brain can be readily transported into blood (Banks et al., 2017; Shi et al., 2016; Shi et al., 2014; Sui et al., 2014), with a small portion contained in anti-L1CAM-captured EVs (Shi et al., 2016; Shi et al., 2014), suggesting that these CNS

disorder-related proteins in brain could also be transported into peripheral blood via EVs. These findings suggest that CNS-derived EVs and their protein and nucleic acid cargos in peripheral blood could serve as potential biomarkers for brain disorders (see section 4 below). The underlying mechanisms of such EV efflux remain to be elucidated, but EVs could move from the CNS to the systemic circulation by direct translocation into capillaries or draining venules, or by passing through interstitial fluid into the CSF and — via the arachnoid granulations and perinasal lymphatics — into the venous system (Fig. 1) (Thompson et al., 2016).

4. Analyses of bloodborne, central nervous system-derived extracellular vesicles

In addition to sparking interest in understanding the potential for EVs to serve as a vehicle for toxic protein clearance and/or brain-periphery communication, the finding that CNS EVs are transported from the brain to the blood led to significant interest in their use as a source of readily accessible CNS biomarkers. Development of traditional biochemical biomarkers for CNS disease has been challenging, due to the difficulty of identifying, isolating, and quantifying molecules that reflect the state of the brain without requiring invasive collection techniques (Keshavan et al., 2017). Use of brain-derived EVs sidesteps this problem, allowing targeting of circulating EVs carrying markers indicating their CNS origin, followed by quantification of their cargo (such as proteins and RNAs), that may be altered in disease states (see section 5 for more details). Extracting EVs from biofluids at the required purity and throughput, however, has been a major challenge (more detailed discussion in section 7 below). One development in this area is the affinity immuno-capture method, in which antibodies against surface markers are used to capture EVs and selection for a specific subpopulation of EVs can be achieved. This approach has been exploited to extract EVs that likely originate from the CNS in peripheral blood, using them to demonstrate that these CNS-derived EVs could serve as a pool of enriched biomarker candidates in a variety of CNS disorders (Table 1) (Abner et al., 2016; Fiandaca et al., 2015; Goetzl et al., 2015a; Goetzl et al., 2015b; Goetzl et al., 2016a; Goetzl et al., 2016b; Goetzl et al., 2018b; Goetzl et al., 2016c; Hamlett et al., 2017; Kapogiannis et al., 2015; Mustapic et al., 2017; Shi et al., 2016; Shi et al., 2014; Winston et al., 2016).

4.1 Extracellular vesicle proteins in Alzheimer disease

In AD, A β peptide 1–42 (A β _{1–42}), total tau (t-tau), and phosphorylated tau (p-tau) not only play critical roles in the disease pathogenesis but are also considered the “core” AD biomarkers in CSF (Lleo et al., 2015). However, the performance of these proteins when measured in blood has been less consistent among studies and robust diagnostic or predictive blood markers for AD have yet to be identified (Henriksen et al., 2014). Goetzl and colleagues used neural cell adhesion molecule (NCAM) first and L1CAM (a probably more CNS-specific EVs surface marker) later to capture likely CNS-derived EVs from human serum or plasma in patients with AD, mild cognitive impairment (MCI or prodromal AD), and controls (Fiandaca et al., 2015; Goetzl et al., 2018a; Goetzl et al., 2015a; Goetzl et al., 2015b; Goetzl et al., 2016a; Kapogiannis et al., 2015; Winston et al., 2016). The authors found that the EV levels of A β _{1–42}, t-tau, and p-tau isoforms were significantly higher in AD

patients compared to controls (Fiandaca et al., 2015), though the alterations in t-tau were disputed by later studies (Guix et al., 2018; Shi et al., 2016). Similar differences were found for progression from MCI (Winston et al., 2016) to dementia or even in pre-clinical cases up to 10 years before onset of AD (Fiandaca et al., 2015). In these studies, a two-step method – first precipitating total EVs with a commercial kit (ExoQuick®), then immunoprecipitating EVs that express L1CAM to generate a subpopulation enriched for EVs of neuronal origin – was used to isolate likely CNS-derived EVs from plasma/serum samples (Mustapic et al., 2017). Although this two-step method is intended to increase the isolation efficiency and purity, possible introduction of contaminating species during the procedure (e.g., use of non-purified thromboplastin-D derived from rabbit brain homogenates for plasma pre-treatment) should be considered and avoided.

In addition to these well-established AD pathogenic proteins, a similar approach has revealed altered levels of a number of proteins implicated in AD, such as phosphorylated insulin receptor substrate-1 (IRS-1) species, lysosomal proteins (e.g., cathepsin-D), heat shock proteins (e.g., HSP70), cellular survival factors (e.g., repressor element 1-silencing transcription factor or REST), low-density lipoprotein receptor-related protein 6 (LRP6), synaptic proteins (e.g., neurogranin), and others in L1CAM-positive EVs in AD patients or subjects examined before the clinical onset of dementia (Goetzl et al., 2015a; Goetzl et al., 2015b; Goetzl et al., 2016a; Kapogiannis et al., 2015; Mustapic et al., 2017). In particular, levels of phosphorylated IRS-1 species in EVs were found to be associated with regional brain atrophy in AD (Mullins et al., 2017). Furthermore, when glutamine aspartate transporter (GLAST) was used to enrich likely astrocyte-derived EVs in plasma, levels of β -site amyloid precursor protein-cleaving enzyme 1 (BACE-1), soluble APP β (sAPP β), glial-derived neurotrophic factor (GDNF) (Goetzl et al., 2016b), and complement effector proteins (Goetzl et al., 2018b) were found to be altered in the GLAST-positive EVs of patients with AD compared to controls, though the astrocytic origin of the GLAST-carrying EVs needs to be further confirmed. All of these results still require validation, but suggest the potential for a blood test to predict development of AD.

4.2 Extracellular vesicle proteins in Parkinson disease

As indicated in section 2 above, α -synuclein oligomerization and aggregation is thought to have a critical role in the etiology of PD (Thomas and Beal, 2007). Recent evidence, including the consistent association of the tau gene (*MAPT*) H1 haplotype with risk of PD (Simon-Sanchez et al., 2009; Zabetian et al., 2007), also suggests that tau plays a role in PD pathogenesis. Both CSF α -synuclein and tau are promising biomarker candidates for PD, while reports on the two proteins in blood are less consistent and promising (Lleo et al., 2015; Shi et al., 2016; Shi et al., 2014). Their levels were assessed in likely CNS-originating L1CAM-containing EVs enriched from plasma of a large PD case-control sample cohort using the immunocapture technique mentioned at the beginning of section 4, which produces an EV sample minimizing contamination from non-EV-associated forms of the target analytes, such as free α -synuclein that is abundantly produced from red blood cells and platelets (Shi et al., 2014). The levels of L1CAM EV-contained α -synuclein and tau were substantially higher in PD patients, suggesting that efflux from the CNS to blood was higher for both proteins (Shi et al., 2016; Shi et al., 2014). Another important finding was that the

diagnostic sensitivity and specificity achieved by plasma EV-contained α -synuclein or tau were comparable to those of their counterpart CSF proteins, and significant associations between L1CAM EV-contained α -synuclein or tau and disease severity or duration were also observed (Shi et al., 2016; Shi et al., 2014).

4.3 Extracellular vesicle proteins in other central nervous system disorders

Down syndrome (DS) is the most common intellectual disability mediated by the trisomy of chromosome 21, and DS patients exhibit AD neuropathology and dementia early in life (Hamlett et al., 2017; Lockstone et al., 2007). Analysis of L1CAM-positive, likely CNS-derived EVs in plasma revealed that neuronal EV levels of $A\beta_{1-42}$ and p-tau species were significantly elevated in individuals with DS compared with age-matched controls at all ages beginning in childhood (Hamlett et al., 2017). These early increases may provide a basis for early intervention as targeted treatments become available. In frontotemporal dementia (FTD), levels of $A\beta_{1-42}$, one isoform of p-tau, REST, and some synaptic proteins in L1CAM-positive EVs were also found to be significantly altered compared to controls (Fiandaca et al., 2015; Goetzl et al., 2015a; Goetzl et al., 2016a), suggesting that changes in the production and/or efflux of these CNS EV-contained proteins might be a common feature in different neurodegenerative diseases. Interestingly, subjects with aging-associated motor decline were also reported to have higher levels of brain-derived neurotrophic factor (BDNF) in circulating L1CAM-positive EVs (Suire et al., 2017). Furthermore, in plasma L1CAM-positive EVs from HIV seropositive individuals, significantly higher levels of high-mobility group box 1, neurofilament light chain, and $A\beta$ proteins were found in cognitively impaired participants compared with neuropsychologically normal individuals, suggesting that these EVs and their protein cargo may be useful for following cognitive impairment and response to therapy in HIV infection (Sun et al., 2017).

4.4 Extracellular vesicle RNAs

Over the past decade, EVs have been shown to have crucial roles in cancer development, including premetastatic niche formation and metastasis (Xu et al., 2018). Cancer cells are now recognized to secrete more EVs than their nonmalignant counterparts, and these EVs carry molecules such as microRNAs, mRNAs, long non-coding RNAs and other RNA species. Therefore, cancer EVs and their RNA cargos isolated from body fluids have strong potential as blood-based or urine-based biomarkers for the diagnosis, prognostication, and surveillance of cancer (Xu et al., 2018).

