Review article

Exosomal miRNAs in central nervous system diseases: biomarkers, pathological mediators, protective factors and therapeutic agents

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A B S T R A C T

Exosomes are small bilipid layer-enclosed extracellular vesicles that can be found in tissues and biological fluids. As a key cell-to-cell and distant communication mediator, exosomes are involved in various central nervous system (CNS) diseases, potentially through transferring their contents such as proteins, lipids and nucleic acids to the target cells. Exosomal miRNAs, which are small non-coding RNAs in the exosomes, are known to be more stable than free miRNAs and therefore have lasting effects on disease-related gene expressions. There are distinct profiles of exosomal miRNAs in different types of CNS diseases even before the onset of irreversible neurological damages, indicating that exosomal miRNAs within tissues and biological fluids could serve as promising biomarkers. Emerging evidence has also demonstrated the pathological effects of several exosomal miRNAs in CNS diseases via specific modulation of disease-related factors. Moreover, exosomes carry therapeutically beneficial miRNAs across the blood-brain-barrier, which can be exploited as a powerful drug delivery tool to help alleviating multiple CNS diseases. In this review, we summarize the recent progress made in understanding the biological roles of exosomal miRNAs as potential diagnostic biomarkers, pathological regulators, and therapeutic targets/drugs for CNS diseases. A comprehensive discussion of the main concerns and challenges for the applications of exosomal miRNAs in the clinical setting is also provided.

Abbreviations: α-SYN, α-synuclein; Aβ, amyloid β-protein; AD, Alzheimer's disease; APP, amyloid precursor protein; APPB2, App binding family b member 2; AUC, area under the curve; BACE1, beta-secretase 1; Bax, Bcl2 associated X protein; BBB, blood brain barrier; Bcl2, B-cell CLL/lymphoma 2; BDNF, brain derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CCL2, C-C motif chemokine ligand 2; CDK5, cyclin dependent kinase 5; CNS, central nervous system; CREB1, CAMP responsive element binding protein 1; CSF, cerebrospinal fluid; CT, computed tomography; CTGF, connective tissue growth factor; dMVBs, degradative multivesicular bodies; ECG, Electrocardiography; EE, early endosome; EGFR, epidermal growth factor receptor; EVs, extracellular vesicles; FOXA2, forkhead box A2; FOXO, forkhead box O1; GBM, glioblastoma; Gli3, glioma-associated oncogene family zinc finger 3; hNSCs, hypothalamic NSCs; Iba1, ionized calcium-binding adapter molecule 1; IGF1, insulin-like growth factor 1; IL-1β, interleukin 1 beta; ILVs, intraluminal vesicles; Lamp2b, lysosome-associated membrane glycoprotein 2b; LE, late endosome; Lrp8, low density lipoprotein receptor-related protein 8; MCAO, middle cerebral artery; MCI, mild cognitive impairment; miRNA, micro ribonucleic acid; MMPs, matrix metalloproteinase regulators; MPS, mononuclear phagocyte system; MRI, Magnetic resonance imaging; mRNA, messenger ribonucleic acid; MS, Multiple sclerosis; MSCs, mesenchymal stromal cells; mTOR, mammalian target of rapamycin; MVBS, multivesicular bodies; NF-κb, nuclear factor kappa b; NSCs, neural stem cells; NTAs, nanoparticle tracking analysis; PANK2, Parkinson disease (autosomal recessive, juvenile 2); PARB, Parkinson disease (autosomal dominant 8); PARK9, Parkinson disease (autosomal recessive 9); PCA, principal component analysis; PD, Parkinson's disease; PDCD4, programmed cell death 4; PDD, Parkinson's disease with dementia; Pde4b, phosphodiesterase 4b; PKM, pyruvate kinase; PSCI, post-stroke cognitive impairment; PTE, Phosphatase And Tensin Homolog; qPCR, real-time quantitative polymerase chain reaction; REST, re1 silencing transcription factor; RISC, RNA-induced silencing complex; ROC, receiver operating characteristic; RRMS, relapsing-remitting MS patients; RVG, Rabies virus glycoprotein; SEC, size exclusion chromatography; SEM, scanning electron microscopy; siRNA, small interfering ribonucleic acid; sMVBs, secretory multivesicular bodies; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SPNc, substantia nigra pars compacta; Stat3, signal transducer and activator of transcription 3; TBI, traumatic brain injury; TEM, transmission electron microscopy; TGFβ, transforming growth factor beta; TGFBR2, transforming growth factor beta receptor 2; TH, tyrosine hydroxylase; TIA, transient ischemic attack; Treg, regulatory T cells; UC, ultracentrifugation; UTR, untranslated region; VaD, vascular dementia; Vamp7, vesicle associated membrane protein 7

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1. Introduction

Intercellular communication is crucial for the proper functions of the central nervous system (CNS) in all multi-cellular organisms. Previously, mechanisms of intercellular communication are thought to primarily involve either soluble factors-mediated ligand-receptor interaction (e.g. signaling pathways) or direct cell-to-cell contacts (e.g. cellular junctions). However, emerging evidence suggests that extracellular vesicles (EVs) released from most eukaryotic cells have key impacts on both neighboring and distant cells, which constitutes a novel form of intercellular communication (Lee et al., 2012). The first report of EVs could be traced back to 1983, when two independent groups discovered multivesicular bodies or multivesicular endosomes (MVBs) released from sheep reticulocytes during the maturation process in vitro (Harding and Stahl, 1983; Pan and Johnstone, 1983). Since then, EVs have been demonstrated to participate in multiple physiological and pathological processes through horizontally transferring molecules, miRNAs, and proteins among cells (Al-Nedawi et al., 2008; Bergsmedh et al., 2001; Ramachandran and Palanisamy, 2012; Ratajczak et al., 2006; Valadi et al., 2007).

Exosomes, originated in the endocytic pathway, are the smallest EVs (40–100 nm in diameter), compared to microvesicles (50–1000 nm in diameter) and apoptotic bodies (500–2000 nm in diameter). Exosomes are formed as intraluminal vesicles (ILVs) in the MVBs of endosomal system, which is different from the biogenesis of other types of EVs (Fig. 1). More specifically, the biogenesis of exosomes starts from endosomes, which could be divided into three different types, including early endosomes, late endosomes, and recycling endosomes (Grant and Donaldson, 2009). Early endosome represents the initial sorting compartment for internalized proteins and other macromolecules in the endocytic vesicles. In this compartment, contents to be recycled are sorted into recycling endosomes (Morelli et al., 2004). The remaining early endosomes mature into late endosomes, also named as MVBs (Stoorvogel et al., 1991). During the maturation process, endosomes initiate inward budding starting from the perimeter membrane into the endosome lumen to form ILVs, which are enriched for tetraspanins CD9 and CD63 (Denzer et al., 2000; Pols and Klumperman, 2009). The fate of late endosomes lies in two different types of MVBs: main MVBs (degradative MVBs, dMVBs) would undergo degradation while the remaining MVBs (secretory MVBs, sMVBs) fuse with the plasma membrane, under the regulation of a number of Rab GTPases (e.g. RAB11, RAB35, RAB27) and SNARE proteins (e.g. VAMP7) (Hsu et al., 2010; Jaiswal et al., 2002; Logan et al., 2006; Rao et al., 2004; Raposo et al., 1996; Savina et al., 2003). The ILVs that are released into the extracellular spaces from sMVBs are referred to as exosomes (Grant and Donaldson, 2009; Mathivanan et al., 2010).

The contents of exosomes vary greatly based on cell types, different physiological and pathological conditions, and other diverse circumstances. In general, exosomes contain a broad spectrum of proteins, lipids, and nucleic acids. Proteins in exosomes include transcription factors, surface receptors, enzymes, signaling intermediates, and exosome-enriched proteins, such as tetraspanins CD9/CD63, Tsg101, Alix, and flotillin-1 (Lee et al., 2011). Lipids in exosomes include sphingomyelin, cholesterol, ganglioside GM3, desaturated lipids, phosphatidylserine, and ceramide (Llorente et al., 2013). Nucleic acids in exosomes include mRNAs, miRNAs, other non-coding RNAs and DNA (Crescielli et al., 2013; Guescini et al., 2010; Mathivanan et al., 2010; Valadi et al., 2007; Xiao et al., 2012). Proteins that are uniquely enriched in exosomes are often listed as markers for exosomes. Although the exact list of proteins is under debate, there is a growing consensus on exosome markers based on their endosomes origination, which include membrane transport and fusion proteins (GTPases, annexins, flotillin), tetraspanins (CD9, CD63, CD81, and CD82), heat shock proteins (Hsc70, and Hsp90), MVB biogenesis-related proteins (Alix and TSG101), and lipid-related proteins and phospholipases (Kalani et al., 2014; Vlassov et al., 2012). In addition to marker proteins, contents of exosomes have been published and continuously updated through multiple databases, such as Exocarta, Vesiclepedia, and EVpedia. These databases provide a great resource for investigators in exosome research (Kalra et al., 2012; Kim et al., 2013; Mathivanan et al., 2012; Mathivanan and Simpson, 2009).

After being released from parent cells, exosomes interact with recipient cells in three distinct modes, each exhibiting different engagements with plasma membrane and releases of contents. (1) Exosomes can be endocytosed into recipient cells through multiple mechanisms (clathrin-mediated endocytosis, caveolin-dependent endocytosis, lipid raft-mediated endocytosis, phagocytosis, and microphinocytosis) and their contents can be released intracellularly. In this way, exosome contents may modulate cellular functions of the recipient cells through direct changes of intracellular protein levels or indirect changes of protein levels by nucleic acids. (2) Exosomes can directly fuse with the

![Fig. 1. The biogenesis, secretion, uptake, and functions of miRNAs-containing exosomes. Exosomes are originated in the endocytic pathway starting with the formation of early endosome (EE) by endocytosis at the plasma membrane. Intraluminal vesicles (ILVs) are formed by the inward budding of membranes of late endosome (LE) and miRNAs, together with other contents, are packaged inside. LE is divided into two populations, degradative multivesicular bodies (dMVB) and secretory multivesicular bodies (sMVB). dMVB are guided to lysosomes for degradation and sMVB are fused with plasma membrane for exosome secretion. Exosomes in extracellular space are uptaken by recipient cells through either fusion or endocytosis, resulting in the releasing of exosome contents into recipient cells. After releasing from exosomes, miRNAs are recruited to RNA-induced silencing complex (RISC) and bind to the 3′UTR of their target genes, therefore manipulating various biological processes of recipient cells.](image-url)
cell membrane and deliver contents to the cytosol of the target cell. Exosome contents may modulate cellular functions similar to what is described in (1). (3) Exosomes may remain attached to the plasma membrane and activate downstream signaling pathways through ligand-receptor interaction.

Exosomes have been reported to be able to regulate various physiological processes in CNS, such as fate determination of neural stem cell (NSCs), homeostasis regulation, and neurogenic niche maintenance in the adult brain (Batiz et al., 2015; Zhang et al., 2018). Importantly, exosomes are involved in the aberrant pathological processes of multiple CNS diseases, including neurodegenerative disorders (e.g. Alzheimer’s disease, Parkinson’s disease, and transmissible spongiform encephalopathies), autoimmune diseases (e.g. multiple sclerosis), and circulation-related diseases (e.g. stroke) (for review see (Pusic et al., 2014; Vella et al., 2008; Wu et al., 2017; Xiao et al., 2017; Zhang and Chopp, 2016)).

Exosome-mediated transfer of microRNAs (miRNAs) has been identified as a novel mechanism of genetic exchange among cells (Valadi et al., 2007). Since the initial discovery, this unique way of genetic exchange has been extensively studied and speculated as a main functional output of exosomes. miRNAs are evolutionarily small conserved (20–24 nucleotides) noncoding antisense RNAs expressed in a variety of cell types and species (Ambros, 2001). miRNAs participate in cellular processes by recognizing and directly binding to the 3’ untranslated region (3’UTR) or open reading frame (ORF) region of target mRNAs, which predominantly silences the expression of the latter either by promoting their degradation or interfering with their translation. In the CNS, miRNAs are reported to regulate a broad range of cellular processes, from normal development and homeostasis to diseases and regeneration (Kuss and Chen, 2008).

