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Roles of extracellular microRNAs in central nervous system

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Abstract

MicroRNAs are small non-coding RNAs containing about 18–25 nucleotides which modulate gene expression post-transcriptionally. Recently, microRNAs have been detected in the extracellular space including a wide range of body fluids. These extracellular miRNAs, often encapsulated in secreted extracellular vesicles, can be transferred into recipient cells and thus inhibits the expression of targeted genes. In view of these findings, a new exosome-based therapeutic approach is invented, which can effectively deliver miRNAs/siRNAs into specific cells. In central nervous system, extracellular miRNAs can not only be used as noninvasive biomarkers for diagnosis of several neurological disorders, but also mediate the intercellular communication between neurons and glial cells. In this review, we will discuss the latest research work regarding the roles of secreted miRNAs in central nervous system and evaluate the potential of exosome-mediated miRNAs/siRNAs delivery in neural therapy.

Keywords: Extracellular microRNA, Body fluid, Extracellular vesicles, Central nervous system

MicroRNAs in the central nervous system

The biogenesis and turnover of miRNAs

MicroRNAs (miRNAs) are 18–25 nucleotide noncoding RNAs that modulates gene expression by posttranscription regulation, which in turn lead to consequent biological functions [1]. Precursor miRNA molecule (pri-miRNA) is originally produced in the nucleus, where it is further processed by a complex of RNase. Afterwards, pre-miRNA is generated and sequentially carried out by exportin 5. Once transported into the cytoplasm, pre-miRNA forms a hairpin structure which is further digested by the RNase Dicer. The cleavage results in a double-stranded small RNA and one of which is the mature miRNA [2, 3]. The strand of mature miRNA is incorporated into RNA-induced silencing complex (RISC), which is known as a multi-protein RNA complex [4]. This is indispensable for their capacity of modulating protein expression, in which a seed sequence (6–8 nucleotides) of the miRNA binds to the 3′ UTR region of mRNAs to repress translation. In mammalian cells, about 30–60% proteins are targeted by miRNAs, among which they are involved in various biological processes that control cell proliferation, differentiation, regeneration, as well as apoptosis [1, 5–7]. In contrary to the biogenesis of miRNAs, the degradation of miRNAs receives limited attention so far. When the concentration of targeted mRNAs is very low, the miRNAs will detach from the RISC and enters into degradation process [8]. The cellular level of miRNAs is controlled by both production and degradation. It is suggested that the period for miRNA degradation is much longer than that of messenger RNA [9]. Furthermore, recent evidences have showed that miRNAs can be steadily exited in the extracellular system which will be discussed in the next chapter [10].

Classical functions of miRNAs in neural system

A large number of miRNAs are expressed in the Central nervous system (CNS), regulating several important proteins which further affects both physiological and pathological process in CNS [11, 12]. It enables us to overview the general effects of miRNAs in CNS by genetic deletion of essential enzymes for miRNA biogenesis. For instance, mice that lack of dicer at E18.5 display abnormal migration of late-born neurons in the cortex as well as affected expansion of oligodendrocyte precursor in the spinal cord [13]. Besides, individual roles of miRNAs

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have also been widely studied. There are several studies suggesting that miR-9 and miR-124 positively regulate neurogenesis [14]. Several miRNAs also show time and space dependent expression pattern during the development of CNS. Schratt et al. has demonstrated that miR-134 is expressed in dendrites in hippocampal neurons, which modulates dendritic spine development by targeting LIMK1 [15]. Among these biological process, there is one thing in common that those miRNAs and targeted mRNAs are both generated in the same cell. The miRNA-mRNA regulation works in a cell-autonomous manner.

Extracellular microRNAs

Extracellular microRNAs in body fluid as disease biomarker

In general thought, RNAs are highly unstable, which can be easily degraded in a very short time after their biogenesis. Until two independent groups (Chen, et al. and Mitchell, et al.) claimed their findings of miRNAs in serum/plasma, it is hard to believe that miRNA can be existed in such environment full of RNAse [16, 17]. These investigations formally start the research of extracellular RNAs. Afterwards, these cell-free miRNAs are detected in more and more body fluids samples such as saliva, urine and even milk [18–20]. Nevertheless, the level of these circulating miRNAs are closely related to a variety of disease processes, including cancers, tissue injuries and even neural degeneration diseases, indicating the potential of circulating miRNAs as non-invasive diagnostic markers for these diseases [21, 22].