In patients with brain tumors (e.g., glioblastomas), as mentioned in section 3 above, EVs containing tumor-specific mRNAs and microRNAs could be detected in peripheral blood (Chen et al., 2013; Garcia-Romero et al., 2017; Noerholm et al., 2012; Skog et al., 2008), and their levels may potentially represent the key information in terms of tumor formation and progression, providing a non-invasive method in brain tumor diagnosis (Skog et al., 2008). The current literature on EV-specific RNAs as biomarkers of other CNS disorders such as neurodegenerative diseases is limited. Studies have identified altered expression of microRNAs in plasma EVs in AD (Cheng et al., 2015; Lugli et al., 2015), but RNAs in likely CNS-derived EVs in blood have yet to be investigated.

5. Translational potential of central nervous system-derived extracellular vesicles in neurological disorder diagnosis

As more is learned about CNS-blood pathways and the characteristics of CNS-derived EVs in blood, new opportunities are arising in developing easy-to-access biomarker assays for CNS disorders (Fig. 2A). To date, the most consistent and best performing CNS disorder molecular markers have been found in the CSF, which is not an ideal biofluid for routine monitoring due to the invasive nature of sample collection by lumbar puncture. Because the collection of peripheral blood is considerably easier than CSF in routine clinical practice, defining circulating biomarkers in blood has numerous advantages. However, despite extensive research, robust diagnostic or predictive blood markers for these diseases remain elusive. Accurately detecting and quantifying those often extraordinarily low-abundance brain-derived or disease-specific proteins in blood is challenging for most current technologies. In this regard, several properties of EVs, particularly those transported from the brain to blood, make them excellent candidates for harboring biomarkers of CNS disorders. Their cargo of protein and nucleic acid is likely to reflect core pathogenic intracellular processes in their originating brain cells, in contrast to biofluids such as blood and CSF, in which the levels of relevant molecules are greatly diluted, and often masked, by the high concentration of less relevant substances, such as biomolecules from peripheral organs (Thompson et al., 2016).

Recent results from multiple laboratories (Table 1) have demonstrated the promise of utilizing CNS cell-specific EVs, which addresses both the problems of the complexity of blood and questions about how or whether blood proteins reflect the brain by specifically targeting proteins in brain-derived plasma EVs, as a platform for a liquid biopsy. In PD, elevated levels of α -synuclein and tau in likely CNS-derived EVs have been observed (Shi et al., 2016; Shi et al., 2014); although the assays did not achieve appropriate sensitivity or specificity for use in clinical diagnosis, they did show potential for further development (Thompson et al., 2016). Other findings hold promise for the development of a marker for early or prodromal AD pathology (Fiandaca et al., 2015; Goetzl et al., 2018a; Goetzl et al., 2015a; Goetzl et al., 2015b; Goetzl et al., 2016a; Goetzl et al., 2016b; Goetzl et al., 2018b; Kapogiannis et al., 2015; Winston et al., 2016). Although these promising findings require further validation in larger independent cohorts, the approach opens a unique “window” into the brain.

6. Bi-directional transport of extracellular vesicles and the potential for extracellular vesicle-based therapies

In addition to transport of EVs from the brain to the blood, accumulating evidence also demonstrates that EVs cross the BBB from the blood to the brain. A recent study (Matsumoto et al., 2017) found that peripherally administered red blood cell-derived EVs could cross the BBB in mice, particularly under inflammatory conditions provoked by peripheral administration of lipopolysaccharide, which is relevant in PD and other neurodegenerative diseases. This transport likely occurs via adsorptive-mediated transcytosis, and EVs that transit the BBB co-localize with brain microglia following

transport into the brain. In agreement with these results, another independent study also demonstrated that hematopoietic cell-derived EVs could transfer functional RNAs from blood to neurons in the brain (Ridder et al., 2014). Although this type of signaling was highly restricted in the healthy animal, inflammatory injuries increased both the frequency of transfer and the range of the neuronal target populations in the brain, suggesting a complex regulation of gene expression in neural cells in response to peripheral inflammation.

These findings raise the possibility that EVs might also prove useful in the development of new treatments for neurological disorders (Fig. 2B) (Lener et al., 2015), and, intriguingly, several studies have used this property of EVs in order to bypass the BBB for drug delivery. As EVs can be modified to carry both small and large molecules, they have the potential to transport bioactive molecules, including microRNAs, siRNAs, and bioactive proteins (Alvarez-Erviti et al., 2011; Cooper et al., 2014; Lee et al., 2014; Sterzenbach et al., 2017), from the periphery into the brain. For example, intravenously injected EVs expressing a neuron-targeting peptide delivered a cargo of small interfering RNA (siRNA) specifically to neurons, microglia, and oligodendrocytes in the brain, resulting in a specific gene knockdown in mice (Alvarez-Erviti et al., 2011). Such a strategy offers a number of advantages as a drug delivery system: EVs can overcome major limitations posed by the BBB and offer good biocompatibility, high targeting specificity, and low immunogenicity (Andaloussi S et al., 2013). Several preclinical studies have demonstrated the therapeutic potential of EVs in CNS disorders. For example, EV-mediated siRNA delivery has been used to diminish expression of key therapeutic targets for AD (BACE1, a protease that initiates A β production by cleaving amyloid precursor protein) and PD (α -synuclein) in mouse models (Alvarez-Erviti et al., 2011; Cooper et al., 2014). More recently, it was reported that engineered exosome producer cells subcutaneously implanted in living mice could consistently deliver cargo mRNA to the brain, and therapeutic catalase mRNA delivery by designer exosomes attenuated neurotoxicity and neuroinflammation in *in vitro* and *in vivo* models of PD (Kojima et al., 2018).

Additionally, endogenous EVs may themselves carry cargos with potential therapeutic properties (Lener et al., 2015). EVs derived from a variety of stem cell types are thought to contain factors that exert regenerative effects (Han et al., 2016). For example, systemic administration of mesenchymal stem cell (MSC)-derived EVs improved functional recovery in rat models of traumatic brain and spinal cord injury, and stroke by enhancing angiogenesis, promoting neurogenesis, and/or reducing inflammation (Huang et al., 2017; Xin et al., 2013; Zhang et al., 2015). Identifying and harnessing the molecules contained within these EVs that are responsible for the beneficial effects observed could prove valuable in creating neuroprotective therapies.

7. Current challenges in analyzing central nervous system-derived extracellular vesicles

7.1 Specificity of likely central nervous system-derived extracellular vesicles.

To examine CNS-derived EVs in peripheral blood, CNS- or brain cell-specific EV markers are needed. L1CAM, developed by our group (Shi et al., 2016; Shi et al., 2014) and adopted by others (Abner et al., 2016; Fiandaca et al., 2015; Goetzl et al., 2018a; Goetzl et al., 2015a; Goetzl et al., 2015b; Goetzl et al., 2016a; Goetzl et al., 2016b; Goetzl et al., 2016c; Hamlett et al., 2017; Kapogiannis et al., 2015; Mustapic et al., 2017; Winston et al., 2016), is probably the best studied CNS EV surface marker to date. Recent proteomic studies demonstrated that L1CAM-containing EVs in plasma have higher concentrations of several markers (e.g., phosphorylated tau, neuron-specific enolase, microtubule associated protein 2, neurofilament light chain, and L1CAM) that are reasonably specific to neurons compared to total plasma EVs (Mustapic et al., 2017), highly suggesting the neuronal (and, to some degree, CNS) origin of these EVs. However, L1CAM is also known to be expressed in certain cancer and specialized cells, including some kidney cells (Kenwrick et al., 2000). Although there is no evidence indicating that L1CAM-containing EVs are secreted from kidney and other peripheral cell types, the CNS- or neuron- specificity of L1CAM-containing EVs in blood needs to be further investigated. Other CNS-specific EV surface markers, including those from different brain cell types (astrocytes, microglia, and neurons) and those from different brain regions that are affected in different neurological disorders, should also be explored to identify biomarker candidates that are more specific to each disease. Thus far, NCAM has been tested (Fiandaca et al., 2015), though it seems to be less CNS-specific than L1CAM. Additionally, GLAST has been utilized to study astrocyte-derived EVs in blood (Goetzl et al., 2016b; Goetzl et al., 2018b), but its CNS- or astrocyte-specificity has not been rigorously tested.