Several unique characters define exosomal miRNAs in CNS homeostasis and diseases. Firstly, exosomal miRNAs are more accessible than cellular miRNA. Exosomes are abundantly present in and can be isolated from various biological fluids with relative ease (e.g. serum) and without the need of overly invasive procedures (Kumar and Reddy, 2016). More importantly, exosomes are highly enriched with miRNAs, compared to their parental cells and cell-free blood (Cheng et al., 2014a). For example, deep sequencing results have demonstrated that the percentages of miRNAs in mapped reads are 3- to 4-folds higher in serum exosomes versus neat serum. Therefore, biological fluid-derived exosomes and exosomal miRNAs have been active targets for biomarker profiling. Secondly, exosomal miRNAs typically have distinct expression patterns, compared to cellular or free miRNAs (Chen et al., 2015; Riancho et al., 2017). During the biogenesis of exosomes, miRNAs are not randomly packaged into exosomes, but selective sorted by multiple mechanisms. For example, due to the high RNA-binding potential of miRNAs, the abundant expression of their target transcripts leads to more miRNAs-miRNA interactions. It can reduce the aggregate of miRNAs that are sorted to exosomes without influencing the cellular miRNA levels (Squadratto et al., 2014). It explained, partially at least, the decrease of mir-15, mir-185-5p, mir-342-3p in the serum exosomes of AD patients since their (predicted) target, APP is highly expressed in the AD brains. The abnormal elevation of APP leads to more miRNAs-miRNA interactions and less free mir-15, mir-185-5p and mir-342-3p to load in exosomes. Besides, miRNAs sorting into exosomes is regulated by Ago2-associated pathway and “chaperone-mediated sorting which requires RNA-binding proteins like hnrNP2 and YBX1 (McKenzie et al., 2016; Melo et al., 2014; Shurtliff et al., 2016; Villarroya-Beltri et al., 2013). Though no current study reported the dysregulation of these RNA-binding proteins, the abnormal abundance of their targets (e.g. miR-223) in CNS disorder patients’ serum exosomes was observed. These findings indicate that these miRNA sorting mechanisms during exosome biogenesis may be specifically interrupted in the pathogenesis and neuroregeneration of CNS disorders (e.g. stroke) (Chen et al., 2017b). Hence, exosomal miRNAs may be more disease-specific than cellular and free miRNAs. Thirdly, CNS-derived exosomal miRNAs may carry information of their parental cells that can be traced to precisely monitor cellular and tissue status in the brain. Exosomes are able to diffuse through the blood brain barrier (BBB) and into the periphery, where they can be selectively captured by cell surface specific antibodies (Goetzl et al., 2015a). For example, neural-derived exosomes (NDE) can be isolated from plasma using a combined approach of precipitation and immunoassortment that targets L1 cell adhesion molecule (L1CAM), which is specifically expressed on neurons (Fiandaca et al., 2015; Goetzl et al., 2015b). This strategy has been used to identify AD-related proteins amyloid beta (Ab) and forms of tau (specifically p-tau) in NDE that distinguish cognitively normal controls from patients with frontotemporal dementia or AD (Fiandaca et al., 2015). It is unknown whether other contents such as miRNAs in blood NDE from AD patients could serve as novel biomarkers. However, the approach has inspired more emerging research to specifically evaluate “primary” exosomal miRNAs that are secreted from injured neural cells in the brain but not those from periphery-derived “secondary” exosomes. These innovative research data may significantly improve the accuracy in the diagnosis of CNS diseases. Fourthly, exosomal miRNAs are more stable than free miRNAs against degradation (Cheng et al., 2014a; Miranda et al., 2016; Valadi et al., 2007). Once packaged into exosomes, miRNAs are cut off from enzymes in biological fluids and protected from RNase treatment. The increased stability of exosomal miRNAs not only helps to identify the temporal changes of exosomal miRNAs expression during disease progression, but also enables exosomal miRNAs to mediate disease-related cell signaling in a more durable way. Lastly, exosomes are novel and promising nanocarriers to deliver siRNAs/miRNAs to CNS. Due to their unique physiological and biological features, exosomes could diffuse across BBB or the choroid plexus, helping exchanges between CNS and peripheral circulation (Johnsen et al., 2014). Exosomes could be further engineered to carry peptides or proteins on their surface, which confers targeting ability to exosomes (Alvarez-Erviti et al., 2011; Ohno et al., 2013; Tian et al., 2014). The fusion of CNS-specific rabies virus glycoprotein (RVG) that specifically binds to the acetylcholine receptor to the lysosomal-associated membrane 541 protein 2b (Lamp2b) could efficiently guide intravenous injected exosomes to neurons in the brain (Alvarez-Erviti et al., 2011). Therefore, exosomal miRNAs could be applied as drugs to be specifically delivered to CNS to modulate the expression of disease-related genes using the same approach, which shed light on the development of new therapeutic strategies for CNS diseases (Alvarez-Erviti et al., 2011; Chen et al., 2015, 2014; Rao et al., 2013).

Investigations on the involvement of exosomal miRNAs in CNS homeostasis and diseases is still in its infancy. There remains significant interest within the scientific community about what exosomal miRNAs can achieve in homeostasis and diseases (Fleschner and Crane, 2017). In this review, we will only discuss the roles of exosomal miRNAs (not cellular or free miRNAs) as biomarkers, pathologica mediator, therapeutic targets, and “drug” delivered by modified exosomes for CNS diseases due to their unique properties.

2. Exosomal miRNAs: sources, isolation, profile analysis and target screening

2.1. The specimens of exosomal miRNAs in the research of CNS diseases

The expression levels of exosomal miRNAs vary among different biological fluids in vivo and culture medium in vitro (Lasser et al., 2012; Wang et al., 2008; Witwer et al., 2013). Regarding in vivo sources, exosomes have been discovered in all of the known biological fluid types, which include blood (plasma or serum), urine, cerebrospinal fluid (CSF), breast milk, pleural effusions, saliva, semen, aqueous humor, and amniotic fluid, etc. (Keller et al., 2011; Liu et al., 2014; Poliakov et al., 2009; Witwer et al., 2013). Exosome contents including
miRNAs are investigated in various biological fluids for the development of biomarkers as well as the identification of potential therapeutic targets of diseases. Establishing relevant biomarkers for CNS diseases has led recent research to unravel exosomal cargo packaging to determine the involvement of exosomal miRNAs in regards to two types of biological fluids: CSF and blood (Chen et al., 2015, 2017a; Chen et al., 2017b; Ebrahimkhani et al., 2017; Leggio et al., 2017; Skog et al., 2008; Wang et al., 2014b).

Produced from arterial blood or by ependymal cells in the choroid plexuses, CSF is in direct contact with the subarachnoid space and the ventricular system of the brain (Gui et al., 2015; Liu et al., 2014; Shi et al., 2015; Sorensen et al., 2016, 2014). The CSF reflects the biochemical changes of the brain, making it the optimal indicator of most CNS diseases. Although exosomal miRNAs isolated from CSF serves as an excellent source in the research of CNS diseases, several limitations narrow its applications in the investigation of exosomal miRNAs as potential biomarkers and pathological mediators in CNS diseases. The first one is the difficulty in sample collection. The invasive processes of CSF collection, including lumbar puncture (commonly used) and cisternal/ventricular puncture (rarely used), may cause dizziness/headache and pose potential damage, which restricts sampling population. Secondly, the limited sample volume of CSF impedes its application in routine screening and testing. The third concern is the reproducibility of exosomal miRNA screening. Significant sample variation is ineluctable owing to the differences of individuals within each group and that of population, age and life styles of donors (e.g. smoke, alcohol, etc.) among various groups. Lastly, its sensitivity in detecting low-abundant miRNAs in exosomes is limited because of the difficulties to acquire large amount of exosomal miRNA.

Beside CSF, plasma/serum is widely used in the diagnosis of diverse diseases including stroke, multiple sclerosis and glioblastoma (Chen et al., 2017a; Gotanda et al., 2016; Ingram et al., 2016; Zhao et al., 2013). As exosomes released from CNS cells robustly diffuse across BBB, CNS disease-related factors could be identified in plasma/serum exosomes. Compared to CSF, plasma/serum is easier accessible, which evades invasive process of sample collection. The abundant sample volume and enrichment of exosomal miRNAs in plasma/serum also enhance the maneuverability and sensitivity in biomarker screening (Yagi et al., 2017). These unique characteristics make circulatory system the most common place for specimen collection. Collecting biological fluid and investigating the diagnostic and pathogenic roles of exosomal miRNAs seem straightforward. However, multiple disadvantages limit its further use in clinical applications. The most serious issue is the specificity of plasma/serum exosomal miRNAs. The circulatory system contains exosomes that potentially originated from all organs and tissues of the body, which could flood the primary disease-specific alteration of exosomal miRNAs with non-specific secondary changes of exosomal miRNAs. Therefore, distinguishing primary information was inherently challenging. Despite the challenges, new techniques continue to improve, helping to overcome this barrier. As we discussed above, NDE could be isolated by immunoadsortion with the specific neuronal antibody L1CAM, which is specifically presented on the surface of NDE (Fiandaca et al., 2015; Goetzl et al., 2015b). Although these groundbreaking works inspired a whole new research direction, the purification of NDE requires further improvement and more tissue- and cell-specific surface proteins are urgently needed to assist in evaluation. For example, the initiation and progression of the majority of CNS diseases are accompanied by neuroinflammation, which requires the activation of microglia, the resident macrophages in the CNS (Blann et al., 2002; Cameron and Landreth, 2010; Hirsch and Hunot, 2009; Rogers et al., 1996). Isolation of microglia-derived exosomes and detection of the specific dysregulated inflammatory miRNAs will be instrumental in establishing the advanced early diagnosis of many CNS disorders (e.g. Alzheimer’s disease) before the onset of irreversible neurological damages. Much research on exosomal miRNAs as biomarkers remains in progress. Plasma, serum, and CSF exosomal miRNA screenings still have a long way to go before overcoming the specificity and reliability concerns. Therefore, identified potential biomarkers through exosomal miRNA analysis must be complemented by other clinical parameters (e.g. MRI) in disease diagnosis and functional validation is necessary to prove the pathological or therapeutic effects of dysregulated exosomal miRNAs in CNS diseases.

In contrast to studies of exosomal miRNAs from biological fluids, in vitro disease models provide an outstanding platform to analyze and modify the contents of exosomes in defined conditions. For example, by specifically manipulating the expression of certain miRNAs in the donor cells, the level of exosomal miRNAs can be modified to examine their function and mechanisms in recipient cells. Although in vitro disease models are easy and compatible for most of functional analyses, it is impossible to perfectly replicate the in vivo situation due to the loss of microenvironment. Thus, information obtained in vitro always needs to be double-checked in animal or human organoid models of diseases (Dinkins et al., 2014; Lee et al., 2017a; Yuyama et al., 2015).

2.2. The isolation of exosomal miRNAs

To date, no standard methodology is established for exosome isolation. Two main approaches are predominantly applied in current studies: ultracentrifugation (UC)-based method and commercially available precipitation-based kits (Helwa et al., 2017; Rekker et al., 2014). Currently, the UC-based method is the most widely used approach, which involves multiple-step differential centrifugation processes (Cheng et al., 2014b; Jeppesen et al., 2014). The sedimentation of different components of EVs varies based on the size, density, shape and the viscosity of different biological fluids and media. The UC-based method utilizes these sedimentation characteristics to first remove cells and large debris in the sample solution. The cell-free sample solution is next centrifuged to remove larger EVs then proceeded to a single high g-force step to pellet exosomes down. However, multiple disadvantages of UC-based approach limited its clinical applicability: (1) this approach cannot separate some non-exosome components (extravesicular proteins complexes/aggregates, lipoprotein particles, etc.) with similar sedimentation characteristics of exosomes; (2) it is time-consuming; (3) limited sample size and volumes can be carried out at a time (Alvarez et al., 2012; Rekker et al., 2014; Witwer et al., 2013). The commercial kit for exosome precipitation, manufactured by multiple brands (Invitrogen, System Biosciences, etc.) provides a robust and fast choice to cover larger size and volumes of samples (Ding et al., 2018). But these precipitation-based kits still face the isolation purity issue by the contamination of other EVs and protein complexes in the exosome pellet. Studies to compare UC-based method and commercial kits suggest that both methods isolate exosomes with similar purity (Helwa et al., 2017). In addition, UC-based method consistently extracts significantly fewer exosomes than commercial kits with diverse starting volumes, while all tested commercial kits generated similar yield of exosomes. Besides the two approaches described above, several alternatives are also utilized in exosome isolation, including size exclusion chromatography (SEC), ultrafiltration, immunoaffinity isolation, microfluidics techniques, and polymeric precipitation (Ding et al., 2018). However, their applications are non-mainstream owing to the issues of low yield, low number of samples, high sample input, being costly and other limitations (Alvarez et al., 2012; Witwer et al., 2013). Recently, cell type-specific exosomes can also be separated using a newly designed immunoprecipitation-based method (Goetzl et al., 2016). This method opens a new window in increasing the specificity of exosome extraction and investigating the role of cell-type specific exosomes in biological and pathological processes.

After isolation, the characterization of exosomes involves morphological test and molecular validation (Ma et al., 2018; Thery et al., 2006, 1999). The most intuitive and classical ways to monitor exosome’s morphology are scanning & transmission electron microscopy (SEM & TEM). With resolution up to less than 1 nm, SEM & TEM are excellent
tools to demonstrate the structure of whole-mounted exosomes in detail. Besides, the morphological feature of exosomes could be captured by indirect approaches, such as dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). By measuring at a fixed angle, DLS can determine the mean size of particles including exosomes. NTA, utilizing the properties of both light scattering and Brownian motion, can also obtain the nanoparticle size distribution. Though both analyses can demonstrate the size of exosomes, NTA examines exosome concentration ad interim, making it a more informative technique than DLS. Notably, the size distribution of exosomes, obtained by both analyses, may not completely match due to the physiological feature of DLS that favors the detection of larger particles over small particles (Ma et al., 2018). Moreover, the presence of exosomes can be detected at molecular level by antibody-based techniques such as western blot and ELISA. As mentioned in introduction, at least 20 protein markers of exosomes have been reported (Thery et al., 2006). Among them, TSG101, Alix, flotillin, Rab5b and CD family (e.g. CD9, CD63, and CD81) are most widely used markers due to their high enrichment in exosomes and extensive expression by a broad variety of cells.

