



Review Article

Molecular signatures in exosomes as diagnostic markers for neurodegenerative disorders

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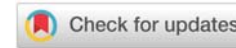
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Abstract

Exosomes are small membrane-bound entities of endocytic origin. These membrane-derived, extracellular vesicles have been shown to be secreted by a number of cell types such as adipocytes, platelets, cardiac progenitor cells, muscle cells, mesenchymal stem cells, lymphocytes, tumor cells, embryonic stem cells, umbilical cord blood-derived cells and cells in the central nervous system including neurons, neuroglial cells etc., These extracellular vesicles contain various protein, lipid, pro-inflammatory cytokines and RNA species whose content is altered under pathological diseased conditions of the CNS. Currently, the techniques available to diagnose neurodegenerative disorders involve analysing the physiological levels of certain proteins in the Cerebrospinal Fluid (CSF) and checking for extracellular senile plaque formation (protein aggregation and accumulation) in the brain using MRI/CT scans. These techniques are quite expensive, invasive and painful in nature as collecting the CSF and accessing the brain area are difficult. In the past few years, there is a growing interest on using exosomes for diagnosis of neurodegenerative disorders due to their easy availability from most of the biological fluids including the blood, urine, saliva, breast milk, semen etc., their extremely high disease-specific bio-molecular signature/profile, the ability of exosomes to cargo a variety of biomolecules in between cells and their capacity to cross the blood-brain barrier. These make them a potential biomarker for neurodegenerative disorder. This review begins with a brief introduction about exosomes and focuses mainly on using exosomes for diagnosing major neurodegenerative diseases like Prion disease, Amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, and Alzheimer's disease.

Abbreviations

AD: Alzheimer's Disease; ALS: Amyotrophic Lateral Sclerosis; APP: β -Amyloidprecursor Protein; A β : Amyloid- β Peptide; A β_{42} (Amyloid- β peptide which is 42 amino acids long); CJD: Creutzfeldt-Jakob Disease; CNS: Central Nervous System; CSF: Cerebral Spinal Fluid; DNA: Deoxy ribonucleic Acid; ESCRT: Endosomal Sorting Complex Required for Transport; GTPase: Guanidine Triphosphatase; HD: Huntington's Disease; HSP: Heat Shock Protein; htt: huntingtin; ILVs: Intraluminal vesicles; Lincrna: Long Intergenic Non-Coding RNA; Incrna: long non-coding RNA; LAMP-1: Lysosome-Associated Membrane Protein 1; Mhtt: Mutant htt; MCI: Mild Cognitive Impairment; miRNA: micro RNA; MRI/CT: Magnetic Resonance Imaging/ Computed Tomography; MRNA: Messenger RNA; MVB: Multivesicular body; nc-RNA: non-coding RNA; NRG1: Neurogranin; PD: Parkinson's Disease; PolyQ: Polyglutamine; PS1: Presenilin-1; PS2: Presenilin-2; PrP: Prion Proteins;

REST: Repressor Element 1-Silencing Transcription Factor; RNA: Ribo Nucleic Acid; rRNA: Ribosomal RNA; Snrna: Small Nuclear RNA; Snorna: Small Nucleolar RNA; mSOD1: Mutant Cu-Zn Superoxide Dismutase1; TARDBP (TDP-43) gene tRNA: Transfer RNA; TSEs: Transmissible Spongiform Encephalopathies

Key messages

In the past decade, progressive research on the exosomes led to the identification of its potential role in intercellular communication by the transfer of various biomolecules including proteins and miRNAs whose contents vary depending from on the physiological to pathological conditions. The major proteins and miRNAs which are involved in neurodegenerative disorders like prion disease, Amyotrophic Lateral Sclerosis (ALS), Huntington disease, Parkinson's disease, Alzheimer's disease etc., are found to be differentially expressed in the exosomes isolated from patients when compared to the healthy

individuals. This makes exosomes as a potential diagnostic biomarker. This review is providing an insight into using exosomes for diagnosing these major neurodegenerative disorders.

Introduction

Exosomes are small cell-derived vesicles whose secretion has been reported for a number of cell types and are present in various biological fluids and Central Nervous System (CNS) tissues. These are the extracellular vesicles that are released from cells due to the fusion of the Multivesicular Body (MVB), an intermediate endocytic compartment, with the plasma membrane. This releases the Intraluminal Vesicles (ILVs) into the extracellular milieu, known as exosomes[1]. Exosomes are known to have various important functions under both physiological and pathological conditions in the CNS, such as the cell-to-cell communication which is done by the transfer of exosomes between neurons [2].

Neurons make up the nervous system which comprises the brain and spinal cord. Neurons lack the ability to reproduce or renew themselves, as a result of which the body is unable to replace them if dead or damaged. Neurodegenerative disorders are chronic and fatigue conditions resulting in the death of neurons causing ataxias or dementias. Dementia refers to a syndrome that is characterized by progressive deterioration of cognitive functions. Few examples of neurodegenerative diseases include Prion disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease (HD), Parkinson's Disease (PD), Alzheimer's Disease (AD) [3].