7.2 Isolation of relatively pure extracellular vesicles for high yield of biomarkers.

EV isolation and characterization remain challenging due to the presence of contaminants with comparable features (Mateescu et al., 2017; Xu et al., 2016). A major challenge in profiling circulating EVs is that differential centrifugation (plus density gradient for purer preparations) is still the “gold” standard, and one of the most frequently used methods for EVs isolation from body fluids (Mateescu et al., 2017; Xu et al., 2016). While effective for general EV preparations, the methodology is cumbersome, requires a large amount of starting material, and is not suitable for high-throughput assays, nor does it provide EV subpopulation (e.g., those containing CNS-specific markers) and individual particle analysis. This method also suffers from generating EV preparations that contain many different types of EVs and even free protein aggregates large enough to be sedimented at the given centrifugal forces (Mateescu et al., 2017; Sodar et al., 2016; Xu et al., 2016). Multiple alternative approaches have been developed (Table 2; see also articles summarizing the current state of knowledge for EV isolation (Mateescu et al., 2017; Minciacchi et al., 2017; Xu et al., 2016)). Mostly polymer-based commercial precipitation products (e.g., Exoquick®) can quickly precipitate EVs from biological fluids (Coumans et al., 2017; Weng et al., 2016), but suffer similar limitations, in that the resulting pellets are likely

contaminated with other particles, large aggregates, associating proteins, etc. (Van Deun et al., 2014). Most studies on CNS-derived EVs in blood have improved on these strategies by utilizing the affinity immunocapture method for EV isolation, which can generate relatively pure EVs, but the yield is usually low (Mustapic et al., 2017; Shi et al., 2014; Xu et al., 2016). Studies (Fiandaca et al., 2015; Mustapic et al., 2017) have been conducted to combine polymer-based precipitation followed by immunocapture to improve yield and purity, but introduction of contaminants during the process is still a concern (Shi et al., 2016).

7.3 Quantification of extremely low abundance central nervous system-derived extracellular vesicle cargos in blood.

The use of blood EVs to yield clinically useful biomarkers for CNS disorders is also hampered by the small numbers of CNS-derived EVs that are secreted by disease-relevant cells and transported into peripheral blood. These difficulties are exacerbated by the complexity and technical limitations of current EV extraction methods in terms of EV yield and purity as mentioned in section 7.2 above. Sensitive assays are needed to quantify the cargos, particularly proteins, in these CNS-derived EVs in blood. For example, measuring tau in L1CAM-containing EVs in plasma (Shi et al., 2016) necessitated the use of newly developed immunoassay technology: Single Molecule Array (Simoa), which makes use of arrays of femtoliter-sized reaction chambers to isolate and detect single protein molecules (Rissin et al., 2011; Rissin et al., 2010). More sensitive and accurate technologies are still needed to analyze individual EVs and their cargos.

8. Next generation tools for extracellular vesicle analysis and future directions

Given the lack of robust and efficient methods to isolate relatively pure EVs and the difficulties involved in quantifying the cargos (particularly proteins), several recent studies have focused on developing novel approaches to purify EVs and analyze individual particles in a more rapid, sensitive, and accurate manner. By using microfluidic devices and trapping EVs with an immune-affinity approach, sophisticated platforms involving on-chip isolation, quantification and characterization (ExoChip) (Kanwar et al., 2014), immunomagnetic exosome RNA (iMER) analysis (Shao et al., 2015), miniaturized micro-nuclear magnetic resonance (μ NMR) microfluidic chip system (Shao et al., 2012), and label-free high-throughput nano-plasmonic exosome assay (nPLEX) using surface plasmon resonance (Im et al., 2014) have been developed to analyze EVs from blood, though these platforms need to be further improved to extend small-scale EV research into high-throughput, routine research and clinical settings.

Nanoparticle tracking analysis (NTA), which allows direct and individual visualization of specific EVs in liquid suspension in real-time and provides size distribution profiles and concentration measurements, has been widely employed in the field. However, NTA faces several limitations, particularly when used for examining particles in biofluids. First, NTA measures particles based on their Brownian movement, and cannot distinguish EVs from other particles. Second, the technology was developed based on monodisperse particle

suspensions, and may not perform optimally on the widely variable particles present in biofluids. To overcome these drawbacks for measuring EVs in body fluids, this technology can also include fluorescent detection (e.g., using fluorescent antibodies against EV surface markers) and thus enables phenotyping of the particles (Dragovic et al., 2011). However, simultaneous measurements of scatter and fluorescence or multiple fluorescent signals are currently unavailable.

Flow cytometric analysis of EVs has also made significant advances. This technology in general cannot detect and quantify particles smaller than 200 nm. Recently, the use of microflow technologies (Apogee) has enabled the direct analysis of EVs as small as ~150 nm (Kibria et al., 2016; Rose et al., 2016). The inclusion of fluorescent detection with small and wide-angle scatter enables microflow technologies to interrogate each particle with fluorescent antibodies, thereby bringing the advantages of traditional flow cytometry to the analysis of EVs and enabling simultaneous measurements of multiple surface markers and even internal markers on a single EV (Minciacchi et al., 2017).

Unfortunately, most of these methods have not been standardized, which makes cross-assay comparisons difficult. It has also prevented the field from specifically defining what EV population has been studied by different investigators. Moving forward, in addition to recently proposed minimal experimental requirements, assay standardization, and transparent reporting (Lener et al., 2015; Lotvall et al., 2014; Mateescu et al., 2017; Van Deun et al., 2017; Xu et al., 2016), we recommend the following:

1. *Identify EV surface markers that are more CNS-specific or specific to brain cell subpopulations or regions:* One can hypothesize that the changes in EV efflux occur even more prominently in the brain regions or cell types/subpopulations specifically affected by various CNS disorders. Thus, identifying more specific EV surface markers could have major impact in this field.
2. *Combine current EV isolation technologies for better yield and purity:* Until a robust technology or assay is developed, it may be a good strategy to combine the immunocapture method with another method to improve target EV yield and purity. For example, a crude preparation step (e.g., filtering, precipitation, or size-exclusion chromatography) may be added before immunocapture, or a concentrating or further purification step added after immunocapture.
3. *Further develop new technologies for analyzing individual EVs and their cargos:* Accurately and reliably analyzing individual EVs with multiple markers (surface and internal) would have numerous advantages, including much reduced contaminating signal/noise, better assessment of the variability, likely reduced sample material requirement, and easier targeting of EV subpopulations. For the last point, many neurological disorders progress through multiple stages, affecting preferentially different regions of the brain (e.g., hippocampus for AD, midbrain for PD) at least at early stages of the diseases, and consequently the cargo in EVs derived from disease-related brain regions or neuronal subpopulations may be more effective and specific disease biomarkers. Therefore, as a good example, EVs carrying both L1CAM and another marker

specific to an AD-related brain region or neuronal subpopulation could be a much better source of effective AD biomarkers in blood than L1CAM-positive EVs in general.

9. Concluding remarks

Brain-derived EVs identified in peripheral blood have shown great potential to harbor disease-specific molecular signatures of CNS disorders, making them an ideal source of biomarkers. Further work is underway to develop techniques that enable high-yield capture of relevant EV populations that are more likely to contain relevant candidate markers and to validate the acquired preliminary results in larger independent cohorts. Keys to success will be the confirmation of the CNS (cell subpopulation or brain region) specificity as well as standardization of EV purification and analysis. Using EVs as novel therapeutic vehicles is also under intensive investigation. This line of research will likely provide easily accessible biomarkers to aid in diagnosis, monitor disease progression, and assess treatment effects, and create novel treatment strategies and drug delivery systems for CNS disorders.

Acknowledgments

J.Z., M.S., and C.P.Z. acknowledge support from the National Institutes of Health (NIH) (U01 NS082137, U01 NS091272, R01 AG056711, and R56 AG057417 to J.Z., R21 NS085425 to M.S., and P50 NS062684 and R01 NS065070 to C.P.Z.).

Abbreviations

Aβ₁₋₄₂	amyloid beta peptide 1–42
AD	Alzheimer disease
APP	amyloid precursor protein
BACE-1	β -site amyloid precursor protein-cleaving enzyme 1
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CSF	cerebrospinal fluid
DS	Down syndrome
EV	extracellular vesicles
GDNF	glial-derived neurotrophic factor
GLAST	glutamine aspartate transporter
HSP70	heat shock protein 70
iMER	immunomagnetic exosome RNA (analysis)

IRS-1	insulin receptor substrate-1
ISEV	International Society of Extracellular Vesicles
L1CAM	L1 cell adhesion molecule
LRP6	low-density lipoprotein receptor-related protein 6
MCI	mild cognitive impairment
μNMR	micro-nuclear magnetic resonance
nPLEX	nano-plasmonic exosome assay
MVB	multivesicular body
NCAM	neural cell adhesion molecule
NTA	nanoparticle tracking analysis
p-tau	phosphorylated tau
PD	Parkinson disease
REST	repressor element 1-silencing transcription factor
siRNA	small interfering RNA
sAPPβ	soluble APP β
SOD1	superoxide dismutase 1
t-tau	total tau
TDP-43	transactive response DNA-binding protein 43 kDa