Unlike proteins, the contamination of free miRNAs in biological fluid is negligible. Ribonucleases are present and active in most of biological fluids including serum, urine and cerebrospinal fluid, which degrades extraneous RNA (Blank and Dekker, 1981). Meanwhile, free miRNAs cannot be recognized by reagents in commercial kits or pelleted by ultracentrifugation because of their small size, further preventing the contamination of free miRNAs. However, miRNAs from non-exosome EVs cannot be excluded by our current techniques. Multiple independent studies suggest that, using either phenol/chloroform-based or phenol/chloroform-free RNA extraction protocols, commercial kits such as ExoQuick-based method obtains higher miRNA yield in biological fluid exosomes, compared to other methods including ultracentrifugation-based approach (Alvarez et al., 2012; Ding et al., 2018; Rekker et al., 2014). This is probably due to the higher recovery efficiencies of exosomal miRNAs using commercial exosome isolation kits compared to the ultracentrifugation method. In addition, the RNA extraction efficiencies of various commercial kits have also been examined, demonstrating various degrees of RNA recovery (Cheng et al., 2014b). Notably, the miRNeasy kit (Qiagen) showed the highest miRNA recovery rate through removing large RNA using the additional RNase MinElute column.

Aside from the yield of total exosomal miRNAs from different exosome and exosomal miRNA extraction methods, the consistency of miRNA profiles among all these methods is also an important factor of consideration for further selection of appropriate exosomal miRNAs extraction methods and data interpretation. Strong correlation of miRNA expression is observed from exosomes isolated by UC-based method and commercial kits (Rekker et al., 2014). However, statistical differences are also found for 17 miRNAs, suggesting the discrepancy in the composition of exosomes isolated with these two methods. Additionally, miRNA levels are also tested among various exosome extraction kits. Surprisingly, although strong correlation of exosomal miRNA profiles can be observed, the recovery of certain miRNAs, such as let-7d, mir-16 and mir-25, exhibits distinct levels among different commercial exosome isolation kits (Ding et al., 2018). The variation of exosomal miRNA recovery could be largely due to the technical differences among exosome isolation kits. Thus, the limitations and variations of all techniques described above led to current situation that no standard procedure is established for exosome and exosomal miRNA extraction, resulting in the difficulties for investigating the exosomal miRNA profiles, identifying the potential biomarkers of diseases, and dissecting the pathological and therapeutic effects of exosomal miRNAs, which is discussed in detail in the rest of our review.

2.3. The profile analysis of exosomal miRNAs

In the past decade, methodologies for miRNA detection and quantification develop rapidly. The techniques for exosomal miRNA profiling used in recent studies are mainly 3 types: qPCR and qPCR-based multiplex assay, deep miRNA sequencing, and chip-based miRNA microarray. Although qPCR-based multiplex assay is less costly, miRNA sequencing and microarray are more rapid and have higher-throughput, making them ideal methods for miRNA profiling at current stage. The advantages and limitations of microarray and deep sequencing for miRNA quantification are widely discussed (Li et al., 2012; Malone and Oliver, 2011). Briefly, microarray is relatively cheaper with less biases, while deep sequencing can detect non-identified sequencing and individual transcript isoforms with higher sensitivity for miRNAs. Therefore, subtle but significant differences are observed for miRNA quantification using these two methods. Besides, both methods face the same issue to detect lowly expressed miRNAs. Thus, the combination of microarray and deep sequencing appears to be the best option to overcome the main concerns of different high-throughput quantification for exosomal miRNAs. However, analysis with a single method is also widely used and accepted.

2.4. The target identification for exosomal miRNAs

Generally, miRNAs achieve their function by guiding the RNAi-induced silenced complex (RISC) to partially complementary sequences in target transcripts, leading to gene suppression by a combination of translation inhibition (Ameres and Zamore, 2013; Bushati and Cohen, 2007). To date, though detailed experimental strategies for miRNA target identification may vary, the logic behind is relatively standardized and fixed (for review see (Thomson et al., 2011)). Basically, the identification of miRNAs involves two steps: putative miRNA targets screening and candidate transcript validation. Putative miRNA targets can be screened through high-throughput gene-expression or proteomic analysis, aiming to obtain negatively regulated transcripts after exogenous expression of a miRNA. By cross-checking with databases for miRNA target prediction (e.g. targetscan, miRanda, and miRDB), candidate mRNAs could be identified. Candidate transcript validation is a gene-specific experimental confirmation process for miRNA targets with well-established techniques, such as luciferase reporter assays, qRT-PCR, and western blot. Among them, luciferase reporter assays have been employed extensively to demonstrate a direct interaction between miRNA and mRNA although it is labor intensive, dependent upon the region chosen for cloning, and sensitive to variances in protocol. In contrast, qRT-PCR and western blot are fast and highly standardized, but they cannot distinguish between direct and secondary miRNA targets, making them less informative.

3. Exosomal miRNAs are potential biomarkers in the diagnosis of CNS diseases

Mounting evidence has demonstrated the distinct profiles of exosomal miRNAs in various diseases in CNS, such as neurodegenerative disorders, multiple sclerosis, stroke, and brain tumors, suggesting exosomal miRNAs may serve as potential biomarkers for diagnosing CNS diseases (Chen et al., 2017a; Chen et al., 2017b; EbrahimiKhani et al., 2017; Leggio et al., 2017; Skog et al., 2008; Wang et al., 2014b). The diagnostic power of differentially expressed exosomal miRNAs in CNS disorders can be evaluated by calculating sensitivity (the presence of “disease” in population with the disease), specificity (the absence of “disease” in population without the disease), positive predictive value (population with a positive result for “disease” to actually have the disease), and negative predictive value (population with a negative result for “disease” to actually not have the disease). Here, we summarize the current advancements in unveiling the profiles and roles of exosomal miRNAs in the diagnosis of CNS diseases (Table 1).
| Disease | Down-regulated miRNAs | Up-regulated miRNAs | AUC | Sensitivity (%) | Specificity (%) | Specimens | Species | References |
|---------|-----------------------|---------------------|-----|----------------|----------------|----------|---------|------------|
| Alzheimer’s disease | miR-193b | – | 71.4 (CSF), 58.8 (Serum) | – | 92.5 | Plasma, Human | Liu et al. (2014) |
| | miR-185-5p, miR-342-3p, miR-141-3p, miR-342-5p, miR-23b-3p, miR-338-3p, miR-3613-3p | – | 0.919 | – | 9.996 (vs PDD) | Plasma, Human | Yang et al. (2018) |
| | miR-135a | 94.4 | 83 | 94.39 (vs PDD) | 100 (vs PDD) | Plasma, Human | Lugli et al. (2015) |
| | miR-384 | 97.2 | 99 | – | – | Plasma, Human | Yang et al. (2018) |
| Parkinson’s disease | miR-1 | 0.920 | 94* | 94* | – | CSF, Human | Gui et al. (2015) |
| | miR-19b-3p | 0.705 | 94* | 94* | – | – | – | – |
| | miR-19b | 0.780 | 93* | 93* | – | – | – | – |
| | miR-409-3p | 0.970 | 90* | 90* | – | – | – | – |
| | miR-10a-5p | 0.900 | 95* | 95* | – | – | – | – |
| | let-7g-3p | 9.5* | – | 95* | – | – | – | – |
| | miR-195 | 0.753 | 68.8 | 77.3 | – | Serum, Human | Cao et al. (2017) |
| | miR-24 | 0.697 | 82.6 | 85 | – | – | – | – |
| | miR-122-5p (RRMS) | – | 0.878 | – | – | Serum, Human | Selmaj et al. (2017) |
| | miR-196b-5p (RRMS) | – | 0.866 | – | – | – | – | – |
| | miR-301-3p (RRMS) | – | 0.681 | – | – | – | – | – |
| | miR-522-5p (RRMS) | – | 0.800 | – | – | – | – | – |
| | let-7i, miR-19b, miR-25, miR-92a | – | – | – | – | Plasma, Human | Kimura et al. (2018) |
| | miR-15b-5p (RRMS) | – | 0.740 | – | – | Serum, Human | Ebrahimkhani et al. (2017) |
| | miR-451a (RRMS) | – | 0.830 | – | – | – | – | – |
| | miR-30b-5p (RRMS) | – | 0.770 | – | – | – | – | – |
| | miR-342-3p (RRMS) | – | 0.820 | – | – | – | – | – |
| | miR-127-3p (PPMS) | – | 0.900 | – | – | – | – | – |
| | miR-370-3p (PPMS) | – | 0.860 | – | – | – | – | – |
| | miR-409-3p (PPMS) | – | 0.810 | – | – | – | – | – |
| | miR-432-5p (PPMS) | – | 0.900 | – | – | – | – | – |
| | miR-486-5p, miR-451a, let-7b-5p, miR-320b, miR-122-5p, miR-215-5p, miR-320d, miR-19b-3p, miR-26a-5p, miR-142-3p, miR-146a-5p, miR-15b-3p, miR-23a-3p, miR-223-3p (INF-β vs naive) | – | – | – | – | Serum, Human | Manna et al. (2018) |
| | miR-126 | – | – | – | – | – | – | – |
| | miR-223 | – | – | – | – | – | – | – |
| | let-7l | – | – | – | – | – | – | – |
| | miR-124 | – | – | – | – | – | – | – |
| | miR-422a (subacute phase group) | – | – | – | – | – | – | – |
| | miR-125b-2-3p (subacute phase group) | – | – | – | – | – | – | – |
| | miR-442a | – | – | – | – | – | – | – |
| | miR-3156, miR-4465, miR-6426, miR-373, miR-7845, miR-4649, miR-4778, miR-1919, miR-6805, miR-4428, miR-2681, miR-6847, miR-4283, miR-6786 (Hypoxia group) | – | – | – | – | – | – | – |
| | miR-21 | – | – | – | – | – | – | – |
| Glioblastoma | miR-6871, miR-1273, miR-6807, miR-5585, miR-1292, miR-6891 (Hypoxia group) | – | – | – | – | – | – | – |
| Stroke | miR-126 | – | – | – | – | – | – | – |
| | miR-223 | – | – | – | – | – | – | – |
| | miR-9 | – | – | – | – | – | – | – |
| | miR-124 | – | – | – | – | – | – | – |
| | miR-422a (acute phase group) | – | – | – | – | – | – | – |
| | miR-3156, miR-4465, miR-6426, miR-373, miR-7845, miR-4649, miR-4778, miR-1919, miR-6805, miR-4428, miR-2681, miR-6847, miR-4283, miR-6786 (Hypoxia group) | – | – | – | – | – | – | – |
| | miR-21 | – | – | – | – | CSF, Human | Shi et al. (2015) |

(continued on next page)
### 3.1. Neurodegenerative disease

#### 3.1.1. Alzheimer’s disease

Alzheimer’s disease (AD), the most common progressive neurodegenerative disorder, is characterized by increased brain inflammation, oxidase stress, memory loss, cognitive decline, and intellectual impairments. It affects approximately 6% of the population over 65 and more than 20% of individuals over 80 years of age, which creates huge social and economic burdens (Danborg et al., 2014). With the progression of AD, symptoms develop from short-term memory loss to difficulties in language, disorientation, loss of motivation, depression, and overall intellectual and cognitive decline (Burns and Iliffe, 2009). One feature of AD is that there is usually a relatively long lag (10 to 20 years) for the first sign of symptoms to occur after the onset of pathological changes in the brain. This lag could serve as an excellent therapeutic window for interventions but unfortunately, most of AD patients are only diagnosed after symptoms develop when pathological changes in the brain are already too advanced to be reversed. The miss of therapeutic window at this asymptomatic stage might also explain the failures of clinical trials that aimed to find cure for AD. Therefore, finding specific accessible biomarker(s) for early diagnosis of AD before the onset of symptoms is crucial and urgent. Biomarkers can act as indicators of pathological progression and pharmacological response to therapeutic interventions in diseases. Until now, there were no peripheral biomarkers that can detect AD early in its pathogenesis. Recent studies suggested exosomal miRNAs can serve as potential biomarkers of AD (Chen et al., 2017a; Trotta et al., 2018). In 2014, Liu et al. observed a decrease in exosomal miR-193b levels in the CSF, serum and plasma in AD patients, compared to healthy controls (Liu et al., 2014). They further examined the sensitivity of exosomal miR-193b as a biomarker of AD, and the positive rates were 71.43% (5/7) and 58.82% (30/51) in the CSF and serum of patients with dementia of the Alzheimer-type, respectively, and 58.14% (25/43) in the serum of patients with mild cognitive impairment. Similar to Liu’s observations, a recent report from Yang et al. also identified that serum exosomal miR-193b, together with miR-135a and miR-384, can serve as potential biomarkers for AD, after comparing with exosomal miRNAs levels in the serum of healthy controls (Yang et al., 2018). The receiver operating characteristic (ROC) curve analysis, which identified optimal cut-off value for these miRNAs, suggested excellent sensitivity & specificity of exosomal miR-135 (94.4% & 94%), miR-193 (92.5% & 83%), and miR-384 (97.2% & 99%) for discriminating AD patients from healthy controls. This cut-off value also yielded convincing positive & negative predictive values for exosomal miR-135 (96% & 95%), miR-193 (87% & 93%), and miR-384 (99% & 97%), suggesting the quality and accuracy of the analysis. Moreover, Cheng et al. investigated the expression profile of circulating exosomal miRNAs in serum from AD patients and healthy controls (Cheng et al., 2015). Among 1419 known miRNAs, the expression of 14 miRNAs (miR-361-5p, miR-30e-5p, miR-93-5p, miR-15a-5p, miR-143-3p, miR-335-5p, miR-106b-5p, miR-101-3p, miR-424-5p, miR-106a-5p, miR-18b-5p, miR-306-5p, miR-20a-5p and miR-582-3p) and 3 miRNAs (miR-1306-5p, miR-342-3p and miR-15b-3p) was significantly up-regulated and down-regulated, respectively. Model validation that incorporates all the 16 selected miRNA markers further acquired promising sensitivity (87%) and specificity (77%) for AD prediction. Later research by Lugli et al. examined plasma exosomal miRNAs in persons with and without AD, in which miRNAs (miR-185-5p, miR-342-3p, miR-141-3p, miR-342-5p, miR-23b-3p, miR-338-3p, and miR-3613-3p) were significantly down-regulated in AD patients; these miRNAs were selected by machine learning experiments (Lugli et al., 2015). The sensitivity of individual down-regulated miRNA as a diagnostic parameter for AD, examined by machine learning analysis, suggested that the accuracy for AD prediction is over 82% in AD patients and 88.6% in control group.