Evidence is accumulating that exosomes play a key role in processes such as coagulation and intercellular signalling. Consequently, there is a growing interest in the clinical applications of exosomes as they can potentially be used for diagnosis, prognosis, therapy and as biomarkers for health and a variety of diseases. First of all, Exosomes carry significant amounts of molecular constituents including proteins, lipids and nucleic acids whose content and composition solely depend and vary with their tissue of origin under pathological conditions [4,5]. Second, exosomes can be concentrated to significantly increase the detection sensitivity and their ability to cross the blood-brain barrier in both the directions [6], helps to overcome the problem of the circulating proteins and nucleic acids getting diluted in the bloodstream (most of them originate from other tissues besides neurons due to the tight regulation of the blood-brain barrier in molecule transport). Third, isolation of exosomes can be done using painless-invasive methods from easily available biological fluids like blood, saliva, urine etc [7-9], making it a potential biomarker for the diagnosis of neurodegenerative disorders.

This review is focused on using exosomes as biomarkers for diagnosing various neurodegenerative disorders. Here, we first provide an overview of exosomes and in the later part; we discuss using exosomes as a diagnostic tool in the context of Prion disease, ALS, HD, PD, and AD.

Exosomes: Origin, composition and functions

Exosomes are lipid bi-membranous entities having a

diameter of 30–120 nm and are of endocytic origin that is present in almost all biological fluids including blood, saliva, semen, urine, breast milk, the culture medium of cell cultures and extracellular matrix bioscaffolds (non-fluid). Invagination of a cell's plasma membrane forms small intracellular vesicles which upon fusion results in early endosomes [10]. On maturation, these early endosomes give rise to the intraluminal vesicles (ILVs) [10,11]. The accumulation of cytoplasmic molecules such as proteins, messenger RNA (mRNA) and small non-coding microRNAs (miRNAs) inside the ILVs is aided by a subset of molecules called endosomal sorting complex required for transport (ESCRT). Accumulation of ILVs in the late endosomes forms a Multivesicular body (MVB). MVBs in turn fuse with the plasma membrane releasing out exosomes [10,12].

Key components of a typical exosome include Normally PrP^c, a protein with adhesion molecules, integrins (β 1, β 2, β 4, ICAM-1), tetraspanins (CD9, CD81, CD63, CD82), lipid rafts (cholesterol, flotilins), immuno regulator molecules (MHC-I, MHC-II, MICA, MICB, ULBP1), membrane transporters (annexins, RabGTPase), heat shock proteins (HSP70, HSP90), cytoskeletal proteins, DNA, mRNAs, mi-RNAs, r-RNAs, t-RNAs, sno-RNAs, snRNAs, Inc-RNAs, Linc-RNAs, nc-RNAs, α -synuclein, alix, TSG101 etc., [13]. Specific databases such as EXOCARTA are available to obtain information regarding the composition of exosomes [14].

Exosomes released from the CNS cells such as neurons, oligodendrocytes etc., play a significant role in the communication between neuron-neuron, neuron-glia and in the regeneration of damaged axons. These endosome-derived vesicles carry specific RNA and protein cargo that regulates certain signal transduction pathways of the recipient cells [2].

Exosomes in disease diagnostics

Prion disease: Prions, meaning proteinaceous infectious particles are composed of prion proteins (PrP), which causes transmissible spongiform encephalopathies (TSEs), including Creutzfeldt-Jakob disease (CJD) in humans [15]. Normally PrP^c, a protein with a well-defined 3D structure is present on the surface membrane of many cells. This protein has been reported to play a significant role in cell-cell adhesion and intracellular signalling [16]. Under pathological conditions, this protein abnormally folds and clumps in the brain forming PrP^{Sc}, leading to the damage of brain tissues [15]. This infectious isoform of PrP (PrP^{Sc}) has the ability to convert normal PrP (PrP^c) into infectious isoforms by causing conformation changes in them [17], making prion disease a progressive and a fatal neurodegenerative disorder.

Dissemination of prions is found to be mediated through exosomal pathways. Exosomes were identified as intercellular carriers of both PrP^{Sc} and PrP^c [18,19]. The ESCRT machinery and the need for ceramide, is significant for exosome biogenesis. Hence, the spread of infectious prions can be controlled by altering the ESCRT complex and decreasing ceramide levels [20]. However, data from certain studies showed that in some cases, the prion infection might not necessarily be associated



