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PERSPECTIVE

Selective secretion of microRNA in CNS system

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EXTRACELLULAR miRNAs ARE PRESENT IN VARIOUS BODY FLUIDS

MicroRNAs (miRNAs) are a diverse class of endogenous small non-coding RNAs, which posttranscriptionally regulate gene expression by interacting with their binding sites in target mRNAs. Recently, several studies have demonstrated that miRNAs are also detectable outside cells, and these miRNAs may be called extracellular miRNAs. Extracellular miRNAs are found in various body fluids, including plasma and serum (Chen et al., 2008), saliva (Park et al., 2009), amniotic fluid (Weber et al., 2010), Bronchial lavage (Molina-Pinelo et al., 2012; Weber et al., 2010), sputum (Xie et al., 2010), tears and urine (Weber et al., 2010), bile (Shigehara et al., 2011), seminal plasma (Wang et al., 2011), breast milk (Zhou et al., 2012), peritoneal fluid (Chen et al., 2012a). Finding the extracellular miRNAs extended the research field of miRNAs, and got a lot of fruitful research findings.

Given the difficulty in accessing brain tissue *in vivo* or postmortem samples, plasma or serum were employed in most studies, which try to find possible biomarkers for various CNS disorders. However, peripheral blood may not closely reflect the alteration of gene expression in the brain. Cerebrospinal fluid (CSF) is another important body fluid which may reflect more special changes of patient brains. Therefore, the miRNAs in CSF become a research focus of biomarkers in CNS diseases. Extracellular miRNAs were found in CSF and were supposed to be potential excellent biomarkers for various CNS disorders. For example, miRNAs in CSF were reported to be suitable biomarker for Alzheimer's disease (Cogswell et al., 2008), primary diffuse large B-cell lymphoma of the central nervous system (Baraniskin et al., 2012) and malignant gliomas (Teplyuk et al., 2012) etc. Recently, Gallego et al. (2012) examined miRNAs in CSF and peripheral blood in patients with psychiatric disorders (chizophrenia). This was the first study that compared miRNA expression profiles in CSF and peripheral blood drawn from the same subjects. Interestingly, they found the levels of 35 miRNAs detected in both CSF and blood samples from all subjects were poorly correlated, which means that miRNAs in CSF may reflect more information of CNS system (Gallego et al., 2012).

THE SOURCE AND SECRETION PATHWAYS OF EXTRACELLULAR miRNAs

One important question is where are these extracellular miRNAs from? The source of such extracellular miRNAs is not clear but three different pathways have been suggested (Chen et al., 2012b): (1) Passive leakage from broken cells; (2) Active secretion via microvesicles, including exosomes and shedding vesicles; (3) Active secretion using a microvesicle-free, RNA-binding protein-dependent pathway. Here, we summarize the latter two different miRNA secretion pathways. Our data and other researchers reported that the majority of microRNAs detectable in serum are concentrated in microvesicles (Gallo et al., 2012). This membrane-enclosed miRNAs secretion may be performed by almost all cell types under both normal and pathological conditions. The contents of microvesicles are very diverse and complicated, including lipids, mRNA, miRNAs and proteins. Previous studies have revealed that microvesicles are capable of specifically targeting to recipient cells to transport proteins, mRNA and lipids (Ratajczak et al., 2006; Ahmed and Xiang, 2011), they can also deliver functionally active miRNAs and proteins to trigger downstream signaling events (Pegtel et al., 2010; Zhang et al., 2010). Usually, in most studies, the microvesicles (including shedding vesicles and exosomes) are isolated together by directly centrifuging the body fluids or culture mediums at a speed more than 100,000 g. Further study may separate the shedding vesicles and exosomes, since they have different origins and possible different

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contents. These combined cargos were delivered to recipient cells as a whole. The surface proteins of microvesicles may determine the destinations of microvesicles. Furthermore, the majority of miRNAs in microvesicles are Ago2 associated, which indicated that these Ago2 equipped miRNAs were functionally active (Arroyo et al., 2011; Turchinovich et al., 2011).

miRNAs can also be secreted in a membrane-free form. Usually, secreted miRNAs were associated with relevant proteins (Wang et al., 2010). Ago2 was found to be the proteins that binding miRNAs in circulating miRNAs. Besides the Ago2 protein, it is reported that high-density lipoproteins (HDL) were involved in a mechanism of intercellular communication by participating the transport and delivery of miRNAs. HDL could bind to exogenous miRNAs and deliver them to recipient cells (Vickers et al., 2011). Very recently, we found that microvesicles-free, Ago2-associated miRNAs are actively released from neuronal cells. Specially, these microRNAs were secreted from a special cell fraction part: the axon terminal part. A panel of miRNAs was found in synaptic vesicles and depolarization could stimulate the secretion of miRNAs via exocytosis pathways, similar to the release of neurotransmitters (Xu et al., 2013).