It is noteworthy that other neurological disorders, such as Parkinson’s disease with dementia (PDD) and vascular dementia (VaD) share many similarities with AD in clinical presentation, neuropathological

| Disease | Up-regulated miRNAs | Down-regulated miRNAs | AUC | Sensitivity (%) | Specificity (%) |
|---------|---------------------|-----------------------|-----|----------------|----------------|
| AD      | let-7a, miR-15b, miR-16, miR-19b, miR-20, miR-21, miR-320 | miR-574-3p | 0.719 | - | - |
|         | miR-320 (High grade group) | miR-21 (High grade group) | 0.738 | - | - |
|         | miR-222 (High grade group) | miR-124 (High grade group) | 0.8 | - | - |
|         | miR-301a | miR-301b | 0.937 | 86.2 | 93.2 |
| Epilepsy| miR-8071 | miR-932 | 0.597 | 90.2 | - |
|         | miR-197-5p | miR-3065-5p | 0.932 | 89.2 | 90.2 |
|         | miR-4322 | miR-342-5p | 0.974 | 98.4 | 91.8 |
|         | miR-6781-5p | miR-3613-5p | 0.841 | - | - |
characteristics, and genetic determinants of risk, etc. (Kalaria, 2016). These overlapping features may negatively affect the diagnostic power of putative biomarkers, making them less likely to be used in AD diagnosis. Interestingly, exosomal miRNAs display high diagnostic power in distinguishing AD from the aforementioned disorders. For example, miR-384, a highly enriched miRNA in serum exosomes of AD patients, has the sensitivity & specificity of 99.07% & 100%, respectively, in discriminating AD patients from PDD patients and 99.10% & 100% in discriminating AD patients from VaD patients (Yang et al., 2018). Despite the excellent diagnosis power, it remains unclear whether miR-384 can detect disease at early stage.

Mild cognitive impairment (MCI), characterized by memory impairment, represents early-stage Alzheimer’s disease (Morris and Cummings, 2005). Studies suggest that AD prevention trials with MCI patients can be a promising strategy for delaying AD when the disease is still in a transitional clinical stage, suggesting the importance in MCI diagnosis (Grundman et al., 2004). Based on a report from Yang et al., the expression of exosomal miR-135a, miR-193b, and miR-384 was similarly modulated in the serum of MCI patients when compared with those of AD patients (Yang et al., 2018). Among these miRNAs, miR-135a exhibits the highest diagnostic accuracy in discriminating MCI patients from control subjects, with the sensitivity & specificity of 90.01% & 95%, respectively. Moreover, ROC curve analysis for the combination of aforementioned three miRNAs yields 0.995 in AUC, 99% in sensitivity, and 95% in specificity, suggesting a strong diagnostic power of exosomal miRNAs in the diagnosis of MCI.

Together, the distinct signatures of exosomal miRNAs indicate their roles as promising biomarkers of in AD. Particularly, those miRNA biomarkers that are changed early in the disease may bring hope to tackle the key challenge for AD treatment.

### 3.1.2. Parkinson’s disease

Parkinson’s disease (PD) is the most prevalent CNS movement disorder and the second most common neurodegenerative disease after AD. The overall frequency of PD ranges from 10 to 18 per 100,000 person-years (Kalai and Lang, 2015). More specifically, the prevalence of PD is approximately 1.8% in persons 65 years of age and older, with an increase from 0.6% for those age 65 to 69 years to 2.6% for those 85 to 89 years (de Rijk et al., 2000d). The symptoms in PD are mainly motor-related, such as resting tremor, rigidity, and loss of postural reflexes, accompanied with non-motor signs including depression, sleep difficulties, dementia, and peripheral impairments (Bernal-Pacheco et al., 2012; Sveinbjornsdottir, 2016). The main pathologic features of PD are the loss of dopaminergic neurons in the substantia nigra pars compacta (SNPC) and the accumulation of protein aggregates of α-synuclein (α-SYN) into Lewy bodies and neurites in the residual dopaminergic neurons (Lees et al., 2009; McGeer et al., 1988). One feature of PD is that the first sign of motor disturbances is not observed until the loss of dopaminergic neurons in the SNPC has reached almost 70% and at least 80% loss of dopamine has taken place in the striatum. Similar to AD, there is a significant lag between the occurrence of clinical symptom and the start of pathological changes in PD patients that could serve as the right therapeutic window for early intervention. Therefore, the exploration for early PD biomarkers becomes an essential aim for PD diagnosis before the onset of symptoms and for neuroprotective therapies that are designed for at-risk populations with the goal to delay or block the ongoing degeneration process. Additionally, the search of PD biomarkers for early diagnosis might lead to the discovery of putative molecular mechanisms and reveal targets for the development of new and more effective therapeutic strategies for this devastating disease.

Similar to AD, exosomal miRNAs present a distinct expression profiles in the CSF of PD patients, compared to that of healthy donors. For example, Gui et al. investigated the global profiling of exosomal miRNAs isolated from CSF in PD patients (Gui et al., 2015). They identified 16 up-regulated exosomal miRNAs and 11 down-regulated exosomal miRNAs in the CSF of PD patients, compared with those in healthy controls. Using independent samples, they found that the expression levels of miR-1 and miR-19b-3p were significantly reduced, while those of miR-153, miR-409-3p, miR-10a-5p, and let-7g-3p were significantly elevated, in CSF exosome samples in PD, when compared with healthy controls. ROC curve analysis determined that the sensitivity and specificity for distinguishing PD patients from control groups were 94% for miR-1, 93% for miR-153, 90% for miR-409-3p, 94% for miR-19b-3p, 95% for miR-10a-5p, and 95% for let-7g-3p, in which miR-409-3p achieved the highest area under the curve (AUC). And the combination of multiple miRNAs (e.g. miR-153 and miR-409-3p) in CSF could enhance the performance of discrimination significantly. Importantly, principal component analysis (PCA) revealed no correlation between exosomal miRNAs differentially expressed in PD and AD patients (Gui et al., 2015). This finding is confirmed with pathway analysis, in which different pathways were identified in PD and AD specific exosomal miRNAs, indicating good accuracy and specificity of CSF exosomal miRNAs in the diagnoses of various neurodegenerative diseases. Two years later, Cao et al. analyzed 24 candidate miRNAs in exosomes from serum of PD patients and healthy donors (Cao et al., 2017). They observed a decrease in the expression levels of miR-19, and an increase in the expression levels of miR-195/miR-24 in PD patients, compared to healthy controls. The ROC curve analysis revealed that the sensitivity and specificity of those three miRNAs in diagnosing PD are: 82.6% and 55%, respectively, for miR-195; 68.8% and 77.5%, respectively, for miR-19b; 81.7% and 85%, respectively, for miR-24. The combination of three miRNAs significantly increased the sensitivity and specificity to 90.0% and 85.3%, respectively. The distinct expression patterns in CSF and serum of PD patients suggest the feasibility of making use of exosomal miRNAs as reliable diagnostic markers for the early diagnosis of PD. Great efforts are in need to develop and standardize the methodological processes for exosomal miRNAs isolation, expression detection and bioinformatics analysis in order to move forward to utilize miRNAs as biomarkers for PD diagnosis in clinical practices in the future.

### 3.2. Multiple sclerosis

Multiple sclerosis (MS), an autoimmune disease of the central nervous system, is the most common cause of neurologic disability in young adults (Nicholas and Rashid, 2013). The pathogenesis of MS may involve the dysfunction of regulatory T (Treg) cells, which facilitates the proliferation and function of inflammatory T cells (Kimura et al., 2016). The imbalance of Treg and inflammatory T cells leads to oligodendrocyte loss, demyelination, and failure to remyelinate. Up until now, there is no single definitive test for MS assessment. Therefore, how to identify a reliable clinical parameter is one of the key challenges for MS diagnosis. The first report of exosomal miRNAs as potential biomarkers of MS came from Selmaj et al. In this study, the investigators utilized small RNA next generation sequencing and identified four miRNAs, miR-122-5p, miR-196b-5p, miR-301-3p, and miR-532-5p, which were significantly down-regulated in the serum exosomes of relapsing-remitting MS patients (RRMS) versus healthy controls (Selmaj et al., 2017). The ROC curve analysis revealed that all four miRNAs provided the best AUC values for discriminating between MS patients in relapse from healthy controls (miR-122-5p: 0.878, miR-196b-5p: 0.866, miR-301a-3p: 0.681, and miR-532-5p: 0.800). Similarly, Kimura et al. also characterized an MS specific exosomal miRNA signature by four over-abundant miRNAs (let-7i, miR-19b, miR-25, and miR-92a) in MS patient plasma versus healthy donors (Kimura et al., 2018). Interestingly, Ebrahimkha et al. used the same technique and identified two sets of differentially expressed miRNAs in RRMS (miR-15b-5p, miR-451a, miR-30b-5p, miR-342-3p) and progressive MS patients (miR-127-3p, miR-370-3p, miR-409-3p, miR-432-5p), compared to healthy controls. The ROC curve and Random Forest analyses indicated the predictive power for RRMS and progressive MS is 66%, versus controls. Additionally, the
combination of 3 or more miRNAs provided a predictive power of 95% for distinguishing RRMS from progressive MS. Progression of MS, as an autoimmune disease, requires the recruitment of dysfunctional T cells from circulatory system. Researches on circulatory system revealed that exosomes in serum or plasma display distinct miRNAs profiles between MS patients and healthy controls, suggesting exosomal miRNAs can serve as a promising diagnostic parameter for MS and its subtypes (Ebrahimkhani et al., 2017).

Besides MS diagnosis, exosomal miRNAs were linked to response to therapy in MS patients, revealing their potential prognostic value (Manna et al., 2018). Screening of serum exosomes from 4 IFN-β-treated RRMS patients and matched treatment-naive controls suggested that 2 miRNAs, miR-22-3p and miR-660-5p, were up-regulated, and 14 miRNAs, miR-486-5p, miR-451a, let-7b-5p, miR-320b, miR-122-5p, miR-215-5p, miR-320d, miR-19b-3p, miR-26a-5p, miR-142-3p, miR-146a-5p, miR-15b-3p, miR-23a-3p, and miR-223-3p, were down-regulated. Though no ROC analyses were done and larger sizes of samples should be recruited for validation purpose, these significantly differentially expressed exosomal miRNAs may serve as a potential prognostic indicator for therapy response or even become a therapeutic target to enhance the curative effect of existing drugs.

3.3. Stroke

Stroke is the second leading cause of death worldwide and a major source of disability in people over 60 years old. Annually, 15 million people suffer from stroke, which causes death or permanent disability in 10 million people. Stroke can be caused by a narrowed/blocked artery (ischemia) or the leaking of a blood vessel (hemorrhagic). The lack of blood supply deprives brain tissues of oxygen, thus leaves brain cells to die within a very short period of time. Therefore, early diagnosis and prompt treatment of stroke is critical in minimizing brain damage and potential complications.