with PrP^{Sc} (i.e.) Transmission of prion infection has occurred even in extremely low levels or in the absence of detectable abnormal Prion Proteins, thereby eliminating the possibility for considering the presence of PrP^{Sc} in exosomes as a potential biomarker for diagnosis of the disease [21,22]. Generally, under pathological conditions, the miRNA expression levels are altered. In case of prion infection, 15 miRNAs were found to be de-regulated; miR-342-3p, miR-320, let-7b, miR-328, miR-128 and miR-139-5p were up-regulated over 2.5 folds and miR-338-3p, miR-337-3p were down-regulated over 2.5 folds. Hence, the prion-induced neurodegeneration is found to be associated with a highly conserved, disease specific deregulation and differential expression pattern of a unique set of miRNAs. Deregulation of certain miRNA seen in prion disease might be a response to the abnormal accumulation of PrP^{Sc} [23,24]. Exosomes that were released from prion-infected neural cells were observed to contain a deregulated miRNA cargo (i.e.) the levels of let-7b, let-7i, miR-128a, miR-21, miR-222, miR-29b, miR-342-3p and miR-424 were increased and the levels of miR-146a was decreased when compared to the miRNA content of exosomes released from normal brain tissues. Therefore, the circulating exosomes released from prion-infected brain tissues have extremely specific, conserved and a distinct miRNA signature that can be exploited for disease diagnostics [25]. Prions have been identified to be present in blood plasma exosomes, and are spread by transfusion of blood. The presence of infectious prions in blood-circulating extracellular vesicles makes plasma exosomes a novel diagnostic tool for prion disease [26,27].

Amyotrophic lateral sclerosis: Amyotrophic lateral sclerosis is a chronic neurodegenerative disease, characterized by progressive muscular paralysis which occurs as a result of motor neuron degeneration (i.e.) death of upper motor neurons in the primary motor cortex and death of lower motor neurons in the corticospinal tracts, brainstem and spinal cord. ALS results due to a dominant mutation in the cytosolic Cu-Zn superoxide dismutase1 (SOD 1) gene and TARDBP (TDP-43) gene. Aggregation of TDP-43 in the cytoplasm is a pathological hallmark of ALS [28]. A distinctive feature that can be observed under pathological conditions include abnormal immunoreactive protein (TDP-43) aggregations in degenerating lower motor neurons and loss of upper motor neurons with intra neuronal ubiquitin-immuno reactive inclusions [29,30].

Exosomes have been reported to play an important role in the secretion and propagation of TDP-43 aggregates. The exosomes secreted from Neuro2a cells and primary neurons in human ALS brains, were reported to have up regulated levels of TDP-43 full length and C-terminal fragment species [28]. Exosomal transfer of mutant Cu-Zn superoxide dismutase 1 (mSOD1) from one cell to another is known to play a significant role in ALS dissemination. The secretion of SOD1 has been studied using stable mouse motor neuron-like NSC-34 cell lines overexpressing human SOD1 (both wild-type hSOD1^{wt} and mutant hSOD1^{G93A}) as ALS cell models. This study resulted in an important finding that SOD1, being a cytoplasmic protein that lacks a signal peptide, depends on exosomes for

its extracellular export [31]. In order to identify distinct RNA profiles from exosomes secreted by mutant cells, a study was conducted, which assessed a set of inflammatory-associated miRNAs in NSC-34 MN-like cells, which were transfected with mutant SOD1(G93A) and the effects produced by their derived exosomes (mSOD1 exosomes) in the activation and polarization of the recipient N9 microglial cells. The results showed that the expression level of inflammatory-associated miR-124 was highly increased in mSOD1 NSC-34 cells and mSOD1 exosomes. Thus, this data suggests that the miR-124 is translocated to the exosomes from mSOD1 MNs and this modulation in mSOD1 exosomal cargo, in turn determines the early and late phenotypic alterations in the recipient N9-microglial cells, making them a promising therapeutic agent in halting microglia activation and associated effects in motor neuron degeneration [32]. Exosomes which were obtained from the blood plasma samples of ALS patients were analysed to identify ALS-associated, specific miRNA signatures. On subjecting the exosomes obtained from ALS patients and healthy controls to next generation sequencing, differentially expressed miRNAs were identified. This data was subsequently validated by droplet digital PCR which showed elevated levels of 5 miRNAs and reduced levels of 22 miRNAs in the exosomes collected from patients with ALS as compared with healthy control subjects. Exosomes that were secreted by ALS brain tissues were found to have a deregulated miRNA profile including miR-9-5p, miR-183-5p, miR-338-3p and miR-1246. MiR-15a-5p and miR-193a-5p. Therefore, exosomes released from ALS brain tissues, which can cross the blood-brain barrier and enter the circulatory system, have highly specific and distinct miRNA signatures making them a potential biomarker for disease diagnostics [33].

Huntington disease: Huntington disease is an autosomal dominant disorder, characterized by neuronal degeneration which is severe mainly in the striatum and the deeper cortical layers. HD is a neurodegenerative condition that is caused by mutations that expands CAG repeats, which is in turn translated into abnormally long stretches of polyglutamine (polyQ) at the N terminus of huntingtin(htt) [34]. The mutant htt (mHtt) and the expanded repeat RNA, aggregates and accumulates in the intra neuronal regions causing toxicity thereby leading to neuron degeneration in HD. HD is caused by deleterious gain-of-function mechanisms conferred on the encoded mutant proteins [35,36].