The source(s) and secretion pathways of secreted miRNAs in CNS system are largely unknown. In this perspective review, we reviewed the advance of research about miRNAs secretion and analyzed the possible source(s) and secretion pathways of secreted miRNAs in CNS system. There may be two kinds of miRNA secretion manners and we reviewed them in a perspective view.

SECRETION OF miRNA IN CNS SYSTEM (MODEL I)

There are many different type cells in CNS system, including neurons, astrocyte, microglia and oligodendrocyte. They may divide into neuronal cells and non-neuronal cells (glial cells). Both neuronal cells and glial cells were reported to be capable of producing microvesicles *in vitro* (Cocucci et al., 2009). Shedding vesicles are derived from cytoplasmic membrane, the diameter and membrane protein markers of shedding vesicles (diameter 100–1000 nm) are different to the exosomes (diameter 20–100 nm). The microRNA profiling of shedding vesicles are still uncharacterized. It is unclear about how the miRNAs are engulfed by plasma membrane for secretion in the form of the shedding vesicles. The selective packaging of miRNAs is still unknown, further study about the miRNA secreted in shedding vesicles form is needed. We only discuss the mechanism of miRNAs selective secretion in exosomes. Exosomes are small membrane vesicles derived from multivesicular bodies (MVBs), and are released to the extracellular by fusion of the outer membrane with the plasma membrane. Many studies have reported that miRNAs and miRNA-loaded RISC (miRISC) are existed in secreted exosomes (Skog et al., 2008; Gibbings et al., 2009). How these miRNAs and miRNA associated proteins are transported to exosomes is still unknown. Gibbings et al. (2009) suggested transporting miRNAs into exosomes may be controlled by GW182, which is required for miRNA function through its association with Ago2, is dramatically enriched in exosomes (Gibbings et al., 2009). However, whether miRNAs and their associated proteins are assembled into miRISC first and then transported into exosomes or they are transported separately into exosomes and then assembled into miRISC is still unknown.

Kosaka et al. (2010) demonstrated that secretion of miRNAs from cultured cells is through a ceramide-dependent secretory pathway and controlled by neutral sphingomyelinase 2 (nSMase2), which is known as a rate-limiting enzyme of ceramide biosynthesis (Kosaka et al., 2010). In fact, a ceramide-dependent secretory mechanism has been suggested to be needed for endosome sorting into the exocytic multivesicular bodies (MVBs) (Trajkovic et al., 2008). Interestingly, the endosomal sorting complex required for transport (ESCRT) system, which has a central role in the accumulation of exosomes to be targeted to lysosomes, is unnecessary for the release of miRNAs (Kosaka et al., 2010). These results suggest a ceramide-triggered, ESCRT-independent mechanism for exosomal miRNA secretion (Chen et al., 2012c).

SECRETION OF miRNA IN CNS SYSTEM (MODEL II)

It still remains elusive about the mechanism of microvesicle-free secreted miRNAs. As mentioned above that HDLs were involved in the transport and delivery of extracellular miRNAs. HDL could bind to exogenous miRNAs and deliver them to recipient cells. However, this study did not assess how microvesicle-free secreted miRNAs were secreted from cells. Very recently, we have found that miRNAs are actively secreted from synaptosomes via exocytosis pathway, as the miRNAs are co-released with neurotransmitters by the fusion of synaptic vesicles with pre-synapse membrane, it is a “microvesicle-free” secretion of miRNAs. However, before the secretion, miRNAs are stored in synaptic vesicles, which are specific membrane vesicles, like exosomes, they also have a selective sorting and packaging protein machineries to collect neurotransmitters. Furthermore, the miRNAs secretion from synaptosomes is a Ca^{2+} dependent event and exocytosis inhibitors (okadaic acid) could inhibit the secretion of miRNAs. Interestingly, we also found that nerve growth factor (NGF) or kainic acid (KA) could regulate the secretion of miRNAs. NGF enhances the KCl-stimulated secretion of miRNAs without altering the spontaneous release of miRNAs during incubation, whereas KA decreased the KCl-stimulated secretion of miRNAs and elevated the spontaneous release of miRNAs during incubation. It is the first study to report that miRNAs are secreted via exocytosis pathway, and in neural system, neuroactive molecules could regulate the miRNAs secretion. It is unknown the proteins responsible for transporting miRNAs into synaptic vesicles. Impor-