References

- Abner EL, Jicha GA, Shaw LM, Trojanowski JQ and Goetzl EJ, 2016 Plasma neuronal exosomal levels of Alzheimer's disease biomarkers in normal aging. *Ann. Clin. Transl. Neurol* 3, 399–403. [PubMed: 27231710]
- Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S and Wood MJ, 2011 Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol* 29, 341–345. [PubMed: 21423189]
- Andaloussi SEL, Mager I, Breakefield XO and Wood MJ, 2013 Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat. Rev. Drug Discov* 12, 347–357. [PubMed: 23584393]
- Banks WA, Kovac A, Majerova P, Bullock KM, Shi M and Zhang J, 2017 Tau Proteins Cross the Blood-Brain Barrier. *J. Alzheimers Dis* 55, 411–419. [PubMed: 27662303]
- Bianco F, Perrotta C, Novellino L, Francolini M, Riganti L, Menna E, Saglietti L, Schuchman EH, Furlan R, Clementi E, Matteoli M and Verderio C, 2009 Acid sphingomyelinase activity triggers microparticle release from glial cells. *EMBO J* 28, 1043–1054. [PubMed: 19300439]
- Budnik V, Ruiz-Canada C and Wendler F, 2016 Extracellular vesicles round off communication in the nervous system. *Nat. Rev. Neurosci* 17, 160–172. [PubMed: 26891626]
- Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G and Bonnerot C, 2005 Exosomal-like vesicles are present in human blood plasma. *Int. Immunol* 17, 879–887. [PubMed: 15908444]

- Chen WW, Balaj L, Liao LM, Samuels ML, Kotsopoulos SK, Maguire CA, Loguidice L, Soto H, Garrett M, Zhu LD, Sivaraman S, Chen C, Wong ET, Carter BS, Hochberg FH, Breakefield XO and Skog J, 2013 BEAMing and Droplet Digital PCR Analysis of Mutant IDH1 mRNA in Glioma Patient Serum and Cerebrospinal Fluid Extracellular Vesicles. *Mol. Ther. Nucleic Acids* 2, e109. [PubMed: 23881452]
- Cheng L, Doecke JD, Sharples RA, Villemagne VL, Fowler CJ, Rembach A, Martins RN, Rowe CC, Macaulay SL, Masters CL and Hill AF, 2015 Prognostic serum miRNA biomarkers associated with Alzheimer's disease shows concordance with neuropsychological and neuroimaging assessment. *Mol. Psychiatry* 20, 1188–1196. [PubMed: 25349172]
- Coleman BM and Hill AF, 2015 Extracellular vesicles--Their role in the packaging and spread of misfolded proteins associated with neurodegenerative diseases. *Semin. Cell Dev. Biol* 40, 89–96. [PubMed: 25704308]
- Cooper JM, Wiklander PB, Nordin JZ, Al-Shawi R, Wood MJ, Vithlani M, Schapira AH, Simons JP, El-Andaloussi S and Alvarez-Erviti L, 2014 Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice. *Mov. Disord* 29, 1476–1485. [PubMed: 25112864]
- Coumans FAW, Brisson AR, Buzas EI, Dignat-George F, Drees EEE, El-Andaloussi S, Emanuelli C, Gasecka A, Hendrix A, Hill AF, Lacroix R, Lee Y, van Leeuwen TG, Mackman N, Mager I, Nolan JP, van der Pol E, Pegtel DM, Sahoo S, Siljander PRM, Sturk G, de Wever O and Nieuwland R, 2017 Methodological Guidelines to Study Extracellular Vesicles. *Circ. Res* 120, 1632–1648. [PubMed: 28495994]
- Dinkins MB, Dasgupta S, Wang G, Zhu G and Bieberich E, 2014 Exosome reduction in vivo is associated with lower amyloid plaque load in the 5XFAD mouse model of Alzheimer's disease. *Neurobiol. Aging* 35, 1792–1800. [PubMed: 24650793]
- Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, Carr B, Redman CW, Harris AL, Dobson PJ, Harrison P and Sargent IL, 2011 Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. *Nanomedicine* 7, 780–788. [PubMed: 21601655]
- Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, Stefanis L and Vekrellis K, 2010 Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *J. Neurosci* 30, 6838–6851. [PubMed: 20484626]
- Faure J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, Grange J, Schoehn G, Goldberg Y, Boyer V, Kirchhoff F, Raposo G, Garin J and Sadoul R, 2006 Exosomes are released by cultured cortical neurones. *Mol. Cell. Neurosci* 31, 642–648. [PubMed: 16446100]
- Feiler MS, Strobel B, Freischmidt A, Helferich AM, Kappel J, Brewer BM, Li D, Thal DR, Walther P, Ludolph AC, Danzer KM and Weishaupt JH, 2015 TDP-43 is intercellularly transmitted across axon terminals. *J. Cell Biol* 211, 897–911. [PubMed: 26598621]
- Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, Laude H and Raposo G, 2004 Cells release prions in association with exosomes. *Proc. Natl. Acad. Sci. U. S. A* 101, 9683–9688. [PubMed: 15210972]
- Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB, Abner EL, Petersen RC, Federoff HJ, Miller BL and Goetzl EJ, 2015 Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. *Alzheimers Dement* 11, 600–607. [PubMed: 25130657]
- Garcia-Romero N, Carrion-Navarro J, Esteban-Rubio S, Lazaro-Ibanez E, Peris-Celda M, Alonso MM, Guzman-De-Villoria J, Fernandez-Carballal C, de Mendivil AO, Garcia-Duque S, Escobedo-Lucea C, Prat-Acin R, Belda-Iniesta C and Ayuso-Sacido A, 2017 DNA sequences within glioma-derived extracellular vesicles can cross the intact blood-brain barrier and be detected in peripheral blood of patients. *Oncotarget* 8, 1416–1428. [PubMed: 27902458]
- George JN, Thoi LL, McManus LM and Reimann TA, 1982 Isolation of human platelet membrane microparticles from plasma and serum. *Blood* 60, 834–840. [PubMed: 7115953]
- Goetzl EJ, Abner EL, Jicha GA, Kapogiannis D and Schwartz JB, 2018a Declining levels of functionally specialized synaptic proteins in plasma neuronal exosomes with progression of Alzheimer's disease. *FASEB J* 32, 888–893. [PubMed: 29025866]

- Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL, Carlson OD, Mustapic M and Kapogiannis D, 2015a Low neural exosomal levels of cellular survival factors in Alzheimer's disease. *Ann. Clin. Transl. Neurol* 2, 769–773. [PubMed: 26273689]
- Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL and Kapogiannis D, 2015b Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. *Neurology* 85, 40–47. [PubMed: 26062630]
- Goetzl EJ, Kapogiannis D, Schwartz JB, Lobach IV, Goetzl L, Abner EL, Jicha GA, Karydas AM, Boxer A and Miller BL, 2016a Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease. *FASEB J* 30, 4141–4148. [PubMed: 27601437]
- Goetzl EJ, Mustapic M, Kapogiannis D, Eitan E, Lobach IV, Goetzl L, Schwartz JB and Miller BL, 2016b Cargo proteins of plasma astrocyte-derived exosomes in Alzheimer's disease. *FASEB J* 30, 3853–3859. [PubMed: 27511944]
- Goetzl EJ, Schwartz JB, Abner EL, Jicha GA and Kapogiannis D, 2018b High complement levels in astrocyte-derived exosomes of Alzheimer disease. *Ann. Neurol* 83, 544–552. [PubMed: 29406582]
- Goetzl L, Darbinian N and Goetzl EJ, 2016c Novel window on early human neurodevelopment via fetal exosomes in maternal blood. *Ann. Clin. Transl. Neurol* 3, 381–385. [PubMed: 27231707]
- Gomes C, Keller S, Altevogt P and Costa J, 2007 Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. *Neurosci. Lett* 428, 43–46. [PubMed: 17942226]
- Gould SJ and Raposo G, 2013 As we wait: coping with an imperfect nomenclature for extracellular vesicles. *J. Extracell. Vesicles* 2, DOI: 10.3402/jev.v3402i3400.20389.
- Grad LI, Yerbury JJ, Turner BJ, Guest WC, Pokrishevsky E, O'Neill MA, Yanai A, Silverman JM, Zeineddine R, Corcoran L, Kumita JR, Luheshi LM, Yousefi M, Coleman BM, Hill AF, Plotkin SS, Mackenzie IR and Cashman NR, 2014 Intercellular propagated misfolding of wild-type Cu/Zn superoxide dismutase occurs via exosome-dependent and -independent mechanisms. *Proc. Natl. Acad. Sci. U. S. A* 111, 3620–3625. [PubMed: 24550511]
- Grey M, Dunning CJ, Gaspar R, Grey C, Brundin P, Sparr E and Linse S, 2015 Acceleration of alpha-synuclein aggregation by exosomes. *J. Biol. Chem* 290, 2969–2982. [PubMed: 25425650]
- Guix FX, Corbett GT, Cha DJ, Mustapic M, Liu W, Mengel D, Chen Z, Aikawa E, Young-Pearse T, Kapogiannis D, Selkoe DJ and Walsh DM, 2018 Detection of Aggregation-Competent Tau in Neuron-Derived Extracellular Vesicles. *Int. J. Mol. Sci* 19.
- Guo BB, Bellingham SA and Hill AF, 2016 Stimulating the Release of Exosomes Increases the Intercellular Transfer of Prions. *J. Biol. Chem* 291, 5128–5137. [PubMed: 26769968]
- Hamlett ED, Goetzl EJ, Ledreux A, Vasilevko V, Boger HA, LaRosa A, Clark D, Carroll SL, Carmona-Iragui M, Fortea J, Mufson EJ, Sabbagh M, Mohammed AH, Hartley D, Doran E, Lott IT and Granholm AC, 2017 Neuronal exosomes reveal Alzheimer's disease biomarkers in Down syndrome. *Alzheimers Dement* 13, 541–549. [PubMed: 27755974]
- Han C, Sun X, Liu L, Jiang H, Shen Y, Xu X, Li J, Zhang G, Huang J, Lin Z, Xiong N and Wang T, 2016 Exosomes and Their Therapeutic Potentials of Stem Cells. *Stem Cells Int* 2016, 7653489. [PubMed: 26770213]
- Harding C, Heuser J and Stahl P, 1983 Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J. Cell Biol* 97, 329–339. [PubMed: 6309857]
- Harrington MG, Fonteh AN, Oborina E, Liao P, Cowan RP, McComb G, Chavez JN, Rush J, Biringer RG and Huhmer AF, 2009 The morphology and biochemistry of nanostructures provide evidence for synthesis and signaling functions in human cerebrospinal fluid. *Cerebrospinal Fluid Res* 6, 10. [PubMed: 19735572]
- Henriksen K, O'Bryant SE, Hampel H, Trojanowski JQ, Montine TJ, Jeromin A, Blennow K, Lonnberg A, Wyss-Coray T, Soares H, Bazenet C, Sjogren M, Hu W, Lovestone S, Karsdal MA and Weiner MW, 2014 The future of blood-based biomarkers for Alzheimer's disease. *Alzheimers Dement* 10, 115–131. [PubMed: 23850333]
- Howitt J and Hill AF, 2016 Exosomes in the Pathology of Neurodegenerative Diseases. *J. Biol. Chem* 291, 26589–26597. [PubMed: 27852825]
- Huang JH, Yin XM, Xu Y, Xu CC, Lin X, Ye FB, Cao Y and Lin FY, 2017 Systemic Administration of Exosomes Released from Mesenchymal Stromal Cells Attenuates Apoptosis, Inflammation, and