Regarding to the findings of circulating miRNAs, the source of these extracellular miRNAs is still unknown. One possible source is the passive leakage from the injury tissue or broken cells, which still lacks direct evidences. It is demonstrated that the exogenous plant miRNAs increase in serum and other tissues after the mice were fed with rice or honeysuckle [23, 24]. These results suggest another explanation that serum miRNAs maybe, at least, part of the result of active secretion from tissue cells.

Secreted microRNAs in extracellular vesicles

Extracellular vesicles (EVs) have small membranous structure, which are secreted from cell to extracellular space in both physiological and pathological conditions. EVs have once been considered as non-functional debris from broken cells [25]. Until recently, a series of investigations show that EVs shedding is involved in intercellular communication [26–28]. EVs are composed of shedding vesicles (SVs) and exosomes, these two groups have different discharging processes as well as their body size [29]. Shedding vesicles are generated during the surface shedding from the plasma membrane (100-500 nm), while the production of exosomes are totally different, which are derived from multivesicular bodies secreted into extracellular space by exocytosis (30-80 nm) [30]. EVs are presented in not only the medium of cell culture but also most part of body fluids, including serum/plasma, saliva, urine as well as milk, which largely overlaps with where secreted miRNAs were found [31]. In addition, it is reported that EVs contains lipids, cytosolic proteins, messenger RNAs and even miRNAs, indicating miRNAs in EVs may be the main source of that found in body fluids [32]. It is suggested that the proportion of miRNA in EVs is about 5% of that in cytoplasm [33].

Functions of secreted microRNAs

The molecules in EVs mentioned above can be transported into the recipient cells leading to further biological functions [22]. MiRNAs are one of these most important molecules enriched in EVs. For instance, embryonic stem cells released EVs that contain large amount of miRNAs, which can be further delivered into the recipient cells in vitro [34, 35]. Once delivered into target cells, miRNAs will show their great capacity in the modulation of protein expression. Zhang et al. have demonstrated that exosomes transfer miR-150 into endothelial cells, which inhibits c-Myb translation in target cells and increase the recipient cell migration [36]. In addition, Yin et al. have showed that miR-214 secreted by tumor cells can enter CD4+ T cells, repressing local expression of PTEN and thus affecting Treg proliferation [37]. Another group suggests that miR-15a, produced in pancreatic β-cells, can enter the bloodstream and contribute to retinal injury [38]. The way of such intercellular miRNA-mRNA regulation has been found in a wide range of biological processes [10]. Additionally, secreted miRNAs may also be involved in fetal–maternal crosstalk as we found that immune-related miRNAs are enriched in colostrum EVs [18, 39, 40]. Furthermore, several studies demonstrated that exosomes derived from placenta mediate the communication between fetus and mother, showing the immune regulatory effects [41, 42]. Moreover, there are evidences that exogenous miRNAs can be absorbed through the gastrointestinal track indicating that extracellular miRNAs may even mediate the interaction between species [43]. Zhang et al. have demonstrated that exogenous plant MIR168a can be absorbed and delivered into the liver of mice fed with rice, where it specifically targets mammalian LDLRAP1 [23]. Zhou et al. provided evidences that after oral administration of honeysuckle, plant MIR2911 can enter the mice tissues, especially lungs, which remarkably inhibited H1N1 viral replication [24]. Together, these results suggest that secreted miRNAs have non-cell autonomous effects which is different with its classical roles inside the cells.

Extracellular microRNAs in the central nervous system

Circulating miRNAs in neurological disorders as diagnostic biomarkers

Since circulating miRNAs within blood and other biofluids can be detected and accurately quantified, they showed great potentials in application of disease diagnosis as non-invasive biomarkers [44, 45]. The panel of serum miRNAs may also be associated with the disease progression for neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS).