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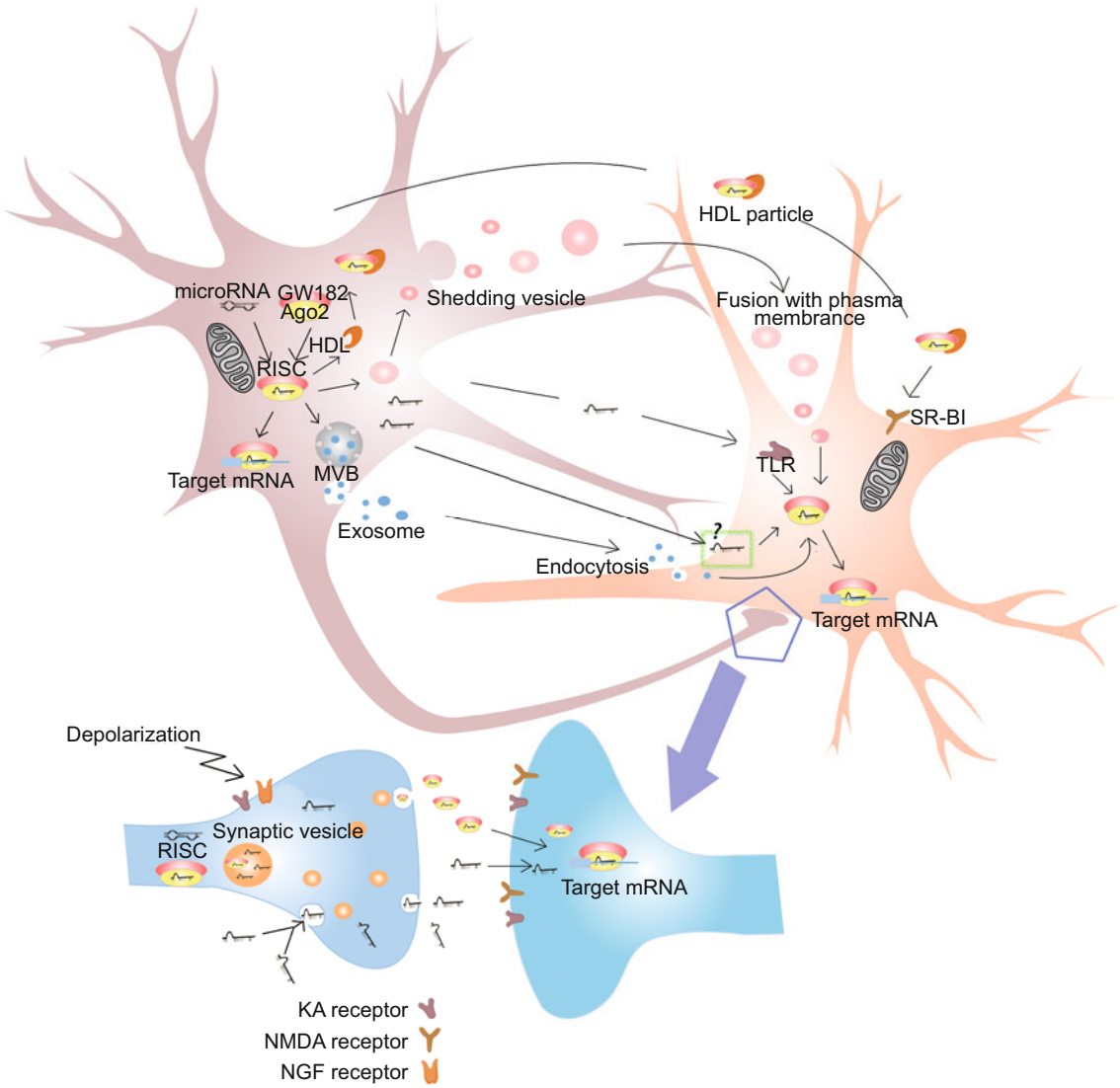

Figure 1. Schematic description of the sorting and release of secreted miRNAs. Mature miRNAs can be transported to the extracellular environment via several different pathways: (1) Exosomes. After fusion of MVBs with the plasma membrane, exosomal miRNAs are released into the circulation accompanying the release of exosomes. (2) Shedding vesicles. MiRNAs are secreted by the process of shedding from the plasma membrane. (3) MV free, associated with RNA-binding proteins. We found that Ago2 associated miRNAs could be secreted from synaptic part after depolarization stimulation. (4) MV free, RNA-binding proteins form? The process of this secretion form is still uncertain. Extracellular miRNAs are up-taken by neuronal cells. Exosomes and shedding vesicles can fuse with the plasma membrane or enter by the process of endocytosis. Protein associated miRNAs are taken up by recipient cells through putative receptors present at the recipient cellular membrane. Extracellular miRNAs may also act as a ligand for Toll-like receptor (TLR) in neuronal cells.