- Promotes Angiogenesis after Spinal Cord Injury in Rats. *J. Neurotrauma* 34, 3388–3396. [PubMed: 28665182]
- Im H, Shao H, Park YI, Peterson VM, Castro CM, Weissleder R and Lee H, 2014 Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. *Nat. Biotechnol* 32, 490–495. [PubMed: 24752081]
- Johnstone RM, Adam M, Hammond JR, Orr L and Turbide C, 1987 Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J. Biol. Chem* 262, 9412–9420. [PubMed: 3597417]
- Kanwar SS, Dunlay CJ, Simeone DM and Nagrath S, 2014 Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes. *Lab. Chip* 14, 1891–1900. [PubMed: 24722878]
- Kapogiannis D, Boxer A, Schwartz JB, Abner EL, Biragyn A, Masharani U, Frassetto L, Petersen RC, Miller BL and Goetzl EJ, 2015 Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. *FASEB J* 29, 589–596. [PubMed: 25342129]
- Kenrick S, Watkins A and De Angelis E, 2000 Neural cell recognition molecule L1: relating biological complexity to human disease mutations. *Hum. Mol. Genet* 9, 879–886. [PubMed: 10767310]
- Keshavan A, Heslegrave A, Zetterberg H and Schott JM, 2017 Blood Biomarkers for Alzheimer's Disease: Much Promise, Cautious Progress. *Mol. Diagn. Ther* 21, 13–22. [PubMed: 27738910]
- Kibria G, Ramos EK, Lee KE, Bedoyan S, Huang S, Samaeekia R, Athman JJ, Harding CV, Lotvall J, Harris L, Thompson CL and Liu H, 2016 A rapid, automated surface protein profiling of single circulating exosomes in human blood. *Sci. Rep* 6, 36502. [PubMed: 27819324]
- Kojima R, Bojar D, Rizzi G, Hamri GC, El-Baba MD, Saxena P, Auslander S, Tan KR and Fussenegger M, 2018 Designer exosomes produced by implanted cells intracerebrally deliver therapeutic cargo for Parkinson's disease treatment. *Nat. Commun* 9, 1305. [PubMed: 29610454]
- Kramer-Albers EM, Bretz N, Tenzer S, Winterstein C, Mobius W, Berger H, Nave KA, Schild H and Trotter J, 2007 Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? *Proteomics Clin. Appl* 1, 1446–1461. [PubMed: 21136642]
- Lachenal G, Pernet-Gallay K, Chivet M, Hemming FJ, Belly A, Bodon G, Blot B, Haase G, Goldberg Y and Sadoul R, 2011 Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. *Mol. Cell. Neurosci* 46, 409–418. [PubMed: 21111824]
- Lee HK, Finniss S, Cazacu S, Xiang C and Brodie C, 2014 Mesenchymal stem cells deliver exogenous miRNAs to neural cells and induce their differentiation and glutamate transporter expression. *Stem Cells Dev* 23, 2851–2861. [PubMed: 25036385]
- Lee TH, D'Asti E, Magnus N, Al-Nedawi K, Meehan B and Rak J, 2011 Microvesicles as mediators of intercellular communication in cancer--the emerging science of cellular 'debris'. *Semin. Immunopathol* 33, 455–467. [PubMed: 21318413]
- Lener T, Gimona M, Aigner L, Borger V, Buzas E, Camussi G, Chaput N, Chatterjee D, Court FA, Del Portillo HA, O'Driscoll L, Fais S, Falcon-Perez JM, Felderhoff-Mueser U, Fraile L, Gho YS, Gorgens A, Gupta RC, Hendrix A, Hermann DM, Hill AF, Hochberg F, Horn PA, de Kleijn D, Kordelas L, Kramer BW, Kramer-Albers EM, Laner-Plamberger S, Laitinen S, Leonardi T, Lorenowicz MJ, Lim SK, Lotvall J, Maguire CA, Marcilla A, Nazarenko I, Ochiya T, Patel T, Pedersen S, Pocsfalvi G, Pluchino S, Quesenberry P, Reischl IG, Rivera FJ, Sanzenbacher R, Schallmoser K, Slaper-Cortenbach I, Strunk D, Tonn T, Vader P, van Balkom BW, Wauben M, Andaloussi SE, Thery C, Rohde E and Giebel B, 2015 Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. *J. Extracell. Vesicles* 4, 30087. [PubMed: 26725829]
- Lleo A, Cavedo E, Parnetti L, Vanderstichele H, Herukka SK, Andreassen N, Ghidoni R, Lewczuk P, Jeromin A, Winblad B, Tsolaki M, Mroczko B, Visser PJ, Santana I, Svenningsson P, Blennow K, Aarsland D, Molinuevo JL, Zetterberg H and Mollenhauer B, 2015 Cerebrospinal fluid biomarkers in trials for Alzheimer and Parkinson diseases. *Nat. Rev. Neurol* 11, 41–55. [PubMed: 25511894]
- Lockstone HE, Harris LW, Swatton JE, Wayland MT, Holland AJ and Bahn S, 2007 Gene expression profiling in the adult Down syndrome brain. *Genomics* 90, 647–660. [PubMed: 17950572]