The classic risk factors of stroke include high blood pressure, tobacco smoking, obesity, diabetes mellitus, etc. Stroke is diagnosed through imaging tests (e.g. CT, MRI), electrical activity tests (e.g. ECG, Evoked Response), and blood flow tests (e.g. ultrasound, angiography). Symptoms of stroke include trouble with speech, numbness, vision impairment, severe headache, and walking difficulty. However, due to the limitation of brain imaging system in detecting small infarcts and similarity in the symptom of different CNS diseases, it is difficult for physicians to make objective assessments in early stroke diagnosis. Therefore, many studies have been carried out for the identification of stroke biomarkers (Hijazi et al., 2016; Vijayan and Reddy, 2016). Approximately 80% of stroke are ischemic stroke. So far, data from basic research and clinical studies demonstrated that circulatory exosomes and miRNAs are closely linked to ischemic vascular diseases (Li et al., 2014; Sepramaniam et al., 2014; Sorensen et al., 2014; Vijayan and Reddy, 2016). Similar to other CNS diseases, exosomal miRNAs, compared to cellular or free miRNAs, are considered as a better biomarker of stroke due to the sensitivity and specificity. For example, the levels of circulating exosomal miR-126 decreased significantly at 3 h post both transient and permanent ischemia, whereas significant reductions in free circulating miR-126 were only detected at 3 h post permanent ischemia but not post transient ischemia (Chen et al., 2015), indicating for a better sensitivity of circulating exosomal miR-126 in diagnosing transient ischemia. Afterwards, Chen et al. observed a significant increase in the levels of exosomal miR-223 in acute ischemic stroke (AIS) patients. ROC analysis to evaluate the diagnostic value of miR-223 revealed that the AUC was 0.859, demonstrating a great sensitivity (84.0%) and specificity (78.8%) of miR-223 for stroke diagnosis (Chen et al., 2017b). In addition to miR-223, levels of miR-9 and miR-124 in serum exosomes were also elevated in AIS patients, compared to healthy controls (Li et al., 2016). AUC for exosomal miR-9 and miR-124 were 0.8026 (95% CI: 0.7235–0.8816) and 0.6976 (95% CI: 0.6506–0.7895), respectively, suggesting that both miRNAs may be sensitive biomarkers to discriminate AIS patients from non-stroke individuals and miR-9 showed a better diagnostic value than miR-124. Besides, exosomal miRNAs may also be a promising tool in distinguishing different types of stroke and different phases of this disease. In 2017, Li et al. reported a significant decrease and increase in the expression levels of plasma exosomal miR-422a and miR-125b-2-3p in the subacute phase group and in the acute phase group, respectively, compared to the healthy controls (Li et al., 2017). ROC analysis showed AUC values were 0.971 for miR-422a (95% CI: 0.927–1.000) and 0.889 for miR-125b-2-3p (95% CI: 0.798–0.980) in the subacute phase group, compared to that in the healthy controls. Additionally, AUC value was 0.769 for miR-422a (95% CI: 0.625–0.914) in the acute phase group, versus that in the healthy donors.

It is worth mentioning that transient ischemic attack (TIA) may have the same symptoms with the ischemic stroke, but with a resolution of these symptoms within 24 h. Due to the complexity and high cost of latest imaging technologies and limited predictive values of existing blood protein markers, how to distinguish TIA from stroke timely and economically remains a significant challenge. Screening on the rat TIA model identified significant up- and down-regulation of the miR-300-3p and miR-122-5p levels, respectively, within 10 min after occlusion of the middle cerebral artery (MCAO) (Li et al., 2018). The sensitivity of those two miRNAs was confirmed by ROC analysis, in which AUC values were 0.960 for miR-122-5p (95% CI: 0.843–1.000) and 0.970 for miR-300-3p (95% CI: 0.902–1.000) at 10 min and 5 min after MCAO, respectively, compared with controls. Although this study suggested exosomal miR-300-3p and miR-122-5p might be used as diagnostic biomarkers for TIA, whether they can distinguish TIA from stroke remains unclear due to the lack of stroke controls.

Taken together, clinical observations and animal studies revealed a strong association of exosomal miRNAs with stroke, implicating their potential utility as blood-based biomarkers in stroke diagnosis, prognosis and treatment monitoring.

3.4. Brain tumor

Brain tumors are generally divided into two types, benign and malignant tumors. Malignant tumors can be further classified into two subtypes, primary (start within the brain) and secondary tumors (spread from other organisms). miRNAs loaded in tumor-originated exosomes represent a potential biomarker for early detection of tumor occurrence and monitoring of tumor cells’ response to treatment. Glioma/glioblastoma (GBM) is the most common and dismal primary brain tumor types in adults with a remarkable ability to alter surrounding cells to facilitate their growth, evasion of immune responses, resistance to chemotherapy, and metastasis. GBMs also hijack surrounding vasculature and stimulate formation of new blood vessels for supply of nutrition. The first report on miRNAs in GBM cells-derived exosomes came from Skog’s group in 2008, in which 11 GBM-enriched miRNAs (let-7a, miR-15b, miR-16, miR-19b, miR-20, miR-21, miR-26a, miR-27a, miR-92, miR-93 and miR-320) were detected in exosomes released from two different primary GBM cells (GBM1 and GBM2) (Skog et al., 2008). Similar expression patterns are observed in GBM cells and exosomes released from these cells. Interestingly, miR-21, one of GBM-enriched miRNAs, is over-expressed in exosomes isolated from GBM patient serum, compared with healthy donors (Shi et al., 2015). ROC curves suggested the AUC for exosomal miR-21 was 0.927 (95% CI: 0.865–0.985), which appeared to be an excellent index to differentiate recurrent glioma patients (n = 70) from healthy donors. Besides, exosomal miR-21 acquires good AUC ratios for the discrimination of high grade glioma (grade III/IV) from lower ones (grade II) or separating grade IV from grade II glioma patients, which are 0.872 (95% CI: 0.817–0.927) and 0.751 (95% CI: 0.681–0.821), respectively. This study is the first indication of exosomal miRNAs as potential biomarker of CNS diseases in general and GBM in particular. Following this work, Manterola et al. identified 2 overexpressed miRNAs, miR-574-3p and...
miR-320, in the exosomes isolated from the serum of GBM patients, compared with paired healthy controls. The differential expression pattern was confirmed by multiple approaches that include miRNA array and qPCR analyses (Manterola et al., 2014). The ROC curve analysis suggested that the combination of those two miRNAs, with another small non-coding RNA, RNU6, exhibited high sensitivity (70%) and specificity (71%) in GBM diagnosis, validating the diagnostic value of circulating exosomal miRNAs. Similarly, serum exosomal miR-301a levels significantly elevated in glioma patients versus healthy controls, correlated with higher pathological grades and lower Karnofsky performance status (KPS) scores (Lan et al., 2018). More importantly, exosomal miR-301a levels were significantly reduced after surgical resection of primary tumors and ascended again during GBM recurrence in patient serum. The strong correlation of serum exosomal miR-301a and glioma is confirmed by ROC analysis. The AUC ratio was 0.937 (95% CI: 0.855–0.987), whereas the sensitivity, specificity, and positive and negative predictive values to distinguish patients with glioma were 86.2%, 93.2%, 95.4% and 66.8%, respectively. Besides, Santangelo et al. observed significant increases in the expression levels of miR-21, miR-222 and miR-124-3p in exosomes from serum of high grade GBM patients, compared with that of low grade GBM patients and healthy controls (Santangelo et al., 2018). ROC curve analysis indicates that either miR-21 (AUC = 0.84, 95% CI: 0.7538–0.9371), miR-222 (AUC = 0.80, 95% CI: 0.6967–0.8980), miR-124-3p (AUC = 0.78, 95% CI: 0.6732–0.8894) alone or the combination of three miRNAs (AUC = 0.87, 95% CI: 0.7885–0.9524) were robust in discriminating patients with GBM from healthy donors. Interestingly, those three miRNAs could also be recruited for distinguishing either GBM from secondary brain metastases or subtypes of GBM, further displaying the sensitivity and specificity of serum exosomal miRNAs as a promising biomarker for GBM diagnosis.

Surprisingly, negative correlation between the expression of exosomal miRNAs in serum and that of miRNAs in GBM tissues is also observed. For example, levels of exosomal miR-320 and miR-574-3p in the GBM patient serum were elevated, compared to healthy controls (Manterola et al., 2014). However, those miRNAs have lower expression levels in GBM tissue in comparison to the normal counterpart and are reported to inhibit the proliferation and invasion of GBM cells (Sun et al., 2015a). Multiple hypotheses could be raised to explain these interesting phenomena: (1) the expression patterns of miRNAs in parent cells and the derived exosomes are distinct, which widely exists in various cell and tissue types; (2) the exosome sources from tissues other than GBM; (3) the techniques among different screening assays are discrepant; (4) there may be some unknown self-protective mechanisms compromising the abnormal miRNA expression in GBM tissue through the circulatory system. Therefore, more comprehensive screenings are needed to clarify the sources of exosomal miRNAs in the circulatory system for novel biomarker identification.

One critical step of GBM diagnosis is to distinguish the subtypes of GBM (invasive vs non-invasive). Knowing the specific GBM subtype is important for the proper design and execution of a therapeutic strategy. Hypoxia is considered as the foremost regulator of glioma development and aggressiveness, since it leads to growth factor expression, tumor angiogenesis, vascular permeability, genetic instability, and apoptosis-resistant cells selection. Zhang et al. examined the miRNA contents in the exosomes released from U87 cells, a GBM cell line, in hypoxia- and normoxia-microenvironments (Zhang et al., 2017a). In this study, distinct expression profiles of exosomal miRNAs between different oxygen concentrations were observed: 14 miRNAs (miR-3156, miR-4486, miR-6826, miR-373, miR-7845, miR-4649, miR-4278, miR-1910, miR-6885, miR-4428, miR-2681, miR-6847, miR-4283, miR-6876) exhibited significantly higher expression levels in hypoxia conditions than in normoxia controls, while 6 miRNAs (miR-6871, miR-1273, miR-6807, miR-5585, miR-1292, miR-6891) have significantly lower expression levels, respectively in hypoxia conditions, compared to normoxia controls. This exosomal miRNA signature may serve as biomarkers to assess the oxygenation status (hypoxia vs normoxia) and aggressiveness of malignant tumors (invasive vs noninvasive).

Emerging evidence has implied the potential roles of exosomal miRNAs as biomarkers and pathological mediators of GBM. These controversial findings are reported, in the aspects of expression profiles and functional analyses, declaring the gaps between current knowledge and the complexity of GBM pathogenesis. Hence, more detailed works are badly needed to fill those gaps to guide GBM diagnosis and treatment.

3.5. Epilepsy

With 65 million people affected worldwide, epilepsy is the third most common chronic neurological disease (Moshe et al., 2015; Thurman et al., 2011). Epilepsy is characterized by recurrent & unprovoked seizures with neurobiological, cognitive, psychological, and social consequences, often with neither a known etiology nor an effective treatment. Epilepsy is highly individual-specific as it could be caused by various genetic or structural modifications in the brain, brain infections, head injuries, strokes, tumors or many comorbid psychiatric disorders (Devinsky, 2003; Piazzini et al., 2001; Schachter, 2009). Because of that, the identification of exosome with diagnostic value for epilepsy is challenging. The only report so far is from Yan et al., who identified 1 up-regulated miRNA (miR-3613-5p) and 5 down-regulated miRNAs (miR-4668-5p, miR-8071, miR-197-5p, miR-4322, and miR-6781-5p) in plasma exosomes from 40 mesial temporal lobe epilepsy with hippocampal sclerosis patients (mTLE-HS), compared with gender and age matched 40 healthy volunteers (Yan et al., 2017). Among them, miR-8071 yields the best diagnostic value for mTLE-HS with 83.33% sensitivity and 96.67% specificity, and is associated with seizure severity.

3.6. Summary

In summary, recent studies revealed encouraging findings in using exosomal miRNAs for diagnosing multiple CNS diseases. Meanwhile, concerns are also raised especially towards the sensitivity and specificity for using exosomal miRNAs as biomarkers. The origins of exosomes and miRNAs in biological fluid are complex, especially for that in the circulatory system. How to technically filtrate and select the disease-specific miRNAs from those serving as general regulators of vascular/ CNS dysfunction is the main concern and challenge for future clinical application of exosomal miRNAs in disease diagnosis. This challenge is technically impossible to solve based on exosome isolation and exosomal miRNAs analysis methodologies at current stage. Hence, the development of more precise and comprehensive screening is a critical focus of ongoing investigations. Another concern is the reproducibility of studies to identify disease-specific exosomal miRNA signatures since mismatches of exosomal miRNA expression profiles have been reported for the same CNS diseases by different literatures. This inconsistency could be caused by multiple reasons. First, different techniques and protocols for exosomal miRNA isolation (e.g. UC- and commercial kit-based isolation) and expression analyses (e.g. next generation sequencing, miRNA microarray and qPCR) demonstrate distinct levels of sensitivity for miRNA detection. Second, significant sample variability is expected because of the highly variable epigenetic and environmental factors among different human populations. Thirdly, due to our limited understanding and the complexity of CNS disorders and stroke, their pathogenesis may be driven by diverse known/unknown risk factors. The different causes of diseases may have differential influences on the expression of exosomal miRNAs. Fourthly, the majority of reviewed investigation determined sensitivity and specificity of exosomal miRNAs in diagnosis by comparing patients’ samples with healthy donors. However, if recruiting people at risk (e.g. smokers or people with hypertension or atherosclerosis), or people with other diseases (e.g. infection) as controls, sensitivity and specificity may decrease.
4. Exosomal miRNAs are contributing factors in the pathogenesis of CNS diseases

As a key post-transcription regulator of gene expression, miRNAs widely participate in the pathogenesis of diseases. Recent findings revealed that exosomal miRNAs could be one key contributing factor in various CNS diseases (Gabriely et al., 2008; Kimura et al., 2018; Liu et al., 2014). Here, we summarized the current findings for the involvement of exosomal miRNAs and their confirmed/predicted target genes in the pathogenesis of CNS diseases (Table 2).