tantly, whether this miRNAs secretion via exocytosis pathway (Non-MV, there is no membrane vesicles released to extracellular) is universal to other cells is still unknown. Perspectively, synaptosomes have the unique features, for examples, synaptosomes are specific isolated neuronal axon fractions existing protein translation and relative self-governed metabolism and excluding the influence of nuclear imported RNA and proteins. Synaptosomes are suitable model to study the mechanism of miRNAs secretion. Further study may take synaptosomes as studying materials to focus on the mechanism of selective miRNAs secretion.

EXTRACELLULAR miRNAs ARE FUNCTIONAL IN RECIPIENT CELLS

Our previous study reported that secreted miRNAs could serve as signaling molecules mediating intercellular communication. In human blood cells and cultured THP-1 cells, miR-150 was selectively packaged into microvesicles and actively secreted. After uptaken by human HMEC-1 cells effectively reduced c-Myb expression and enhanced cell migration in HMEC-1 cells. *In vivo* studies confirmed that intravenous injection of THP-1 microvesicles signifi-

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cantly increased the level of miR-150 in mouse blood vessels. Importantly, microvesicles isolated from the plasma of patients with atherosclerosis contained higher levels of miR-150, and they more effectively promoted HMEC-1 cell migration, which is supposed to be involved in atherosclerosis (Zhang et al., 2010). In 2012, Cantaluppi et al. demonstrated that microvesicles released from endothelial progenitor cells (EPCs) enhanced human islet vascularization during islet transplantation. They found that pro-angiogenic miR-126 and miR-296 miRNAs containing microvesicles favored insulin secretion, survival and revascularization of islets transplanted in SCID mice. If adding the specific antagomiRs of miR-126 and miR-296, the microvesicles will lose the pro-angiogenic effect (Cantaluppi et al., 2012). Katare et al. reported that miR-132 secreted by pericyte progenitor cells (SVP) acts as a paracrine activator of cardiac healing through inhibition of Ras-GTPase activating protein and methyl-CpG-binding protein-2, which are validated miR-132 targets (Katare et al., 2011). Grange et al. reported that the MVs released from the $CD105^+$ cell fraction in human renal cell carcinoma more effectively trigger angiogenesis and promote the formation of a premetastatic niche. Furthermore, they characterize of $CD105^+$ MVs and define a set of pro-angiogenic mRNAs and microRNAs implicated in tumor progression and metastases (Grange et al., 2011). miR-133b secreted by multipotent mesenchymal stromal cells can enter into neural cells and contribute to neurite outgrowth (Xin et al., 2012). Taken together, these studies demonstrated that some extracellular miRNAs are actively secreted from cells and can serve as a versatile communication tools playing important roles in horizontal transfer of information between cells (Chen et al., 2012b).

UNDERSTANDING THE PHYSIOLOGICAL AND PATHOLOGICAL ROLES OF SECRETED miRNAs

Secreted miRNAs derived from different cells have recently been identified, including but not limited to immune cells, stem cells, adipocytes, neuronal cells and blood cells. These findings suggest that the original cells of secreted miRNAs are diverse. The miRNA profiling is different when the cells are under different status. Furthermore, the extracellular miRNAs existed in different forms as above discussed. Therefore, these secreted miRNAs existing in diverse forms and possessing various compositions are versatile tools in cell communication. The secreted miRNAs could be delivered to specific type cells far away from the donor cells in microvesicles forms though circulating system or other body fluids (like endocrine). The naked miRNAs secreted in microvesicles-free manner might not be as specific as the miRNAs secreted in microvesicles, and they might be uptaken by adjacent cells, (like paracrine). Interestingly, extracellular miRNAs may be a signal molecular in some extent, for example, it is reported that let-7 can activate the RNA-sensing Toll-like receptor (TLR) 7 and induce neurodegeneration through neuronal TLR7, which contributes to the spread of CNS damage (Lehmann et al., 2012). In summary, this new form of intercellular communication by extracellular miRNAs may promote our understanding of signal and molecule transfer between cells. The elucidation of this novel information transfer system will be important in understanding many physiological and pathological processes.

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