- Lotvall J, Hill AF, Hochberg F, Buzas EI, Di Vizio D, Gardiner C, Gho YS, Kurochkin IV, Mathivanan S, Quesenberry P, Sahoo S, Tahara H, Wauben MH, Witwer KW and Thery C, 2014 Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *J. Extracell. Vesicles* 3, 26913. [PubMed: 25536934]
- Lugli G, Cohen AM, Bennett DA, Shah RC, Fields CJ, Hernandez AG and Smalheiser NR, 2015 Plasma Exosomal miRNAs in Persons with and without Alzheimer Disease: Altered Expression and Prospects for Biomarkers. *PLoS ONE* 10, e0139233. [PubMed: 26426747]
- Mateescu B, Kowal EJ, van Balkom BW, Bartel S, Bhattacharyya SN, Buzas EI, Buck AH, de Candia P, Chow FW, Das S, Driedonks TA, Fernandez-Messina L, Haderk F, Hill AF, Jones JC, Van Keuren-Jensen KR, Lai CP, Lasser C, Liegro ID, Lunavat TR, Lorenowicz MJ, Maas SL, Mager I, Mittelbrunn M, Momma S, Mukherjee K, Nawaz M, Pegtel DM, Pfaffl MW, Schiffrers RM, Tahara H, Thery C, Tosar JP, Wauben MH, Witwer KW and Nolte-'t Hoen EN, 2017 Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - an ISEV position paper. *J. Extracell. Vesicles* 6, 1286095. [PubMed: 28326170]
- Matsumoto J, Stewart T, Sheng L, Li N, Bullock K, Song N, Shi M, Banks WA and Zhang J, 2017 Transmission of alpha-synuclein-containing erythrocyte-derived extracellular vesicles across the blood-brain barrier via adsorptive mediated transcytosis: another mechanism for initiation and progression of Parkinson's disease? *Acta Neuropathol. Commun* 5, 71. [PubMed: 28903781]
- Medina M and Avila J, 2014 The role of extracellular Tau in the spreading of neurofibrillary pathology. *Front. Cell. Neurosci* 8, 113. [PubMed: 24795568]
- Minciacchi VR, Zijlstra A, Rubin MA and Di Vizio D, 2017 Extracellular vesicles for liquid biopsy in prostate cancer: where are we and where are we headed? *Prostate Cancer Prostatic Dis* 20, 251–258. [PubMed: 28374743]
- Mora EM, Alvarez-Cubela S and Oltra E, 2015 Biobanking of Exosomes in the Era of Precision Medicine: Are We There Yet? *Int. J. Mol. Sci* 17.
- Mullins RJ, Mustapic M, Goetzl EJ and Kapogiannis D, 2017 Exosomal biomarkers of brain insulin resistance associated with regional atrophy in Alzheimer's disease. *Hum. Brain Mapp* 38, 1933–1940. [PubMed: 28105773]
- Mustapic M, Eitan E, Werner JK Jr., Berkowitz ST, Lazaropoulos MP, Tran J, Goetzl EJ and Kapogiannis D, 2017 Plasma Extracellular Vesicles Enriched for Neuronal Origin: A Potential Window into Brain Pathologic Processes. *Front. Neurosci* 11, 278. [PubMed: 28588440]
- Ngolab J, Trinh I, Rockenstein E, Mante M, Florio J, Trejo M, Masliah D, Adame A, Masliah E and Rissman RA, 2017 Brain-derived exosomes from dementia with Lewy bodies propagate alpha-synuclein pathology. *Acta Neuropathol. Commun* 5, 46. [PubMed: 28599681]
- Noerholm M, Balaj L, Limperg T, Salehi A, Zhu LD, Hochberg FH, Breakefield XO, Carter BS and Skog J, 2012 RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls. *BMC Cancer* 12, 22. [PubMed: 22251860]
- Pan BT and Johnstone RM, 1983 Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell* 33, 967–978. [PubMed: 6307529]
- Pan BT, Teng K, Wu C, Adam M and Johnstone RM, 1985 Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J. Cell Biol* 101, 942–948. [PubMed: 2993317]
- Polanco JC, Scicluna BJ, Hill AF and Gotz J, 2016 Extracellular Vesicles Isolated from the Brains of rTg4510 Mice Seed Tau Protein Aggregation in a Threshold-dependent Manner. *J. Biol. Chem* 291, 12445–12466. [PubMed: 27030011]
- Potolicchio I, Carven GJ, Xu X, Stipp C, Riese RJ, Stern LJ and Santambrogio L, 2005 Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13 in neuropeptide catabolism. *J. Immunol* 175, 2237–2243. [PubMed: 16081791]
- Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P and Simons K, 2006 Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. U. S. A* 103, 11172–11177. [PubMed: 16837572]

- Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ and Geuze HJ, 1996 B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med* 183, 1161–1172. [PubMed: 8642258]
- Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P and Ratajczak MZ, 2006 Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* 20, 847–856. [PubMed: 16453000]
- Record M, Subra C, Silvente-Poirot S and Poirot M, 2011 Exosomes as intercellular signalosomes and pharmacological effectors. *Biochem. Pharmacol* 81, 1171–1182. [PubMed: 21371441]
- Ridder K, Keller S, Dams M, Rupp AK, Schlaudraff J, Del Turco D, Starmann J, Macas J, Karpova D, Devraj K, Depboylu C, Landfried B, Arnold B, Plate KH, Hoglinger G, Sultmann H, Altevogt P and Momma S, 2014 Extracellular vesicle-mediated transfer of genetic information between the hematopoietic system and the brain in response to inflammation. *PLoS Biol* 12, e1001874. [PubMed: 24893313]
- Rissin DM, Fournier DR, Piech T, Kan CW, Campbell TG, Song L, Chang L, Rivnak AJ, Patel PP, Provuncher GK, Ferrell EP, Howes SC, Pink BA, Minnehan KA, Wilson DH and Duffy DC, 2011 Simultaneous detection of single molecules and singulated ensembles of molecules enables immunoassays with broad dynamic range. *Anal. Chem* 83, 2279–2285. [PubMed: 21344864]
- Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, Piech T, Patel PP, Chang L, Rivnak AJ, Ferrell EP, Randall JD, Provuncher GK, Walt DR and Duffy DC, 2010 Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat. Biotechnol* 28, 595–599. [PubMed: 20495550]
- Rose JA, Wanner N, Cheong HI, Queisser K, Barrett P, Park M, Hite C, Naga Prasad SV, Erzurum S and Asosingh K, 2016 Flow Cytometric Quantification of Peripheral Blood Cell beta-Adrenergic Receptor Density and Urinary Endothelial Cell-Derived Microparticles in Pulmonary Arterial Hypertension. *PLoS ONE* 11, e0156940. [PubMed: 27270458]
- Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, Jackson B, McKee AC, Alvarez VE, Lee NC and Hall GF, 2012 Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J. Biol. Chem* 287, 3842–3849. [PubMed: 22057275]
- Shao H, Chung J, Balaj L, Charest A, Bigner DD, Carter BS, Hochberg FH, Breakefield XO, Weissleder R and Lee H, 2012 Protein typing of circulating microvesicles allows real-time monitoring of glioblastoma therapy. *Nat. Med* 18, 1835–1840. [PubMed: 23142818]
- Shao H, Chung J, Lee K, Balaj L, Min C, Carter BS, Hochberg FH, Breakefield XO, Lee H and Weissleder R, 2015 Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. *Nat. Commun* 6, 6999. [PubMed: 25959588]
- Shi M, Kovac A, Korff A, Cook TJ, Ghingina C, Bullock KM, Yang L, Stewart T, Zheng D, Aro P, Atik A, Kerr KF, Zabetian CP, Peskind ER, Hu SC, Quinn JF, Galasko DR, Montine TJ, Banks WA and Zhang J, 2016 CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. *Alzheimers Dement* 12, 1125–1131. [PubMed: 27234211]
- Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, Ghingina C, Li Y, Aro P, Dator R, He C, Hipp MJ, Zabetian CP, Peskind ER, Hu SC, Quinn JF, Galasko DR, Banks WA and Zhang J, 2014 Plasma exosomal α -synuclein is likely CNS-derived and increased in Parkinson's disease. *Acta Neuropathol* 128, 639–650. [PubMed: 24997849]
- Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Kruger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB and Gasser T, 2009 Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet* 41, 1308–1312. [PubMed: 19915575]
- Simon D, Garcia-Garcia E, Gomez-Ramos A, Falcon-Perez JM, Diaz-Hernandez M, Hernandez F and Avila J, 2012 Tau overexpression results in its secretion via membrane vesicles. *Neurodegener. Dis* 10, 73–75. [PubMed: 22269430]

- Simpson RJ, Kalra H and Mathivanan S, 2012 ExoCarta as a resource for exosomal research. *J. Extracell. Vesicles* 1, 18374.
- Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr., Carter BS, Krichevsky AM and Breakefield XO, 2008 Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat. Cell Biol* 10, 1470–1476. [PubMed: 19011622]
- Sodar BW, Kittel A, Paloczi K, Vukman KV, Osteikoetxea X, Szabo-Taylor K, Nemeth A, Sperlagh B, Baranyai T, Giricz Z, Wiener Z, Turiak L, Drahos L, Pallinger E, Vekey K, Ferdinandy P, Falus A and Buzas EI, 2016 Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection. *Sci. Rep* 6, 24316. [PubMed: 27087061]
- Sterzenbach U, Putz U, Low LH, Silke J, Tan SS and Howitt J, 2017 Engineered Exosomes as Vehicles for Biologically Active Proteins. *Mol. Ther* 25, 1269–1278. [PubMed: 28412169]
- Street JM, Barran PE, Mackay CL, Weidt S, Balmforth C, Walsh TS, Chalmers RT, Webb DJ and Dear JW, 2012 Identification and proteomic profiling of exosomes in human cerebrospinal fluid. *J. Transl. Med* 10, 5. [PubMed: 22221959]
- Stuendl A, Kunadt M, Kruse N, Bartels C, Moebius W, Danzer KM, Mollenhauer B and Schneider A, 2016 Induction of alpha-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. *Brain* 139, 481–494. [PubMed: 26647156]
- Sui YT, Bullock KM, Erickson MA, Zhang J and Banks WA, 2014 Alpha synuclein is transported into and out of the brain by the blood-brain barrier. *Peptides* 62, 197–202. [PubMed: 25278492]
- Suire CN, Eitan E, Shaffer NC, Tian Q, Studenski S, Mattson MP and Kapogiannis D, 2017 Walking speed decline in older adults is associated with elevated pro-BDNF in plasma extracellular vesicles. *Exp. Gerontol* 98, 209–216. [PubMed: 28843509]
- Sun B, Dalvi P, Abadjian L, Tang N and Pulliam L, 2017 Blood neuron-derived exosomes as biomarkers of cognitive impairment in HIV. *AIDS* 31, F9–f17. [PubMed: 28692534]
- Thomas B and Beal MF, 2007 Parkinson's disease. *Hum. Mol. Genet* 16 Spec No. 2, R183–194. [PubMed: 17911161]
- Thompson AG, Gray E, Heman-Ackah SM, Mager I, Talbot K, Andaloussi SE, Wood MJ and Turner MR, 2016 Extracellular vesicles in neurodegenerative disease - pathogenesis to biomarkers. *Nat. Rev. Neurol* 12, 346–357. [PubMed: 27174238]
- Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ and Lotvall JO, 2007 Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol* 9, 654–659. [PubMed: 17486113]
- Van Deun J, Mestdagh P, Agostinis P, Akay O, Anand S, Anckaert J, Martinez ZA, Baetens T, Beghein E, Bertier L, Bex G, Boere J, Boukouris S, Bremer M, Buschmann D, Byrd JB, Casert C, Cheng L, Cmoach A, Daveloose D, De Smedt E, Demirsoy S, Depoorter V, Dhondt B, Driedonks TA, Dudek A, Elsharawy A, Floris I, Foers AD, Gartner K, Garg AD, Geeurickx E, Gettemans J, Ghazavi F, Giebel B, Kormelink TG, Hancock G, Helsmoortel H, Hill AF, Hyenne V, Kalra H, Kim D, Kowal J, Kraemer S, Leidinger P, Leonelli C, Liang Y, Lippens L, Liu S, Lo Cicero A, Martin S, Mathivanan S, Mathiyalagan P, Matusek T, Milani G, Monguio-Tortajada M, Mus LM, Muth DC, Nemeth A, Nolte-'t Hoen EN, O'Driscoll L, Palmulli R, Pfaffl MW, Primdal-Bengtson B, Romano E, Rousseau Q, Sahoo S, Sampaio N, Samuel M, Scicluna B, Soen B, Steels A, Swinnen JV, Takatalo M, Thaminy S, Thery C, Tulkens J, Van Audenhove I, van der Grein S, Van Goethem A, van Herwijnen MJ, Van Niel G, Van Roy N, Van Vliet AR, Vandamme N, Vanhauwaert S, Vergauwen G, Verweij F, Wallaert A, Wauben M, Witwer KW, Zonneveld MI, De Wever O, Vandesompele J and Hendrix A, 2017 EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. *Nat. Methods* 14, 228–232. [PubMed: 28245209]
- Van Deun J, Mestdagh P, Sormunen R, Cocquyt V, Vermaelen K, Vandesompele J, Bracke M, De Wever O and Hendrix A, 2014 The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. *J. Extracell. Vesicles* 3.
- Vella LJ, Greenwood DL, Cappai R, Scheerlinck JP and Hill AF, 2008 Enrichment of prion protein in exosomes derived from ovine cerebral spinal fluid. *Vet. Immunol. Immunopathol* 124, 385–393. [PubMed: 18501435]