Exosomes are found to be associated with the spread of mHtt (polyQ proteins) and expanded HD-associated repeat RNA [37]. Studies conducted on in vivo models (involving a co-culture of the SH-SY5Y human neuroblastoma cell lines and mouse neural stem cells with 143 CAG repeat fibroblasts) and in vitro models (newborn wild-type mice) suggests that, under pathological conditions, exosomes cargo the mHtt in between cells, thereby transmitting HD [38]. There exists a hypothesis that mutant proteins involving mHtt and various other miRNA that are found in exosomes from a wide variety of sources highly intersect with the HD datasets. This increased overlap of mutant proteins and miRNA from both exosome and HD datasets suggests both mechanistic and biomarker links [39].

Parkinson's disease: Parkinson's disease is the second most common and chronic neurodegenerative disease after Alzheimer's. Parkinson's neuropathological hallmarks can be characterized by a progressive loss of dopaminergic neurons in the substantia nigra, along with the presence of intracellular inclusions of aggregated α -synuclein (deposits of ubiquitinated protein in neural cytosol) termed Lewy bodies. Mitochondrial dysfunction has also been proposed to play an important role in the pathogenesis of PD [40-42].

On evaluating the levels of α -synuclein in exosomes, which are relatively more specific to the CNS, it was found that the concentration of cerebrospinal fluid (CSF) exosomal α -synuclein was lower in PD patients when compared to healthy controls. In contrast, it has also been reported that the concentrations of plasma exosomal α -synuclein are higher in PD patients compared to the controls [43]. Exosomes have also been found to be present in the saliva of PD patients and the levels of α -synOlig, α -synOlig/ α -synTotal in salivary exosomes are comparatively higher in PD patients than in controls [44]. Blood plasma levels of exosomes derived from various sources such as neurons, astrocytes and oligodendrocytes were quantified in diseased patients and healthy controls to formulate a correlation between the exosome levels and PD. Results showed that the plasma levels of neuron-derived exosomes (characterized using the biomarkers CD81 and SNAP25) were significantly higher in PD compared to controls [45]. On analysing the proteomic data of exosomes isolated from serum samples of PD patients, it was found that 23 proteins including Syntenin 1 were differentially abundant in Parkinson's patients [46]. MicroRNA profiling was performed on exosomal miRNA isolated from CSF of Parkinson's patients and the data showed that there was a significant up-regulation in 16 exosomal miRNAs including miR-10a-5p, let-7g-3p, miR-153 and miR-409-3p and 11 exosomal miRNAs including miR-1 and miR-19b-3p were under regulated in PD CSF when compared to healthy controls [47]. Global microRNA expression profiles were obtained from circulating plasma exosomal miRNA in diseased patients and healthy controls, as a result 13 most differentially-expressed miRNAs in PD, namely, miR-548b-3p, miR-1307, miR-647, miR-505, miR-192*, miR-626, miR-506, miR-1826, miR-222, miR-572, miR-671-5p, miR-1225-5p and miR-9* were identified. Data show that under pathological conditions, there is a significant over expression of exosomal miRNA-331-5p and the levels of exosomal miR-505 were significantly lower in PD Plasma compared to controls. k-TSP algorithm has been used to identify 9 pairs of PD-predictive miRNA classifiers, namely, miR-1307/miR-632, miR-1225-5p/miR-891b, miR-579/miR-708*, miR-647/miR-99a*, miR-1826/miR-450b-3p, miR-506/miR-1253, miR-488/miR-518c*, miR-192*/miR-485-5p and miR-200a/miR-455-3p. The above-mentioned pairs can be interpreted as, for the first pair miR-1307/miR-632, if the expression of miR-1307 is significantly higher than that of miR-632, then the patient has PD else they are normal. A similar interpretation pattern can be applied for the rest of the miRNA k-TSP pairs as well [48,49]. This data as a whole suggests that exosomes play a significant role in the pathogenesis of PD by acting as an intercellular carrier of α -synuclein and various miRNAs, thus can be used as a potential diagnostic biomarker for PD.

Alzheimer's disease: Alzheimer's is a chronic, progressive and most common neurodegenerative disorder which causes irreversible damage and death of neurons, mainly in the cortex and hippocampus. On average, two thirds of all dementia cases (42 to 81 percentage) are Alzheimer's types [40]. The pathological hallmarks of AD usually include the deposition of β -amyloid fibrils (encoded by A β PP) and neurofibrillary tangles, composed of abnormally hyperphosphorylated microtubule binding protein tau (encoded by MAPT). The deposits of the Amyloid- β peptide (A β) in the form of extracellular senile plaques is the cleavage product of β -amyloid precursor protein (APP) by α -, β - and γ - secretases. In particular, the γ - secretase cleaves APP into A β_{42} (Amyloid- β peptide which is 42 amino acids long) which has pathogenetic significance as it forms the toxic insoluble fibrils that deposit and accumulates as extracellular senile plaques causing AD. In short, Alzheimer's is associated with mutations in either one of the three proteins namely, APP, presenilin-1 (PS1) or presenilin-2 (PS2) (the catalytic subunit of γ - secretase is composed either of PS1 or PS2) [50,51].