In PD patients, the profiling of serum miRNA revealed that miR-1, miR-22p, and miR-29a were significantly reduced compared with healthy controls. In addition, the level of miR-16-2-3p, miR-26a-2-3p, and miR-30a in serum can tell whether these PD patients receive treatment [46]. Later, it is demonstrated that five serum miRNAs can make a distinction between PD patients and normal controls, while another research group [47], Dong et al. even showed that 4-miRNA panel in serum help to distinguish different stages of PD patients from normal individuals [48]. In addition to the differential expression of circulating miRNAs, Kasandra et al. also detected potential novel miRNAs in blood and cerebrospinal fluid from AD and PD patients. In their investigation, the level of extracellular miRNAs detected in body fluids showed remarkable changes with different illnesses status, which indicates those extracellular miRNAs finger prints may help the diagnosis of the disease at different stages [49]. While in the case of AD, four serum miRNAs including miR-31, miR-93, miR-143, and miR-146a are significantly reduced compared to normal controls [50]. Another work revealed serum miR-223 as a promising diagnostic marker for AD. Additionally, the differential expression of miR-125b and miR-223 together may assist the early diagnosis of AD [51]. One research about ALS model reveals that miR-206 is up-regulated in skeletal muscles as well as plasma [52]. Furthermore, investigation of two intendent cohorts of ALS patients demonstrated that two circulating miRNAs (miR-4299 and miR-4649-5p) were markedly altered [53]. Besides, there are also evidences showing the association between circulating miRNAs and magnetic resonance imaging measurement of multiple sclerosis (MS) severity indicating that serum miRNAs are also significantly changed in MS patients. The alteration of serum miRNA levels could help to the evaluation of MS subtype and progression [54, 55].

Except for neurodegenerative diseases, circulating miRNAs were also used as biomarkers in acute neural injury, brain tumors and even neuropsychiatric disorders. Recently, a panel of serum miRNAs were found to differentiate mild and severe traumatic brain injury (TBI) patients [56]. In addition, elevated level of secreted miRNAs in serum is strongly related to the pathogenesis of ischemic stroke [57]. Another study in in 2017 by Wu et al. demonstrated that a panel of 3-miRNAs in serum can clearly distinguish ischemic stroke from transient ischemic attack patients [58]. In middle cerebral artery occlusion rat model, the differential expression of serum miRNAs provide strong advantage in evaluating the severity of neural injury during stroke pathology [59]. High-grade gliomas are the most aggressive and devastating brain tumors. Circulating miRNAs are appealing biomolecules which may facilitate the diagnosis of such malignant gliomas. In blood of glioblastoma patients, compared with controls, miR-128 overexpression has been identified [60]. Furthermore, Regazzo et al. suggested that serum miRNAs are potentially applicable in the diagnosis of malignant gliomas, which can precisely tell the differences between glioblastoma and slow-growing gliomas [61]. The alteration of circulating miRNAs has also been linked with several neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia. Vasu et al. have demonstrated that thirteen serum miRNAs are significantly changed in ASD patients, among which five miRNAs are enough to help the differential diagnosis of ASD [62]. In the investigation of schizophrenia patients, it is also reported that plasma miRNAs are abnormally expressed in disease group compared with healthy controls, indicating the great potential of circulating miRNAs in evaluating the disease progression [63]. Taken together, these investigations suggest that circulating miRNAs are promising biomolecules for the differential diagnosis of neurological disorders.

Role of extracellular miRNAs in physiological and pathological condition in CNS

Substantial evidence indicates that EVs, especially exosomes produced via cell exocytosis, can transport messenger RNAs, miRNAs as well as proteins into target cells, mediating the intercellular communication [32]. In the central nervous system, both neurons and glial cells can release EVs, which has been considered to be a new mode to maintain homeostasis [64].

In healthy neurons, EVs play an important role in local and possibly interneuronal exchanging of small biomolecules. In one specific scenario, both synaptic RNAs and proteins can be transported across the synapse via exosomes, which further modulates synaptic plasticity [65]. In addition, Xu et al. showed that synaptosomes can release and uptake miRNAs in different physiological conditions, indicating the miRNAs secretion in synapse may be a novel mode of communication between neurons [66]. Moreover, it is also indicated that synaptic vesicles contain miRNAs, which indicates the role of secreted miRNAs in modulating local protein translation

at synaptic terminals [67]. Neurons can not only secret miRNAs but also react with extracellular miRNAs as it is reported that miRNAs in extracellular space can bind to neuronal TLR7 and thus activate nociceptor neurons [68].

There are also abundant miRNAs in exosomes derived from astrocyte, which showed different expression pattern from that of parent cells, indicating a selective package of miRNAs from cytoplasm into exosomes [69]. Those packaged miRNAs may mediate neuron-glia interaction both in physiological and pathological condition. Carlos et al. proposed that miRNAs in astrocytic exosomes can be delivered into neuronal cells, which may contribute to the regulation of neural plasticity [70]. Another study reveals that miR-34a in shedding vesicles generated from astrocyte can be delivered into dopaminergic neurons, and thus enhanced neuronal loss under neurotoxic stress by downregulation of BCL-2 in target cells [33]. Furthermore, it is also reported that astrocytic exosomes can transfer miRNAs into metastatic tumor cells, which inhibit the expression of PTEN and prime brain metastasis outgrowth in vivo [71].