### 4.1. Neurodegenerative diseases

#### 4.1.1. Aging-the beginning of age-related diseases

Neural aging as a progressive loss of function involves central and peripheral neural cells including neurons and NSCs (Chang and Guarente, 2013; Satoh et al., 2013). It promotes neurodegeneration and impairs neurogenesis, leading to age-related diseases such as AD, PD, Huntington’s disease, and amyotrophic lateral sclerosis (Boerrigter et al., 1992; Coppede and Migliore, 2010). Key changes in neural aging include the decrease in brain volume and gray matter loss in the medial frontal cortical regions, which can cause cognitive impairment in the elderly (Bergfield et al., 2010; Courchesne et al., 2000; Peters et al., 2008; Salthouse, 2009; Sowell et al., 2004). Emerging evidence has implied that miRNAs regulate pathways and interconnected signaling cascades that are the basis for the cognitive decline and neurodegenerative disorders during aging in humans and nonhuman primates (Persengiev et al., 2012). Recently, Zhang et al. reported a significant decline in miRNA expression levels in exosomes secreted from mid-aged hypothalamic NSCs (htNSCs), compared to young controls (Zhang et al., 2017b). The administration of htNSC-derived exosomes via the hypothalamic third-ventricle cannula in mid-aged mice led to the maintenance of NSCs pool, reduction of neuroinflammation and alleviation of cognitive impairment, suggesting speed of aging can be partially controlled through exosomal miRNAs.

#### 4.1.2. Alzheimer’s disease

As one of the most complex diseases, the pathogenesis of AD remains vague and contentious. Currently, multiple molecular mechanisms were proposed to link to the pathogenesis of AD. For example, one commonly accepted mechanisms starts from the formation of Aβ, which clusters into amyloid plaque (Benilova et al., 2012). The amyloid plaques on the blood vessels and on the outside surface of neurons of the brain cause irreversible neuron death and dementia eventually. Besides the Aβ cascade, Tau protein aggregation, aging, oxidative stress, neuroinflammation and gene mutation (e.g. Trem2) also participate in the AD neuropathology, neurodysfunction, and neurodegeneration (Carmona et al., 2018; Galpern and Lang, 2006; Lu et al., 2014; Markesbery, 1997; Venegas et al., 2017).

In 2014, Liu et al. demonstrated that exosomal miR-193b levels were negatively correlated with Aβ42 only in the CSF, but not in the serum, of patients with AD (Liu et al., 2014). They further demonstrated that...
miR-185-5p directly bind to the transcripts corresponding to amyloid precursor protein (APP), implying loss of miR-192/195-mediated APP inhibition might be one potential mechanism of AD. It is important to emphasize that the global expression analyses of miRNAs in different biological fluid provide valuable information for pathological effect prediction of exosomal miRNAs in AD, not only for development of potential biomarkers. For example, Cheng et al. reported the reduction of mir-15 levels in the circulating exosomes released from AD patients versus healthy controls (Cheng et al., 2015). Recent reports have demonstrated mir-15/107 family may associate with the progression of AD in two ways: (1) mir-15 may directly target AD-related genes (e.g. APP and BACE1) and inhibit the expression of the latter; (2) mir-15 may control the abnormal phosphorylation of Tau and APP by regulating the expression of p35/CDK5 (Moncini et al., 2017; Parsi et al., 2015). Thus, it is highly possible that exosomes lack of miR-15 participate in the accumulation of APP in AD. Another interesting exosomal miRNA is miR-342-3p, since (1) its expression is high in brain and plasma; (2) it is one of miRNAs with highest reduction (~60%) in the plasma of AD patients compared with healthy controls; (3) this reduction is consistently reported by multiple research groups using different types of samples from AD patients (Cheng et al., 2015; Liang et al., 2007; Lugli et al., 2015). The databases for miRNA target prediction revealed multiple important target genes of mir-185-5p and miR-342-3p which are tightly linked to AD, such as APP, LRP8 (an ApoE receptor) and APPB2 (APP binding family b member 2) (Jaeger and Pietrzik, 2008; Li et al., 2005). Taking this information together, exosomal miRNAs could mediate the pathogenesis of AD through regulating the expression and activities of APP, Tau and BACE1.

Besides the classic risk factors discussed above, neuroinflammation is another key character of AD (Cameron and Landreth, 2010; Heneka et al., 2015; Heneka and O’Banion, 2007; Sardi et al., 2011). Microglia is the first line of immune defense and a main mediator of neuroinflammation in multiple CNS diseases including AD (Heneka et al., 2013; Wu et al., 2013). Once activated into a pro-inflammatory phenotype, microglia secret exosomes enriched with Aβ, Tau and IL-1β, which facilitates the neuronal death and spread of AD (Asai et al., 2015; Trota et al., 2018). Interestingly, mir-3613-3p, whose expression levels are significantly lower in exosomes isolated from AD patient plasma, are predicted to bind to the 3’UTR of IL-1β and inhibit the translation of the latter, revealing the association of exosomal miRNAs to AD-related neuroinflammation (Lugli et al., 2015). With the help of studies on exosomal miRNAs, it is highly possible to locate new therapeutic targets and develop innovative strategies for AD treatment in the near future.

4.1.3. Parkinson’s disease

There is no study that conclusively identified the mechanisms for exosomal miRNAs in the pathogenesis of PD. However, multiple techniques and databases have implicated the potential mechanisms of exosomal miRNAs-mediated effects on PD pathogenesis. Gui et al. applied DIANA-miPath on the differentially expressed exosomal miRNAs in PD (Gui et al., 2015). In their study, KEGG pathway analysis identified significant enrichment of neurotrophin signaling and dopaminergic synapses, both of which are key components in the progression of PD. Similarly, Cao et al. searched the predicted targets of dysregulated exosomal miRNAs in the CSF of PD patients on Targetscan database (Cao et al., 2017). Interestingly, Parkin RBR E3 ubiquitin protein ligase PARK2/PARK8 and PARK9, whose transcripts are targeted by miR-19b and miR-195/miR-24, respectively, are found closely associated with the oxidative stress, dopamine secretion and other biological changes (Heman-Ackah et al., 2013; Vinish et al., 2011). All these abnormal biological activities lead to the early onset of PD. Following these studies, more and interesting prediction could be made for the roles of dysregulated exosomal miRNAs in PD. For example, the Targetscan database implies mir-409-3p, an up-regulated miRNA in PD CSF exosomes, might target CREB1 (cAMP responsive element binding protein 1). CREB1, a cellular transcription factor that regulates the expression of neuronal genes (e.g. TH) and neurotrophic factors (e.g. BDNF), plays an important part in preventing the progression of multiple neurodegenerative disorders including PD. Consequently, miR-409-CREB1 axis may serve as an essential element in the pathogenesis of PD. Meanwhile, it is worthwhile to emphasize the existence of mismatch between the expression signature of exosomal miRNAs and their putative functions in PD. For example, Doxakis has shown that miR-153 could repress the translation of a-SYN in vitro (Doxakis, 2010). However, the levels of miR-153 were significantly higher in exosomes from the CSF of PD patients versus controls (Gui et al., 2015). These inconsecutive reports demonstrate the complexity of PD and the juvenility of current techniques. Thus, more detailed studies unveiling the role of exosomal miRNAs in PD pathogenesis are badly needed. With expanding knowledge in miRNA profiling and target validation, new ways are being blazed for exploring the roles of exosomal miRNA as pathological mediators of PD and therapeutic targets of PD treatment.

4.2. Multiple sclerosis

The involvement of exosomal miRNAs in the pathogenesis of MS is also under extensive investigation. For example, Kimura et al. observed that circulating exosomal let-7i is markedly increased in MS patients and negatively regulates Foxp3+ Treg cell frequency and function by suppressing the expression of insulin-like growth factor 1 (IGF1) receptor and transforming growth factor beta (TGFβ) receptor 1 in MS patients, compared to healthy donors (Kimura et al., 2018). The gain of function studies demonstrated the let-7i specifically inhibits the differentiation of Treg cells, but not other types of T cells, such as Th1 and Th17 cells. They further identified IGF1/TGFβ signaling pathways as downstream factors in the let-7i-mediated reduction of Treg cell frequency and function. In a chronic inflammatory disease such as MS, TGFβ is an essential regulator in the differentiation and maturation of Treg. Interestingly, multiple dysregulated exosomal miRNAs (e.g. miR-25, miR-92a) in MS are reported to have an impact on the activity of TGFβ pathway through targeting CDKN1A/p21 and BCL2L11/Bim (Kan et al., 2009; Wong et al., 2010).

One key pathological characteristic of MS is the demyelination in CNS, caused by the apoptosis of oligodendrocytes (Stadelmann et al., 2011). Currently, multiple signaling pathways have been identified to regulate the myelination, including IGF and Wnt signaling pathways (Fancy et al., 2009; Gveric et al., 1999). Several differentially expressed exosomal miRNAs in MS are predicted to target those pathways through computational algorithms. For example, mir-15b-5p, which levels are significantly down-regulated in RRMS patients serum exosome, is predicted to target the 3’UTR of IGF1, a key factor for maintaining myelination and enhancing remyelination (Mason et al., 2003). Besides, let-7 and miR-122 are predicted to repress Wnt pathway via inactivation of Wnt and Frizzled, the ligand and receptor of Wnt signaling pathway (Cai et al., 2017; Jin et al., 2016; Wang et al., 2014a). Thus, these confirmed and predictive studies reveal the close association of exosomal miRNAs with the pathological process of MS. In summary, as one of the easily accessible and abundant biological fluids, the circulatory system is widely investigated to explore the roles of exosomal miRNAs as biomarkers and pathological mediators of MS, which is a rapidly growing field.

4.3. Stroke

Although distinct expression profiles of exosomal miRNAs are observed in the circulatory system of stroke patients, the mechanisms how exosomal miRNAs regulate the pathogenesis of stroke remain unknown. With the information from circulatory miRNAs, the involvement of exosomal miRNAs in the pathological processes of stroke can be foreseen. For example, mir-125b-2-3p that is down-regulated in exosomes from subacute stroke patients, may target the transcripts of Egf1 (Hypoxia-Inducible Factor Prolyl Hydroxylase 2), a HIF1α suppressor
(Li et al., 2016). Without the neuroprotective effects of HIF1α, more severe brain injury and less stroke recovery could be observed, demonstrating one potential mechanism for miR-125b-2-3p-mediated neuronal death in stroke (Baranova et al., 2007; Li et al., 2016).

Since stroke may cause acute damage of the brain, understanding the etiological origins is indispensable for stroke prevention. A plenty of works have denoted the affiliation of miRNAs to risk factors of ischemic stroke, the predominant type of stroke. For example, miRNAs may regulate atherosclerosis (miR-21, miR-126), hyperlipidemia (miR-33, miR-125a-5p), hypertension (miR-155), and plaque rupture (miR-222, miR-210) (Rink and Khanna, 2011). Interestingly, exosomal miR-126, but not free one in plasma is dysregulated in stroke patients, suggesting a potential role of exosomal miR-126 in atherosclerosis diagnosis.

4.4. Brain tumor

4.4.1. Primary tumor-glioma/glioblastoma

Until recently, several pilot works have unveiled the role of exosomal miRNAs in the pathogenesis of GBM. The study on exosomal miR-21, a GBM patient serum exosome enriched miRNAs, indicates that the invasion, migration, proliferation, and survival of GBM cells are regulated by miR-21-mediated inhibition of matrix metalloproteinase regulators (MMPs) through malignancy suppressors RECK and TIMP3 (Gabriely et al., 2008). Apart from that, miR-21 protects and promotes the growth of GBM cells by targeting the transcripts of PTEN/FOXO1/EGFR/PDCD4, decreasing Bax/Bcl2 ratio, and inhibiting the activity of Caspase-3/9, which indicates that miR-21 positively participates in modulating GBM through multiple mechanisms (Lei et al., 2014; Shi et al., 2010, 2015; Tokudome et al., 2015; Zhou et al., 2010). Beside miR-21, the serum exosomal miR-301 is also up-regulated in glioma patients, as we mentioned above (Lan et al., 2019). The in vitro study suggested that miR-301 could activate the AKT and FAK signaling pathways by targeting PTEN in glioma cells, implying the involvement of exosomal miR-301 in the progression of glioma. Additionally, van der Vos et al. recently confirmed the uptake of GBM released exosomes by recipient microglia/macrophages in the brain, as well as confirming the transportation of miRNAs in exosome to recipient cells and distant communication of cells through microenvironment (van der Vos et al., 2016v).

However, since different GBM cell lines and experimental conditions were utilized in research, the outcome of exosomal miRNAs’ involvement in GBM can vary, which increases the difficulty in the interpretation of results and the selection of therapeutic targets. For example, miR-373, an up-regulated exosomal miRNA in U87 cells after hypoxic treatment, is expected to enhance the growth and invasiveness of GBM cells (Zhang et al., 2017a). But on the contrary, Wei et al. reported that miR-373 inhibited the migration and invasion of U251 cells, another GBM cell line, by repressing CD44 and TGFBR2 expression (Wei et al., 2016).