Quantification of the levels of various AD related proteins in plasma neuronal derived exosomes (NDEs) led to a finding that the levels of P-S396-tau, P-T181-tau and A β 1-42 in plasma NDEs were extremely high whereas, the levels of neurogranin (NRGN) and the Repressor Element 1-Silencing Transcription factor (REST) were significantly reduced in Alzheimer's patients and patients with Mild Cognitive Impairment (MCI) converting to AD when compared to those in cognitively normal controls and patients with a stable MCI [52,53]. Also, the plasma NDE levels of cathepsin D, ubiquitinated proteins and lysosome-associated membrane protein 1 (LAMP-1) were significantly higher and the levels of heat-shock factor-1, heat-shock protein 70, and low-density lipoprotein receptor-related protein 6 were significantly lower in AD patients when compared to healthy controls [54]. On measuring the expression levels of Micro RNA in plasma exosomes, it was found that there was a significant difference in the expression levels of 20 miRNAs namely, miR-185-5p, miR-23b-3p, miR-29b-3p, miR-125b-5p, miR-138-5p, miR-24-3p, miR-139-5p, miR-150-5p, miR-152-3p, miR-338-3p, miR-342-3p, miR-342-5p, miR-548at-5p, miR-141-3p, miR-659-5p, miR-3613-3p, miR-4772-3p, miR-3916, miR-5001-3p and miR-3065-5p among the AD group, especially, miR-342-3p was significantly down-regulated in Alzheimer's patients [55]. Studies indicate that the overexpression of microRNA - miR-193b significantly represses the mRNA and protein expression of APP. Thus, the level of exosomal miR-193b in blood and CSF was found to be significantly lowered in Alzheimer's patients as compared to the controls [56]. Release of the above-mentioned Alzheimer's related proteins and miRNAs in plasma NDEs occur approximately 10 years prior to the onset of the disease, making neuronal derived exosomes a novel biomarker for Alzheimer's diagnosis.

Conclusion

In the last decade, there is a growing interest in using exosomes as a potential disease diagnostic tool. Initially, waste material disposal from cells was considered as the only known

function of exosomes. However, progressive research on these extracellular vesicles led to the identification of its intercellular communication ability that transfers various biomolecules including proteins and RNA from one cell to another whose contents vary from physiological to pathological conditions (Figure 1). Their ability to cross the blood-brain barrier and their high disease-specific biomolecular signature or profile provide us with information regarding the status of the CNS. Although there has been a great development made in exosome isolation and analysis techniques, further studies and advanced research works are required regarding the use of exosomes in disease diagnosis and therapeutics.

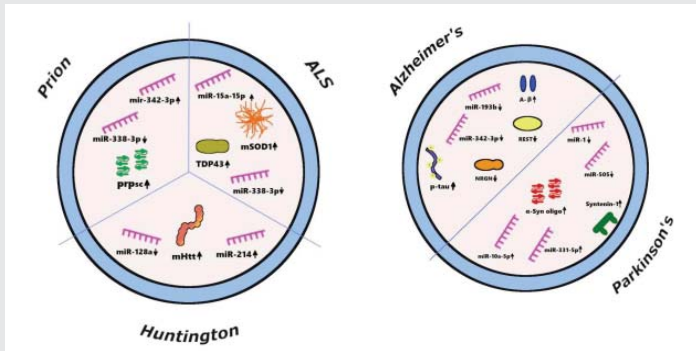


Figure 1: Representation of differentially expressed miRNA and protein biomarkers in exosomes for diagnosis of neurodegenerative disorders.

↑ - Signifies up-regulated biomolecules, ↓ - Signifies down-regulated biomolecules