- Vella LJ, Sharples RA, Lawson VA, Masters CL, Cappai R and Hill AF, 2007 Packaging of prions into exosomes is associated with a novel pathway of PrP processing. *J. Pathol* 211, 582–590. [PubMed: 17334982]
- Weng Y, Sui Z, Shan Y, Hu Y, Chen Y, Zhang L and Zhang Y, 2016 Effective isolation of exosomes with polyethylene glycol from cell culture supernatant for in-depth proteome profiling. *Analyst* 141, 4640–4646. [PubMed: 27229443]
- Winston CN, Goetzl EJ, Akers JC, Carter BS, Rockenstein EM, Galasko D, Masliah E and Rissman RA, 2016 Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimers Dement (Amst)* 3, 63–72. [PubMed: 27408937]
- Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG and Chopp M, 2013 Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. *J. Cereb. Blood Flow Metab* 33, 1711–1715. [PubMed: 23963371]
- Xu R, Greening DW, Zhu HJ, Takahashi N and Simpson RJ, 2016 Extracellular vesicle isolation and characterization: toward clinical application. *J. Clin. Invest* 126, 1152–1162. [PubMed: 27035807]
- Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW and Simpson RJ, 2018 Extracellular vesicles in cancer - implications for future improvements in cancer care. *Nature reviews. Clinical oncology* 15, 617–638.
- Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borrás FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, Colás E, Cordeiro-da Silva A, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NH, Hendrix A, Kierulf P, Kokubun K, Kosanovic M, Kralj-Iglic V, Kramer-Albers EM, Laitinen S, Lasser C, Lener T, Ligeti E, Line A, Lipps G, Llorente A, Lotvall J, Mancek-Keber M, Marcilla A, Mittelbrunn M, Nazarenko I, Nolte-’t Hoen EN, Nyman TA, O’Driscoll L, Olivan M, Oliveira C, Pallinger E, Del Portillo HA, Reventos J, Rigau M, Rohde E, Sammar M, Sanchez-Madrid F, Santarem N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van der Grein SG, Vasconcelos MH, Wauben MH and De Wever O, 2015 Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* 4, 27066. [PubMed: 25979354]
- Yuyama K, Sun H, Mitsutake S and Igarashi Y, 2012 Sphingolipid-modulated exosome secretion promotes clearance of amyloid-beta by microglia. *J. Biol. Chem* 287, 10977–10989. [PubMed: 22303002]
- Yuyama K, Sun H, Sakai S, Mitsutake S, Okada M, Tahara H, Furukawa J, Fujitani N, Shinohara Y and Igarashi Y, 2014 Decreased amyloid-beta pathologies by intracerebral loading of glycosphingolipid-enriched exosomes in Alzheimer model mice. *J. Biol. Chem* 289, 24488–24498. [PubMed: 25037226]
- Zabetian CP, Hutter CM, Factor SA, Nutt JG, Higgins DS, Griffith A, Roberts JW, Leis BC, Kay DM, Yearout D, Montimurro JS, Edwards KL, Samii A and Payami H, 2007 Association analysis of MAPT H1 haplotype and subhaplotypes in Parkinson’s disease. *Ann. Neurol* 62, 137–144. [PubMed: 17514749]
- Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A and Xiong Y, 2015 Effect of exosomes derived from multipotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. *J. Neurosurg* 122, 856–867. [PubMed: 25594326]
- Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G and Amigorena S, 1998 Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat. Med* 4, 594–600. [PubMed: 9585234]

Highlights

- Extracellular vesicles (EVs), including exosomes and (shedding) microvesicles, play critical roles in normal central nervous system (CNS) function and neurological disorders.
- EVs may travel between the CNS and peripheral circulation.
- Brain-derived EVs found in peripheral blood contain molecular signatures (proteins, RNAs, etc.) specific to the cells of origin and have potential clinical utility as biomarkers for CNS disorders.
- Modified EVs have potential as vehicles for the delivery of therapeutic agents into the CNS.
- Further development, optimization, and standardization of EV isolation and analysis techniques are required to enable interlaboratory data comparison and for the advancement of their clinical utility.

Figure 1, Diagram of possible EV transport mechanisms from the central nervous system to peripheral blood.

A) Direct translocation of EVs into capillaries or draining venules via the BBB; **B)** EVs passing through interstitial fluid into the CSF and then the venous system via the arachnoid granulations; **C)** EVs are transported into the perinasal lymphatics, with or without passage through the CSF, and then into the venous system. BBB, blood-brain barrier; CSF, cerebrospinal fluid; EV, extracellular microvesicle.

Figure 2, Potential clinical applications utilizing the bidirectional transfer of EVs between the brain and peripheral blood.

A) CNS EVs carrying disease-specific molecular signatures of CNS disorders can be transported from the brain to peripheral blood and isolated/analyzed based on CNS- or brain cell-specific surface markers. Their cargo of protein and nucleic acid is likely to reflect core pathogenic intracellular processes in their originating brain cells, and could serve as a novel biomarker source for CNS disorders. **B)** EVs carrying signal molecules can be transported via the BBB or other pathways from peripheral blood into the brain to target specific cell types. EVs, particularly those capable of transiting the BBB, may be modified to deliver therapeutic molecules in a variety of CNS conditions. BBB, blood-brain barrier; CNS, central nervous system; EV, extracellular microvesicle.

Table 1

Selected published studies using EVs as a source of biomarkers for CNS disorders.