4.4.2. Secondary tumor

Metastasis, the most common cause of brain cancer, means the spreading of tumor cells to the brain from another tissue in the body to form secondary brain tumor (Bos et al., 2009; Fox et al., 2011). The progress of brain metastases at distant locations requires disseminated tumor cells to adapt to the microenvironments of metastatic sites (Quail and Joyce, 2013). As tumor cells exhibit highly metabolic activities, they have the capacity to modify the microenvironment through releasing exosomes (D’Asti et al., 2012). In 2015, Fong et al. demonstrated that breast tumor cells secreted miR-122, a master regulator of glucose metabolism, via exosomes predominantly (Fong et al., 2015). The exosomal miR-122 further targeted pyruvate kinase (PKM) and repressed glucose consumption in niche cells to promote metastasis in brain. Additionally, the brain microenvironment may contain exosomal miRNAs enhancing metastasis. For example, Zhang et al. identified miR-19a, a member of the oncogenic miR-17~92 cluster, is highly enriched in astrocyte-derived exosomes (Zhang et al., 2015). miR-19 targeted PTEN and down-regulated the expression of the latter. The loss of PTEN in brain metastatic tumor cells elevated the secretion of CCL2, which recruited Ibα-expressing myeloid cells to reciprocally enhance the proliferation and survival of brain metastatic tumor cells. In summary, multiple mechanisms have been identified by independent research groups for exosomal miRNA-mediated brain metastasis, revealing the importance of exosomal miRNAs in pathogenesis and treatment of brain tumors.

4.5. Epilepsy

Due to the complexity of the etiology of epilepsy and many factors, the pathogenesis of epilepsy, especially the involvement of exosomal miRNAs, is unclear. There are a few miRNAs functional studies in epilepsy that the knockdown of miR-34a (Hu et al., 2012), miR-132 (Jimenez-Mateos et al., 2011), miR-134 (Jimenez-Mateos et al., 2012), and miR-184 (McKieran et al., 2012) influenced neuronal death, caused by status epilepticus, with none target gene identified. However, these miRNAs were not picked out by plasma exosomal miRNA profiling from mTLE-HS patients and healthy donors, leaving the exosomal miRNA-associated mechanisms of epilepsy a new field to be investigated.

4.6. Summary

Taken together, the expression profiling and functional analyses from recent reports reveal the involvement of exosomal miRNAs in the pathogenesis of CNS diseases, which implies new therapeutic targets. However, the biological effects of exosomal miRNAs in pathological processes of CNS diseases remain poorly investigated. This could be due to multiple reasons. First, the field of study on exosomal miRNAs in pathogenesis or pathology of CNS and circulatory diseases is still like a newly discovered and uncultivated land. The knowledge for exosomes in general and exosomal miRNAs in particular is very limited, which, restricts the design and amelioration of exosome-based techniques including content modification and drug delivery. Second, owing to the complexity of diseases, only a small portion of genes are identified to be involved in the pathogenesis of CNS diseases. It increases the degree of difficulty in discovering key exosomal miRNAs in CNS diseases since miRNAs achieve their functions by inhibiting translation of their target genes. Third, although potential target genes for dysregulated exosomal miRNAs could be predicted by the aid of newly established database, a general target prediction is not sufficient to unveil and illustrate the potential mechanisms of exosomal miRNAs in CNS diseases as miRNAs may bind to and silence distinct down-stream genes in a tissue or cell type-specific manner. Thus, the screening and validation of direct binding between miRNAs and their putative candidates are necessary. Considering the time consuming nature of these experiments, validation of candidate miRNA target genes will remain a challenging task. Fourth, the lack of perfect animal models for CNS diseases is also a limiting factor. Currently, the development of animal models for CNS diseases is majorly through drug administration, surgery or gene editing. Unfortunately, these approaches can only mimic partial pathology features or cover small proportions of risk factors of diseases. Therefore, the results generated in animal models via the modification of certain miRNAs levels in exosomes may fail to reflect the real pathological effects of exosomal miRNAs. Similar concerns are also tenable for in vitro and ex vivo disease models. Hence greater efforts are urgently needed to overcome these limitations and extend our knowledge in these fields.

5. Exosomal miRNAs are potential protective factors in the neural regeneration

The progression of CNS disorders is often accompanied with neural regeneration aiming to protect and repair existing neural cells or to
replace the dying cells. As a key element of CNS microenvironment, exosomes greatly contribute to neuroprotective and neuroregenerative processes. For example, exosome secretion is enhanced in the brain of the patients suffered from Down syndrome. This increase of exosomes may be intended as a protective mechanism to alleviate neuronal endosomal abnormalities (Gauthier et al., 2017). Emerging evidence has implied that endogenous exosomal miRNAs can be potential protective factors in the restoration/regeneration of CNS after an apparent brain injury. For example, differentiating or matured neural cells-secreted exosomes that abundantly containing certain miRNAs (e.g. miR-125). These miRNAs confer the neural potential to non-neural stem cells (e.g. MSCs) (Lachenal et al., 2011; Takeda and Xu, 2015). Similarly, our group has observed that NSCs secret exosomes highly enriched with miR-9 and miR-21, which promotes neuronal differentiation \textit{in vitro}. These data, together with previous reports (Ma et al., 2019), imply that miRNAs in endogenous cell-derived exosomes may have the potential in activating neurogenesis to treat various CNS diseases. Thus, the neuroprotective effects of endogenous exosomal miRNAs can be interpreted with studies on \textit{in vitro} disease models, although we are lack of direct indication \textit{in vivo}.

5.1. Stroke

Brain ischemic preconditioning (IPC) with transient and sublethal ischemic episode is well known to increase cerebral resistance to subsequent ischemic cerebral injury (Thushara Vijayakumar et al., 2016). One possible mechanism for the protective effects of IPC may associate with astrocytes that secrete exosomes enriched with miR-92b (Xu et al., 2019). Exosome-shuttled miR-92b from IPC astrocytes protects neurons against oxygen and glucose deprivation in a \textit{in vitro} model of cerebral ischemia. Interestingly, Ji et al. found that serum levels of exosomal miR-9 and miR-124 were elevated in AIS patients (Ji et al., 2016). Both of these miRNAs are highly expressed in CNS and positively associated with neurogenesis (Madeline et al., 2017; Sun et al., 2015b). The upregulation of miR-9 could significantly promote neuronal survival and neurite outgrowth in an \textit{in vitro} ischemic model, suggesting a potential role of exosomal miR-9 in neuroregeneration (Nampoothiri and Rajanikut, 2019). miR-9 is known to target apoptotic signaling such as BCL2L11 or regenerative signaling such as VEGF. With miR-9 abundantly expressed in neurogenic regions of the brain, it stands to reason that utilization of such inherent properties could lead to significant neuroregeneration post ischemic stroke. Beside the neuroregenerative capacity (Yang et al., 2017), exosomal miR-124 was considered as a suppressor for pro-inflammatory responses (Yang et al., 2019). These reports suggest that the elevation of exosomal miR-124 may mediate post stroke neuronal function recovery by enhancing neurogenesis and alleviating neuroinflammation, though it may also lead to peripheral immunosuppression, the proximate mediator of increased risk for pneumonia and septicemia (Ji et al., 2016).

5.2. Traumatic brain injury

Traumatic brain injury (TBI), the most common cause of injury-induced death and long-term disability worldwide, results in cognitive and physical functioning impairments (McKee and Luken, 2016). TBI is reported to alter miRNAs expression \textit{in situ} (hippocampus) (Redell et al., 2009) and peripherally (Redell et al., 2010). The investigation of the therapeutic benefits of exosomal miRNAs in TBI is recently initiated. In 2017, Huang et al. mimicked the \textit{in vivo} immune response situation post TBI in an \textit{in vitro} model (Huang et al., 2018). They incubated microglia with TBI mouse brain extracts and observed a significant increase in the levels of miR-124-3p in microglia-derived exosomes. These miR-124-3p-enriched exosomes repressed mTOR signaling pathway through directly inhibiting Pde4b expression, therefore promoting the transition of pro-inflammatory M1 microglia to anti-inflammatory M2 microglia and neurite outgrowth \textit{in vitro}. The intravenous administration of these exosomes to TBI mice exerted a protective effect on repressing neuroinflammation and improving neurologic outcomes. This work indicates the protective effects of immune cells in promoting neural regeneration post TBI through exosomal miRNA secretion \textit{in vivo}. Additionally, multiple highly enriched miRNAs in CNS-derived exosomes including miR-9 were up-regulated 24 h post TBI in hippocampus (Redell et al., 2009), revealing their association with enhancing neurogenesis and function recovery.

5.3. Summary

Studies on endogenous exosomal miRNAs have revealed their great neuroprotective potentials in acute brain damage. However, \textit{in vivo} evidence of neuroprotection has been limited. This is due to several reasons. First, as we mentioned in section 4 “Exosomal miRNAs are contributing factors in the pathogenesis of CNS diseases”, studies on exosomes and their containing miRNAs are typically limited in scale and lack comprehensive approaches in the investigations. Second, advances in technologies are ever more powerful to identify minuscule changes of endogenous exosomal miRNAs than before. High throughput sequencings have identified thousands of differentially expressed miRNAs in CNS diseases. The wealth of information requires time-consuming experiments to pinpoint the beneficial versus harmful effects of these miRNAs in the pathogenesis of CNS diseases. At present, only some well-investigated highly conserved miRNAs are being investigated. Further research needs to be performed on those less conserved and more distinct miRNAs, which will help fill the gap of knowledge and ease the difficulties in differentiating neuroprotective exosomal miRNAs from pathogenesis-associated ones. Third, even for these well-studied miRNAs, their roles in CNS diseases are often not clear-cut. For example, miR-124 has hundreds of confirmed/predicted target genes with various expression patterns and locations. Therefore, although the elevation of exosomal miR-124 enhances M2 polarization of microglia to inhibit neuroinflammation in CNS, it may also cause peripheral immunosuppression. This attribute of miRNAs requires that examination of the involvement of miRNAs in neuroprotection should base on detailed tissue-, cell-, or even organelle-specific approaches to circumvent the undesired side effects.

Hence, \textit{in vitro} studies have provided evidence for the involvement of endogenous exosomal miRNAs in neural regeneration and functional recovery, though \textit{in vivo} validation is urgently needed. Ongoing investigations using \textit{in vivo} approaches are rapidly extending our understanding on the biology and clinical utilities of exosomal miRNAs.

6. Exogenous exosomal miRNAs are therapeutic “drug” in the treatment of CNS diseases

Due to its highly selective permeability, the BBB rigorously restricts the transportation of large molecules and almost all small molecules to the brain from other organs (Almutairi et al., 2016). For example, the BBB restricts the penetration of more than 98% drugs into the brain. These drugs may improve therapy of various CNS diseases, such as anticancer drugs paclitaxel, doxorubicin, methotrexate, and vincristine (Pardridge, 2012). Different invasive techniques have been developed and or are under development, to overcome the BBB, such as neurosurgery, osmotic/biochemical opening of the BBB, and various formulations of nanoparticles (Abbott, 2013; Pardridge, 2012; Peng et al., 2013). However, those techniques also have inherent problems in terms of drug delivery, such as adverse events caused by nanotoxicity, re-duction in efficacy and rapid drug clearance by mononuclear phagocyte system (MPS). Inspiringly, several reports indicated that exosomes have the capacity to cross BBB and overcome the immune privileged status for the minimization of drug clearance by MPS (Alvarez-Erviti et al., 2011; Haney et al., 2015; Kojima et al., 2018; Yang et al., 2015). More importantly, exosomes can reach and convey proteins and RNAs into the brain through intranasal, intravenous, intraperitoneal and
### 6.1. Naturally enriched exosomal miRNAs from selected cells as therapeutic drugs

#### 6.1.1. Multiple sclerosis

As the demyelination of gray matter is one important component of MS pathogenesis, to enhance the oligodendrocyte-based remyelination becomes a promising therapeutic strategy of MS. Recently, evidence showed that parabiotic exposure of aged animals to the youthful ones promoted differentiation of oligodendrocyte precursors into mature myelin-producing cells and improved the recovery of demyelination (Ruckh et al., 2012). Following this study, Pusic and Kraig reported that miR-219, which is deficient in MS lesion, is enriched in circulating exosomes in youth and in environment enrichment conditions (Junker et al., 2009; Pusic and Kraig, 2014). They further identified that immune cells including blood mononuclear cells, T cells, B cells and blood dendritic cells were the origins of these miR-219-enriched exosomes (Pusic et al., 2016). These miR-219-enriched exosomes facilitated oligodendrocyte differentiation, enhanced myelination and regulated the inflammatory response in brain slice culture, suggesting that exosomal miRNAs, released by endogenous immune cells under pathological or environment enrichment conditions, exhibit promising therapeutic potential in neural regeneration and treatment of MS in the future (Pusic and Kraig, 2014; Pusic et al., 2016).

#### 6.1.2. Stroke

Since 2001, the intravenous administration of multipotent mesenchymal stromal cells (MSCs) has been proved to enhance functional recovery in experimental stroke models (Chen et al., 2001). However, only a very small portion of MSCs enter the brain after intravenous administration, excluding the possibility that abundant MSCs integrate into the brain to replace damaged cells and reconstruct neural circuitry. Recently, a novel mechanism has been discovered to explain the robust functional recovery after MSCs administration that MSCs could release exosomes which transfer miRNAs to parenchymal cells and thereby modulate the neural cell gene expression and protein production which enhance neurite outgrowth (Xin et al., 2012, 2014). For example, miR-133b in MSCs exosomes can improve functional recovery, promote axonal plasticity and neuronal processes remodeling in the ischemic boundary zone, confirmed by the knock-in and knock-down approaches (Xin et al., 2013). Further mechanistic study revealed that MSCs exosomes were transferred to adjacent astrocytes and neurons, and increased the expression of connective tissue growth factor (CTGF) in astrocytes that facilitated neurite remodeling and functional recovery (Xin et al., 2013). Hence, exosomal miRNAs, released by exogenous cells such as MSCs, showed promising neural regenerative capacity including the improvement of neural function and neurite outgrowth in stroke.