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References

- Edgar JR (2016) What are exosomes, exactly? BMC Biol 14: 46. [Link: https://bit.ly/2MwvtWC](https://bit.ly/2MwvtWC)
- Kuo WP, Amphornrat J, Thilemann S, Goebels S, Nave K (2013) Neurotransmitter-Triggered Transfer of Exosomes Mediates Oligodendrocyte - Neuron Communication. PLoS Biol 11: e1001604. [Link: https://bit.ly/3cxQ2Px](https://bit.ly/3cxQ2Px)
- Dening T, Sandilyan MB (2015) Dementia : definitions and types. Nurs Stand 29: 37-42. [Link: https://bit.ly/3cvqQsZ](https://bit.ly/3cvqQsZ)
- Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, et al. (2015) Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. Alzheimer's Dement 11: 600-607. [Link: https://bit.ly/2ABlglp](https://bit.ly/2ABlglp)
- Saman S, Kim W, Raya M, Visnick Y, Miro S, et al. (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. [Link: https://bit.ly/2UdjUKL](https://bit.ly/2UdjUKL)
- García-romero N, Carrión-navarro J, Esteban-rubio S, Fernández-carballal C, de Mendivil AO, et al. (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. [Link: https://bit.ly/3cA5AT5](https://bit.ly/3cA5AT5)
- Aryani A, Denecke B (2016) Exosomes as a Nanodelivery System : a Key to the Future of Neuromedicine? Mol Neurobiol 53: 818-834. [Link: https://bit.ly/3dC3UJY](https://bit.ly/3dC3UJY)
- Boukouris S, Mathivanan S (2015) Exosomes in bodily fluids are a highly stable resource of disease biomarkers. Proteomics Clin Appl 9: 358-367. [Link: https://bit.ly/3cA5GtV](https://bit.ly/3cA5GtV)
- Kawikova I, Askenase PW (2015) Diagnostic and therapeutic potentials of exosomes in CNS diseases. Brain Res 1617: 63-71. [Link: https://bit.ly/2XwSqlw](https://bit.ly/2XwSqlw)
- Emanueli C, Shearn AIU, Angelini GD, Sahoo S (2015) Exosomes and exosomal miRNAs in cardiovascular protection and repair. Vascul Pharmacol 71: 24-30. [Link: https://bit.ly/3gOZKAu](https://bit.ly/3gOZKAu)
- Stoorvogel W, Strous GJ, Geute HJ, Oorschot V, Schwartz AL (1991) Late Endosomes by Maturation Derive from Early Endosomes. Cell 65: 417-427. [Link: https://bit.ly/3eSyXBu](https://bit.ly/3eSyXBu)
- Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, et al. (1996) B lymphocytes secrete antigen-presenting vesicles. J Exp Med 183: 1161-1172. [Link: https://bit.ly/3gXP0A3](https://bit.ly/3gXP0A3)
- Mathivanan S, Fahner CJ, Reid GE, Simpson RJ (2012) ExoCarta 2012 : database of exosomal proteins, RNA and lipids. Nucleic Acids Res 40: 1241-1244. [Link: https://bit.ly/2zUyDUf](https://bit.ly/2zUyDUf)
- Keerthikumar S, Chisanga D, Ariyaratne D, Saffar H AI, Anand S, et al. (2016) ExoCarta : A Web-Based Compendium of Exosomal Cargo. J Mol Biol 428: 688-692. [Link: https://bit.ly/2AJnYLP](https://bit.ly/2AJnYLP)
- Collinge J (2001) Prion Diseases of Humans and Animals : Their Causes and Molecular Basis. Annu Rev Neurosci 24: 519-550. [Link: https://bit.ly/2UsUIWv](https://bit.ly/2UsUIWv)
- Solis GP, Schrock Y, Geiss C, Luncz L, Thomanetz V, et al. (2009) Regulation of Embryonic Cell Adhesion by the Prion Protein. PLoS Biol 7: e55. [Link: https://bit.ly/3cGMWJq](https://bit.ly/3cGMWJq)
- Baldwin MA, Pan KM, Nguyen J, Huang Z, Groth D, et al. (1994) Spectroscopic characterization of conformational differences between PrPC and PrPSc: an α -helix to β -sheet transition. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 343: 435-441. [Link: https://bit.ly/2UbFvDy](https://bit.ly/2UbFvDy)
- Alais S, Simoes S, Baas D, Lehmann S, Darlix JL, et al. (2008) Mouse neuroblastoma cells release prion infectivity associated with exosomal vesicles. BIOL CELL 100: 603-615. [Link: https://bit.ly/3dFdkEE](https://bit.ly/3dFdkEE)
- Fevrier B, Vilette D, Archer F, Loew D, Faigle W, et al. (2004) Cells release prions in association with exosomes. Proc Natl Acad Sci U S A 101: 9683-9688. [Link: https://bit.ly/2MuYzZ7](https://bit.ly/2MuYzZ7)
- Vilette D, Laulagnier K, Huor A, Alais S, Simoes S, et al. (2015) Efficient inhibition of infectious prions multiplication and release by targeting the exosomal pathway. Cell Mol Life Sci 72: 4409-44027. [Link: https://bit.ly/370m7P2](https://bit.ly/370m7P2)
- Barron RM, Campbell SL, King D, Bellon A, Chapman KE, et al. (2007) High Titers of Transmissible Spongiform Encephalopathy Infectivity Associated with Extremely Low Levels of PrPSc in Vivo. J Biol Chem 282: 35878-35886. [Link: https://bit.ly/374FmqQ](https://bit.ly/374FmqQ)
- Deslys JP, Robain O, Jaegly A, Beringue V, Peyrin J, et al. (1997) Transmission of the BSE Agent to Mice in the Absence of Detectable Abnormal Prion Protein. Science 275: 402-405. [Link: https://bit.ly/2Ud2Wfy](https://bit.ly/2Ud2Wfy)
- Saba R, Goodman CD, Huzarewich RLCH, Robertson C, Booth SA (2008) A miRNA Signature of Prion Induced Neurodegeneration. PLoS one 3: e3652. [Link: https://bit.ly/307kT33](https://bit.ly/307kT33)
- Montag J, Hitt R, Opitz L, Schulz-schaeffer WJ, Hunsmann G, et al. (2009) Upregulation of miRNA hsa-miR-342-3p in experimental and idiopathic prion disease. Mol Neurodegener 4: 36. [Link: https://bit.ly/30a6WBp](https://bit.ly/30a6WBp)
- Bellingham SA, Coleman BM, Hill AF. Small RNA (2012) deep sequencing reveals a distinct miRNA signature released in exosomes from prion-infected neuronal cells. Nucleic Acids Res 40: 10937-10949.