In microglia, secreted miRNAs also play key roles in mediated neuron-glia communication. EVs shed from M1 polarized microglia contain high level of miR-375, which inhibits the expression of PDK1 and increases neuronal injury in recipient cells [72]. Besides, pro-inflammatory miRNAs which include miR-146a and miR-155 are also increased in EVs derived from those M1 polarized cells, indicating the possible role of secreted miRNAs in the dissemination of inflammatory responses in brain [73].

In addition to the exosomes derived from normal cells, one study provided direct visual evidence that extracellular vesicles produced by glioblastoma deliver miR-21 into microglia and decrease the targeted mRNA level of c-Myc in vivo [74]. Nevertheless, secreted miRNAs in exosomes can even contribute to the communication between brain and blood. Systemic inflammation induced an increase of pro-inflammatory miRNAs in EVs derived from choroid plexus, which are received by glial cells, enhancing the downstream inflammatory responses [75]. Another work shows that environmental enrichment stimulates the production of pro-myelinating exosomes that contain high level of miR-219 from immune cells, which further promote CNS myelination [76].

Together, these results suggest a distinctive role of secreted miRNAs in mediating intercellular communication in CNS as well as the interaction between blood and brain.

Therapeutic potential of secreted miRNAs/siRNAs in neurological disorders

Over the last decades, EVs, especially exosomes have been used to deliver small functional molecules in the therapy for several diseases including neurodegenerative disorders [32]. Exosomes are emerging as mediators not only of neurodegeneration, but also of neuroprotection. They were shown to be involved in the regeneration and recovery after peripheral neural injury as well as neuronal damages in CNS [77]. Furthermore, their capability to cross the blood–brain barrier provides us great advantage to use them as delivery vehicles for neurological disorders [78, 79]. In one breakthrough study, wood's group used self-derived exosome from dendritic cells, which carry a fusion protein that links Lamp2b with the rabies virus glycoprotein (RVG) peptide with neuron specificity, to deliver siRNA into brain through intravenously injection. Those engineered exosomes showed great capacity in crossing blood–brain barrier and delivery of exogenous siRNA into neural cells, which results in a specific knockdown of BACE1 [78]. Newly studies also demonstrate that exosomes based therapy can alleviate neuroinflammation, increase neurogenesis and angiogenesis, which further improve spatial learning after TBI in animal models [80–82]. Another encouraging series of findings suggested that the expression level of miR-133b in MSCs significantly upregulated after exposing to ischemic conditions, which can be further transmitted into neurons and astroglia by MSC-derived exosomes, consequently promoting neurite growth and recovery of brain function [83–85]. In addition to the effect of secreted miRNAs on neurite remodeling, exosomal miRNAs also have the potential to modulate neuronal differentiation. It is demonstrated that miR-124 can be delivered into neural precursor cells (NPCs) through exosome, which downregulated the protein level of Sox9 and promoted the neurogenesis from the NPCs [86]. These studies together provide some methodology references and enlightenments for the exploration of extracellular miRNAs delivery strategy in the CNS.

Conclusion

The study of extracellular miRNAs in CNS is an exciting area that has aroused strong research interest. In addition to their great potential in the differential diagnosis of neurological disorders, secreted miRNAs represent a novel mode of intercellular communication in both physiological and pathological conditions, suggesting a new level of complexity in information transmission and processing within the neural system. Nevertheless, the transport of exogenous miRNAs into recipient cells by exosomes also suggests their application in the delivery of RNA-based therapeutics. It is of great significance to make deeper understanding of extracellular miRNAs mediated intercellular communication as well as mechanisms of their package, release and uptake, which will improve diagnostic and therapeutic strategy in CNS diseases.

Abbreviations
AD: Alzheimer's disease; ALS: Amyotrophic lateral sclerosis; ASD: Autism spectrum disorder; CNS: Central nervous system; EVs: Extracellular vesicles; MS: Multiple sclerosis; MSCs: Mesenchymal stem cells; NPCs: Neural precursor cells; PD: Parkinson's disease; RISC: RNA-induced silencing complex; RVG: Rabies virus glycoprotein; SVs: Shedding vesicles; TBI: Traumatic brain injury

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LL conceived the original idea. LL and WJ co-wrote and co-edited the final version of the manuscript. Both authors read and approved the final manuscript.

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