### 6.2. Selectively loaded miRNAs in exosomes as therapeutic drugs

In the past decade, various types of drugs have been packaged into exosomes for delivery purposes. The first report is published on 2011, that Alvarez-Erviti et al. demonstrated the delivery of siRNAs through exosomes into the mouse brain by tail vein injection (Alvarez-Erviti et al., 2011). They observed that a significant decline in mRNA and intracranial administration, which indicates the high flexibility and compatibility of exosome-based drug delivery in treating CNS diseases (Table 3) (Alvarez-Erviti et al., 2011; Haney et al., 2015; Lang et al., 2017; Lee et al., 2017b). Nowadays, two different approaches have been utilized to investigate the potential therapeutic application of exosomal miRNAs as drugs to treat CNS disorders. The first one is to directly administer exosomes which already contain therapeutic beneficial miRNAs. Another way is to package selected miRNAs that abolish disease-associated genes or enhance neuroregenerative factors, into exosomes for in vivo studies.
protein levels of BACE1, a therapeutic target in AD, initiating the exploration of exosome-based drug delivery for CNS diseases. Following their works, multiple groups used exosomes as the drug transporter for other types of CNS diseases. For example, Haney et al. developed catalase-load exosomes, which transport catalase, a redox enzyme that was specifically down-regulated in PD, to the brain efficiently through intranasal injection (Haney et al., 2015). In the same year, Yang et al. reported that anticancer drug could be delivered to zebrafish brain by exosomes, which successfully block/delay the progression of cancer (Yang et al., 2015). All these studies strongly support the profound therapeutic effects of exosome-mediated drug delivery in CNS disease.

Similar to siRNAs, the functional transfer of miRNAs through exosomes suggested exosomes as an effective carrier to deliver miRNA for treating CNS disorders (Montecallo et al., 2012). These groundbreaking works are reported from three separate groups at almost the same time.

6.2.1. Neurodegenerative diseases

In almost all pioneer studies of exosome-mediated miRNA transferring for treating neurological disorders, miR-124 was selected due to its unique position in the regulatory hierarchy of CNS development and disorders (Sun et al., 2015b). For example, miR-124, highly and specifically expressed in CNS, could 1) promote neurogenesis by targeting Baf53A (Yoo et al., 2009), Lhx2 (Sanuki et al., 2011), and SCP1 (Visvanathan et al., 2007), 2) inhibit tumor growth by repressing Cdk6 (Silber et al., 2008), Pnp12 (Conti et al., 2012), and Rock1 (An et al., 2013), and 3) regulate neuroimmunity by down-regulating C/EBP-α (Ponomarev et al., 2011) and p38a (Lawson et al., 2013). In 2017, Lee et al. generated miR-124-expressing HEK293 cell line to collected miR-124-enhanced exosomes (Exo-miR-124) (Lee et al., 2017b). These exosomes were injected into the bilateral striatum of Huntington’s disease mouse model. Though no behavioral improvement was observed, Exo-miR-124 administration significantly repressed the expression of transcript corresponding to REST, a key pro-neural gene repressor, suggesting the potential role in activating neurogenesis in degenerating CNS (Lee et al., 2017b).

6.2.2. Brain tumor

Similar miR-124 loading approaches are also utilized to examine the practicability of exosome-mediated miRNA delivery in treating glioma (Lang et al., 2017; Sharif et al., 2018). Exo-miR-124 co-culture significantly reduces the viability, clonogenicity, migration, and chemosensitivity enhancement of glioma cells in vitro. Animals implanted with Exo-miR-124-treated glioma stem cell in the brain or Exo-miR-124 treatment post glioma cells implantation exhibit longer living time, compared with their matched controls. Mechanistic studies further revealed that miR-124 inhibits glioma growth by silencing CDK6 and FOXA2, key genes maintaining cell cycle and glioma cell lipid metabolism, respectively. Besides miR-124, other miRNAs were employed as therapeutic “drugs” in exosome-based delivery strategies for CNS diseases. In 2013, Katakowski et al. packaged miR-146 into exosomes by ectopic expressing miR-146 in MSCs (Katakowski et al., 2013). Exosomes carrying miR-146 significantly inhibit glioma growth in vitro and in vivo, probably though down-regulating EGFR and NF-kb expression.

6.2.3. Stroke

In 2017, Yang et al. loaded miR-124 into MSCs-derived exosomes, which target injured region in ischemia model mouse after intravenous injection (Yang et al., 2017). Exo-miR-124 treatment reduces the proportions of Sox2+ & Nestin+ NSCs in the ischemic cortex at 5-day post ischemia. Besides, the number of DCX+ neuronal precursor cells increase two folds versus controls, suggesting Exo-miR-124 promotes adult neurogenesis post ischemia via biasing NSCs into neuronal cells. As miR-17-92 cluster plays an important role in mediating NPCs’ proliferation and cell death resistance after stroke (Liu et al., 2013), miR-17-92 was loaded in MSCs-derived exosomes (Exo-miR-17/92) that were intravenously administered into MCAO rats (Xin et al., 2017). Exo-miR-17/92 treatment significantly enhanced neuroplasticity and functional recovery after stroke, which was likely achieved through inhibiting the expression of PTEN, a miR-17-92 target, and activating mTOR signaling pathway (Xin et al., 2017).

6.2.4. Traumatic brain injury

As mentioned above, Huang et al. demonstrated that cultured microglia secreted miR-124 containing exosomes, which repressed neuroinflammation and promoted neurite outgrowth post TBI in vitro and in vivo (Huang et al., 2018), suggesting miR-124 could also be a promising “drug” for TBI treatment. The therapeutic effects of Exo-miR-124, derived from MSCs, on TBI were examined by Yang et al. (Yang et al., 2019). The tail vein injection of Exo-miR-124, 1-day post trauma, promoted the polarization of microglia into anti-inflammatory phenotype (M2), neurogenesis in the hippocampal region, and neurological function recovery within 1 week. The improvement of TBI outcome is probably achieved by miR-124-mediated repression of TLR-4 signaling pathway, which leads to M2 polarization and neuroprotection.

Thus, the exosome-based delivery strategy for miRNAs, especially for miR-124 is widely applied to various CNS disorder models in vitro and in vivo. Inspiring results are seen to converge over time, mapping out a bright future for the preclinical and clinical application of exosomal miRNAs in treating CNS diseases.

6.3. Targeted therapeutic delivery for exosomal miRNAs

One key challenge for using exosomes as the delivery system lies in the precise targeting of specific regions and cell types. The majority of current in vivo studies for the effects of exosome-based drug delivery on CNS diseases did not address the cellular localization of drug-loaded exosomes administrated intracranially, intravenously or intraperitoneally. Recently, an interesting phenomenon is reported that exosomes released by NSCs in neonatal subventricular zone selectively target microglia (Morton et al., 2018). This observation can be due to two reasons: (1) different cell types in CNS have distinct capacity in acquiring exosomes and (2) exosomes are capable to select the recipient cells. Multiple reports have demonstrated that the capacity and speed of phagocytosis play key roles in the efficiency of exosome up-taking by various types of cells or tissues, which explains the primary internalization of NSC-released exosomes by microglia. Therefore, exosomes can serve as an excellent cargo to transport miRNAs to microglia in brain to manipulate the inflammatory response in CNS diseases. Inspiringly, with newly developed approaches, exosomes are also engineered to carry ligands, thus exosomes can be guide to specific receptor-expressing cells. One well-established strategy utilizes CNS-specific rabies viral glycoprotein (RVG) peptide that specifically binds to the acetylcholine receptor (Alvarez-Erviti et al., 2011). RVG is fused with Lamp2b, a well-known exosomal membrane protein, and expressed on the surface of exosomes without affecting the physical properties of these modified exosomes. After tail vein delivery, RVG-expressing exosomes specifically target neuronal cells and abundantly enriched in CNS (Yang et al., 2017). These modified exosomes display significantly higher delivery efficiency versus unmodified ones after systemic administration, leading to better therapeutic outcomes by exosomal miRNA delivery.

6.4. Summary

Exosome-based miRNA delivery exhibits potential capability to alter BBB integrity (Zhao and Zlokovic, 2017). For example, exosomes enriched with miR-101 (Mishra and Singh, 2013) and miR-155 (Lopez-Ramirez et al., 2014) negatively regulate BBB function, while exosomes with specific loading of miR-132 (Xu et al., 2017) and miR-125a-3p (Reijerkkerk et al., 2013) may enhance barrier tightness. Thus, the administration of exosomes with high abundance of specific miRNAs may become a next generation miRNA-based therapy that improves from the
traditional direct application of miRNAs as therapeutic drugs. This new approach to modulate BBB permeability may be utilized to treat autoimmune diseases in CNS (e.g. MS) or to facilitate CNS penetration of other drugs. Aside from clinical applications, exosomal miRNAs will remain a focus of investigations in pre-clinical research, similar to siRNA (Alvarez-Erviti et al., 2011). Unlike siRNAs, miRNAs have capacity to target transcripts corresponding to multiple genes. Exosome-based delivery of miRNAs could be more powerful in regulating certain biological processes but less specific in manipulating the expression of gene of interest, which requires systematical and rigorous experimental design to achieve specific goals of pre-clinical studies.

With rapid expansion of studies and trials on exosome-based delivery of siRNAs or miRNAs, concerns are also raised. The first one is due to the complexity to determine and monitor the contents of exosomes derived from different cells. More detailed studies are demanded to investigate how to specifically load only desired “drugs” through exosomes and remove unnecessary components at the same time. Furthermore, it is critical to verify whether the RVG-Lamp2b fusion-protein induces adaptive immunity after repeated administration. Moreover, alternative targeting strategies are needed since the acetylcholine receptor markedly declines in brains affected by AD, narrowed the RVG-based targeting for clinical use in AD (van den Boorn et al., 2011). Lastly, exosomes derived from various sources have been applied as miRNA carrier without detailed comparison for their safety, BBB permeability, or loading and delivery efficiency, which, requires further investigation.

7. Conclusions and future directions

The field of exosomal miRNA research is still in its infancy, particularly those on CNS diseases. Observations from independent groups have demonstrated distinct expression profiles of exosomal miRNAs in various CNS diseases. With the help of those pioneer studies, the rationality and feasibility to recruit exosomal miRNAs as potential biomarkers have been widely discussed by the scientific community. Recent findings suggest high sensitivity and specificity in utilizing biological fluid exosomal miRNAs as early biomarkers of certain CNS diseases, especially before the onset of irreversible neurological damages. However, as discussed previously, the lack of knowledge for the pathogenesis of diseases and methodology for filtrating disease-specific exosomal miRNAs and enhancing the reproducibility of exosomal mRNA screening are main barriers to the application of exosomal miRNAs in clinical settings.

As a newly developed research field, the roles and mechanisms of exosomal miRNAs in CNS diseases are under extensive investigation. Growing evidence has demonstrated that exosomal miRNAs might be a double-edged sword in CNS diseases. On the one hand, studies have shown that exosomal miRNAs are directly or indirectly involved in the pathogenesis of CNS diseases. For instance, exosomal miR-193, whose levels decrease only in the serum of AD patients but not that of patients with other CNS diseases, specifically targets APP. The lack of exosomal miR-193 is likely associated with the abnormal accumulation of Aβ, a leading cause of AD. On the other hand, exosomal miRNAs also participate in neural regeneration after brain injury. miRNAs in exosomes derived from endogenous or exogenous cells are widely involved in enhancing myelination and neuroplasticity, repressing inflammatory response, and promoting neurogenesis. Hence, to expand our knowledge in exosomal mRNA-mediated effects on CNS diseases progression and regeneration is essential for the discovery of new therapeutic targets and development of new drugs and therapeutic strategies.

Fortunately, pioneer works using exosomes for the siRNAs/miRNAs delivery have been established in CNS, along with other organs. Due to their biological characteristics (e.g. low toxicity and unresponsiveness), exosomes are recruited as cargos passing through BBB. Exosomes can be engineered to transport miRNAs to certain regions or specific types of cells using multiple approaches, such as RVG-Lamp2b fusion. Significant achievements have been reported that exosomes can deliver miRNAs of interest to certain types of cells, which leads to promoting neurogenesis and inhibiting tumor growth in animal models of CNS diseases. However, how to precisely monitor exosomes and their contents are still challenging in the application of exosomal mRNA as therapeutic “drugs”.

Taken together, with better understanding of the expression patterns and pathological roles of exosomal miRNAs, the recruitment of exosomal miRNAs as promising biomarkers in the diagnosis and therapeutic targets/agents in the treatment of CNS diseases is a near possibility. In addition, the extension of our knowledge in exosomes in general and exosomal miRNAs in particular will shed light on the development of new therapeutic strategies involving highly efficient exosome-based miRNAs delivery system to bypass the BBB and immune responses.

Declaration of Competing Interest

The authors declare no conflict of interests regarding the publication of this paper.

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Appendix A. The Peer Review Overview

The Peer Review Overview associated with this article can be found in the online version, at doi:https://doi.org/10.1016/j.pneurobio.2019.101694.

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