26. Saá P, Yakovleva O, Castro J De, Vasilyeva I, Paoli SH De, et al. (2014) First Demonstration of Transmissible Spongiform Encephalopathy-associated Prion Protein (PrP TSE) in Extracellular Vesicles from Plasma of Mice Infected with Mouse-adapted Variant Creutzfeldt-Jakob Disease by in Vitro. *J Biol Chem* 289: 29247-29260. [Link: https://bit.ly/3cB6vm0](https://bit.ly/3cB6vm0)
27. Berrone E, Corona C, Mazza M, Costassa EV, Faro M Lo, et al. (2015) Communication Detection of cellular prion protein in exosomes derived from ovine plasma. *J Gen Virol* 96: 3698-3702. [Link: https://bit.ly/3dNbyBD](https://bit.ly/3dNbyBD)
28. Iguchi Y, Eid L, Parent M, Soucy G, Bareil C, et al. (2016) Exosome secretion is a key pathway for clearance of pathological TDP-43. *Brain* 139: 3187-3201. [Link: https://bit.ly/2BsawfN](https://bit.ly/2BsawfN)
29. Wijesekera LC, Leigh PN (2009) Amyotrophic lateral sclerosis. *Orphanet J Rare Dis* 4: 3. [Link: https://bit.ly/2U8NC3C](https://bit.ly/2U8NC3C)
30. Hardiman O, Al-chalabi A, Chio A, Corr EM, Logroscino G, et al. (2017) Amyotrophic lateral sclerosis. *Nat Rev Dis Primers* 3: 17085
31. Gomes C, Keller S, Altevogt P (2007) Evidence for secretion of Cu , Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. *Neurosci Lett* 428: 43-46. [Link: https://bit.ly/2XzPsg4](https://bit.ly/2XzPsg4)
32. Pinto S, Cunha C, Barbosa M, Vaz AR, Brites D (2017) Exosomes from NSC-34 cells transfected with hSOD1-G93A are enriched in mir-124 and drive alterations in microglia phenotype. *Front Neurosci* 11: 273. [Link: https://bit.ly/308RJAq](https://bit.ly/308RJAq)
33. Saucier D, Wajnberg G, Roy J, Beauregard AP, Chacko S, et al. (2019) Identification of a circulating miRNA signature in extracellular vesicles collected from amyotrophic lateral sclerosis patients. *Brain Res* 1708: 100-108. [Link: https://bit.ly/3dAh7DI](https://bit.ly/3dAh7DI)
34. Sugars KL, Rubinsztein DC (2003) Transcriptional abnormalities in Huntington disease. *Trends Genet* 19: 233-238. [Link: https://bit.ly/2AGd8X4](https://bit.ly/2AGd8X4)
35. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, et al. (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet* 36: 585-595. [Link: https://bit.ly/3eT74Jw](https://bit.ly/3eT74Jw)
36. Petersén Á, Mani K, Brundin P (1999) Recent advances on the pathogenesis of Huntington's disease. *Exp Neurol* 157: 1-18. [Link: https://bit.ly/3cCw7iB](https://bit.ly/3cCw7iB)
37. Zhang X, Abels ER, Redzic JS, Margulis J, Finkbeiner S, et al. (2016) Potential Transfer of Polyglutamine and CAG-Repeat RNA in Extracellular Vesicles in Huntington's Disease: Background and Evaluation in Cell Culture. *Cell Mol Neurobiol* 36: 459-470. [Link: https://bit.ly/2XxDDXN](https://bit.ly/2XxDDXN)
38. Jeon I, Cicchetti F, Cisbani G, Lee S, Li E, et al. (2016) Human-to-mouse prion-like propagation of mutant huntingtin protein. *Acta Neuropathol* 132: 577-592. [Link: https://bit.ly/2XDUHvr](https://bit.ly/2XDUHvr)
39. Wang JKT, Langfelder P, Horvath S, Palazzolo MJ (2017) Exosomes and Homeostatic Synaptic Plasticity Are Linked to Each other and to Huntington ' s , Parkinson ' s , and Other Neurodegenerative Diseases by Database-Enabled Analyses of Comprehensively Curated Datasets. *Front Neurosci* 11: 149. [Link: https://bit.ly/3gXDDI8](https://bit.ly/3gXDDI8)
40. Nussbaum R, Ellis C (2003) Alzheimer's disease and Parkinson's disease. *N Engl J Med* 348: 1356-1364.