Study	EV isolation	EV validation	Study cohort	EV analytes and outcomes*
Shi et al., 2014	Differential centrifugation and anti- L1CAM immunocapture	TEM, WB [†]	PD: 267 HC: 215	α -synuclein: PD $\uparrow$; associated with UPDRS motor in PD
Fiandaca et al., 2015	ExoQuick precipitation and anti- NCAM immunocapture	NTA	AD/MCI: 28/29 HC for AD: 57 FTD: 16 HC for FTD: 16	A β ₁₋₄₂ : AD/MCI $\uparrow$, FTD $\uparrow$ t-tau: AD/MCI $\uparrow$, FTD n.d. p-tau (T181P): AD/MCI $\uparrow$, FTD $\uparrow$ p-tau (S396P): AD/MCI $\uparrow$, FTD n.d.
			AD at 2 time points (pre-clinical and AD diagnosis): 24	A β ₁₋₄₂ : preclinical $\uparrow$, AD $\uparrow$ compared to HC; AD $\uparrow$ compared to preclinical t-tau: preclinical n.d., AD n.d. p-tau (T181P): preclinical $\uparrow$, AD $\uparrow$ compared to HC; AD n.d. compared to preclinical p-tau (S396P): preclinical $\uparrow$, AD $\uparrow$ compared to HC; AD n.d. compared to preclinical
Goetzl et al., 2015b	ExoQuick precipitation and anti- L1CAM immunocapture	NTA (described in Fiandaca et al., 2015)	AD/MCI: 8/18 HC for AD: 26 FTD: 16 HC for FTD: 16	lysosomal proteins (cathepsin D, LAMP-1, etc.): AD/MCI $\uparrow$, FTD n.d. (except for cathepsin D, FTD $\uparrow$); AD/MCI $\uparrow$ compared to FTD HSP70: AD/MCI $\downarrow$, FTD $\downarrow$; AD/MCI $\uparrow$ compared to FTD
			AD/MCI at 2 time points (pre-clinical and AD/MCI diagnosis): 11/9	lysosomal proteins (cathepsin D, LAMP-1, etc.): preclinical $\uparrow$, AD/MCI $\uparrow$ (except for cathepsin D, FTD $\uparrow$) HSP70: preclinical $\downarrow$, AD/MCI $\downarrow$ All analytes: AD/MCI n.d. compared to preclinical
Goetzl et al., 2015a	ExoQuick precipitation and anti- L1CAM immunocapture	NTA (described in Fiandaca et al., 2015)	AD/MCI: 8/16 HC for AD: 24 FTD: 10 HC for FTD: 10	cellular survival factors (LRP6, HSF1, and REST): AD/MCI $\downarrow$, FTD n.d. (except for REST, FTD $\uparrow$)
			AD/MCI at 2 time points (pre-clinical and AD/MCI diagnosis): 9/7	cellular survival factors (LRP6, HSF1, and REST): preclinical $\downarrow$, AD/MCI $\downarrow$; AD/MCI $\downarrow$ compared to preclinical
Kapogiannis et al., 2015	ExoQuick precipitation and anti- L1CAM immunocapture	NTA (described in Fiandaca et al., 2015)	AD/MCI: 10/16 HC for AD: 26 FTD: 16 HC for FTD: 16	P-Ser312-IRS-1: AD/MCI $\uparrow$, FTD $\uparrow$ P-pan-Tyr-IRS-1: AD/MCI $\downarrow$ FTD n.d.
			AD at 2 time points (pre-clinical and AD diagnosis): 22	P-Ser312-IRS-1: preclinical $\uparrow$, AD $\uparrow$; AD n.d. compared to preclinical P-pan-Tyr-IRS-1: preclinical $\downarrow$, AD $\downarrow$; AD n.d. compared to preclinical
Shi et al., 2016	Differential centrifugation and anti- L1CAM immunocapture	TEM, WB (described in Shi et al., 2014), and NTA [†]	PD: 91 AD: 106 HC: 106	t-tau: PD $\uparrow$, AD n.d.; associated with disease duration in PD
Winston et al., 2016	ExoQuick precipitation and anti- L1CAM immunocapture	TEM, NTA	AD: 10 MCI converting to AD: 20 stable MCI: 20 HC: 10	A β ₁₋₄₂ , p-tau (T181P), p-tau (S396P): AD $\uparrow$, converting MCI $\uparrow$ compared to HC and stable MCI NRGN, REST: AD $\downarrow$, converting MCI $\downarrow$ compared to HC and stable MCI
Goetzl et al., 2016a	ExoQuick precipitation and anti- L1CAM immunocapture	NTA, WB, and TEM (described in Fiandaca et al., 2015 and Mustapic et al., 2017)	AD: 12 HC for AD: 12 FTD: 16 HC for FTD: 16	synaptic proteins (NRGN, synaptotagmin, synaptopodin, synaptophysin, GAP43, etc.): AD $\downarrow$ FTD $\downarrow$ (except for GAP43, FTD n.d.); AD $\downarrow$ compared to FTD

Study	EV isolation	EV validation	Study cohort	EV analytes and outcomes*
				Synaptopodin, synaptotagmin, and synaptophysin: associated with MMSE or ADAS-cog
			AD at 2 time points (pre-AD and AD dementia): 9 FTD at 2 time points (pre- FTD and FTD dementia): 10	Synaptotagmin, synaptophysin, and NGRN: pre-AD ↓, pre-FTD ↓; AD ↓ compared to pre-AD, FTD n.d. compared to pre-FTD Synaptopodin and GAP43: pre-AD ↓, pre- FTD n.d.; AD ↓ compared to pre-AD, FTD ↓ compared to pre-FTD
Goetzl et al., 2016b	ExoQuick precipitation and anti- GLAST immunocapture	NTA (described in Fiandaca et al., 2015 and Mustapic et al., 2017)	AD/MCI: 12 HC for AD: 10 FTD: 14 HC for FTD: 10	BACE-1, sAPPβ: AD/MCI ↑, FTD n.d. GDNF: AD/MCI ↓, FTD n.d.
Hamlett et al., 2017	ExoQuick precipitation and anti- L1CAM immunocapture	NTA, WB, and TEM (described in Fiandaca et al., 2015 and Mustapic et al., 2017)	DS: 47 HC: 37	Aβ ₁₋₄₂ , p-tau (T181P), p-tau (S396P): DS ↑
Goetzl et al., 2018b	ExoQuick precipitation and anti-GLAST (ACSA-1) immunocapture	NTA, WB, and TEM (described in Goetzl et al., 2016b and Goetzl et al., 2016a)	Early AD/MCI: 28 HC: 28	IL-6, TNF-α, and IL-1β: AD/MCI ↑ Complement effector proteins (C1q, C4b, C3d, factor B, factor D, etc.): AD/MCI ↑ Complement regulatory proteins (CD59, DAF, etc.): AD/MCI ↓
			AD at 2 time points (pre-AD and AD dementia): 16	Complement effector proteins, IL-6, TNF-α, and IL-1β: AD ↑ compared to pre-AD Complement regulatory proteins: AD ↓ compared to pre-AD ↓

* ↑, higher; ↓, lower; n.d., no difference compared to HC except where indicated otherwise.

† The brain-to-blood transportation of EVs was confirmed in mice.

Aβ₁₋₄₂, amyloid beta 1–42; ACSA-1, astrocyte cell surface antigen-1 (i.e., GLAST); AD, Alzheimer disease; ADAS-cog, AD assessment scale-cognitive subscale; BACE-1, β-site amyloid precursor protein-cleaving enzyme 1; CNS, central nervous system; DAF, decay-accelerating factor; DS, Down syndrome; EV, extracellular vesicle; FTD, frontotemporal dementia; GAP43, growth-associated protein 43; GDNF, glial-derived neurotrophic factor; GLAST, glutamine aspartate transporter; HC, healthy control; HSF1, heat-shock factor 1; HSP70, heat-shock protein 70; IL, interleukin; IRS-1, insulin receptor substrate 1; L1CAM, L1 cell adhesion molecule; LAMP-1, lysosomal-associated membrane protein 1; LRP6, low-density lipoprotein receptor-related protein 6; MCI, mild cognitive impairment; MMSE, mini-mental state examination; NRGN, neurogranin; NCAM, neural cell adhesion molecule; NTA, nanoparticle tracking analysis; p-tau, phosphorylated tau; PD, Parkinson disease; REST, repressor element 1-silencing transcription factor; sAPPβ, soluble amyloid precursor protein β; t-tau, total tau; TEM, transmission electron microscopy; TNF-α, tumor necrosis factor-α; UPDRS, unified Parkinson disease rating scale; WB, Western blot.

Table 2

Advantages and limitations of methods to isolate extracellular (micro)vesicles.

Method for EVs isolation and principle		Purity*	Yield*	Advantages (A) / Disadvantages (D)
(Differential) Ultracentrifugation	Size, shape, and density (when used with sucrose cushion or density-gradient) based isolation	+ (regular); +++ (density-gradient)	++ (regular); + (density-gradient)	A: No sample pre-treatment required; Large sample capacity (regular); Density-gradient generally reduces contaminants (current "gold" standard) D: Requires special equipment; Cumbersome, long run time, and labor intensive; High speed centrifugation may damage EVs; Mixed EV population and contamination from non-EV protein aggregates (regular)
(Ultra-) Filtration	Size based isolation; generally membrane-based, specialized designs include flow field-flow fractionation, hydrostatic filtration dialysis, etc.	++	++	A: Generally fast, easy to use; May have large sample capacity D: Some designs may require special equipment; Risk of EV loss due to pressure-induced deterioration and vesicle trapping to membrane
Size exclusion chromatography	Size based	++	++	A: Easy to use; Isolation of specific size of EVs with high purity; Moderate sample capacity D: Sample loss during isolation; Time consuming; May require special equipment
Precipitation	Mostly polymer-based (e.g., ExoQuick™ and Total Exosome Isolation™); can be chemical or other types (e.g., lectins) of precipitation	+	+++	A: Easy to use; no specialized equipment required D: Can be time consuming; no separation of different types of vesicles; Contamination from non- EV proteins, nucleic acids
Immunoaffinity capture	Antibodies against specific EV surface markers	+++	+	A: Isolation of specific EVs with relatively high purity; possibility to enrich EV subpopulations D: Time consuming; Specific antibodies required, high reagent cost
Microfluidic methods	Devices include acoustic nanofilter, nanowire on micropillars, or an immuno- affinity approach to isolate EVs	+ to ++	+ to ++	A: Easy to use; May be fast and able to isolate specific EVs in some designs D: Usually low sample capacity; Lack of large scale tests on clinical samples

*
+, low; ++, medium; +++, high.