41. Eriksen JL, Wszolek Z, Petrucelli L (2005) Molecular Pathogenesis of Parkinson Disease. *Arch Neurol* 62: 353-357. [Link: https://bit.ly/2BAT5Kc](https://bit.ly/2BAT5Kc)
42. Gandhi S, Wood NW (2005) Molecular pathogenesis of Parkinson's disease. *Hum Mol Genet* 14: 2749-2755. [Link: https://bit.ly/3dzYPBT](https://bit.ly/3dzYPBT)
43. Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, et al. (2014) Plasma exosomal α -synuclein is likely CNS - derived and increased in Parkinson's disease. *Acta Neuropathol* 128: 639-650. [Link: https://bit.ly/3eTDtQo](https://bit.ly/3eTDtQo)
44. Cao Z, Wu Y, Liu G, Jiang Y, Wang X, Wang Z, et al. (2019) α -synuclein in salivary extracellular vesicles as a potential biomarker for Parkinson's disease. *Neurosci Lett* 696: 114-120. [Link: https://bit.ly/2Y66Tnk](https://bit.ly/2Y66Tnk)
45. Ohmichi T, Mitsunashi M, Tatebe H, Kasai T, Ali El-Agnaf OM, et al. (2019) Quantification of brain-derived extracellular vesicles in plasma as a biomarker to diagnose Parkinson's and related diseases. *Park Relat Disord* 61: 82-87. [Link: https://bit.ly/3eQVkv](https://bit.ly/3eQVkv)
46. Tomlinson PR, Zheng Y, Fischer R, Heidasch R, Gardiner C, et al. Identification of distinct circulating exosomes in Parkinson ' s disease. *Ann ClinTranslNeurol* 2: 353-361. [Link: https://bit.ly/2XAkRil](https://bit.ly/2XAkRil)
47. Gui Y, Liu H, Zhang L, Lv W, Hu X (2015) Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease. *Oncotarget* 6: 37043-37053. [Link: https://bit.ly/2MuZWXL](https://bit.ly/2MuZWXL)
48. Khoo SK, Petillo D, Kang UJ, Resau JH, Berryhill B, et al. (2012) Plasma-based circulating microRNA biomarkers for Parkinson's disease. *J Parkinsons Dis* 2: 321-331. [Link: https://bit.ly/2Mwzn4u](https://bit.ly/2Mwzn4u)
49. Yao YF, Qu MW, Li GC, Zhang FB, Rui HC (2018) Circulating exosomal miRNAs as diagnostic biomarkers in Parkinson's disease. *Eur Rev Med Pharmacol Sci* 22: 5278-5283. [Link: https://bit.ly/2MxVYgU](https://bit.ly/2MxVYgU)
50. Murphy MP, LeVine III H (2010) Alzheimer's Disease and the β -Amyloid Peptide. *J Alzheimers Dis* 19: 311-323. [Link: https://bit.ly/2XzGtM3](https://bit.ly/2XzGtM3)
51. Selkoe DJ (2001) Alzheimer's Disease : Genes , Proteins , and Therapy. *Physiol Rev* 81: 741-766. [Link: https://bit.ly/2ACL6fh](https://bit.ly/2ACL6fh)
52. Winston CN, Goetzl EJ, Akers JC, Carter BS, Rockenstein EM, et al. (2016) Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimer's Dement Diagnosis* 3: 63-72. [Link: https://bit.ly/2Xy1M0g](https://bit.ly/2Xy1M0g)
53. Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Schwartz JB, et al. (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.
54. Goetzl EJ, Schwartz JB, Abner EL, Petersen RC, Miller BL, et al. (2015) Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. *Neurology* 85: 40-47. [Link: https://bit.ly/2MzMjX6](https://bit.ly/2MzMjX6)
55. Lugli G, Cohen AM, Bennett DA, Shah RC, Fields CJ, et al. (2015) Plasma exosomal miRNAs in persons with and without Alzheimer disease: Altered expression and prospects for biomarkers. *PLoS One* 10: e0139233. [Link: https://bit.ly/3eTCv6l](https://bit.ly/3eTCv6l)
56. Liu CG, Song J, Zhang YQ, Wang PC (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. *Mol Med Rep* 10: 2395-2400. [Link: https://bit.ly/3gVp0tl](https://bit.ly/3gVp0tl)