

# Circulating MicroRNAs: A Novel Class of Potential Biomarkers for Diagnosing and Prognosing Central Nervous System Diseases

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Received: 6 February 2013 / Accepted: 13 April 2013 / Published online: 30 April 2013  
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**Abstract** As a class of important endogenous small noncoding RNAs that regulate gene expression at the posttranscriptional level, microRNAs (miRNAs) play a critical role in many physiological and pathological processes. It is believed that miRNAs contribute to the development, differentiation, and synaptic plasticity of the neurons, and their dysregulation has been linked to a series of diseases. MiRNAs exist in the tissues and as circulating miRNAs in several body fluids, including plasma or serum, cerebrospinal fluid, urine, and saliva. There are significant differences between the circulating miRNA expression profiles of healthy individuals and those of patients. Consequently, circulating miRNAs are likely to become a novel class of noninvasive and sensitive biomarkers. Although little is known about the origin and functions of circulating miRNAs at present, their roles in the clinical diagnosis and prognosis of diseases make them attractive markers, particularly for tumors and cardiovascular diseases. Until now, however,

there have been limited data regarding the roles of circulating miRNAs in central nervous system (CNS) diseases. This review focuses on the characteristics of circulating miRNAs and their values as potential biomarkers in CNS diseases, particularly in Alzheimer's disease, Huntington's disease, multiple sclerosis, schizophrenia, and bipolar disorder.

**Keywords** Circulating microRNAs · Central nervous system diseases · Biomarker

## Abbreviations

A $\beta$	$\beta$ -Side amyloid
AD	Alzheimer's disease
AMPA	$\alpha$ -Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
BACE1	$\beta$ -Side amyloid precursor protein cleaving enzyme 1
BD	Bipolar disorder
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
GAP	GTPase-activating protein
GRIA	AMPA glutamate receptor subunit
HD	Huntington's disease
HTT	Protein Huntingtin
JUN	Jun proto-oncogene
LIMK1	Lim-domain containing protein kinase 1
miRNA	MicroRNA
MS	Multiple sclerosis
NMDA	<i>N</i> -methyl-D-aspartate
PTPN1	Protein tyrosine phosphates, nonreceptor type 1
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
REST	RE1-silencing transcription factor
SPTLC	Serine palmitoyltransferase long chain

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**Electronic supplementary material** The online version of this article (doi:10.1007/s10571-013-9940-9) contains supplementary material, which is available to authorized users.

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## Introduction

MicroRNAs (miRNAs) are ~22 nucleotides long, single-stranded, nonprotein-coding RNA molecules that regulate protein expression levels either by promoting mRNA degradation or by attenuating protein translation at the posttranscriptional level. Since the first miRNA (*lin-4*) was found in 1993 (Lee et al. 1993), miRNAs have elicited worldwide interest because of their characteristic functions and modes of action. Specific miRNAs have been revealed to be engaged in a variety of biological processes, such as cell development, proliferation, differentiation, and apoptosis (Li et al. 2010). In human beings, it has been estimated that there are >1,000 miRNAs in the genome that regulate ~60 % of all protein-coding genes (Friedman et al. 2009; Siomi and Siomi 2010). Most mRNA targets contain multiple miRNA binding sites, and each miRNA can regulate multiple genes. Therefore, the dysregulation of miRNA levels might perturb the expression of many genes, thereby playing a key role in the occurrence of diseases.

One of the main challenges in modern medicine is treating central nervous system (CNS) disorders which are characterized by complex mechanisms and are difficult to cure. Common CNS disorders include neurodevelopmental disorders, neurodegenerative disorders, neuroimmunological disorders, neuro-oncological disorders and other psychiatric disorders, such as schizophrenia, bipolar disorder (BD), major depression and autism spectrum disorders (Qureshi et al. 2010). It is difficult to cure these CNS diseases primarily because of the complexity of their pathomechanisms and the difficulty of achieving an accurate diagnosis at an early stage. For example, once patients are diagnosed with Parkinson's disease because of the presence of dyskinesia, their dopaminergic neurons have already degenerated by over 60 %. Therefore, finding biomarkers that allow for an accurate diagnosis at an early stage is crucial for curing CNS disorders. For example, certain specific proteins in the cerebrospinal fluid (CSF) have been identified in recent years as potential biomarkers for both the early diagnosis and prognosis of Alzheimer's disease (AD). It has been shown that A $\beta$ 42 and tau protein levels in the CSF, as well as the ratios of tau/A $\beta$ 42 and p-tau/A $\beta$ 42, can be used not only in differential diagnosis but also to predict conversion and the rate of progression from cognitive normalcy to mild dementia and severe impairment (Holtzman 2011; Fagan and Perrin 2012).

Although the detailed etiologies of many CNS disorders are still poorly understood, the defects of epigenetic mechanism may be revealed as risk factors for the occurrence of many diseases. In particular, the critical effects of miRNAs in the CNS have been recognized, and the expression patterns of characteristic miRNA profiles have

been implicated in modulating various neurologic- disease states, which could identify whether the tissue is in a normal condition or in various disease stages (Hebert and De Strooper 2009). As a subset of miRNAs, special circulating miRNAs that are found in body fluids have been implicated in diagnosing and prognosing various disorders. In this review, we discuss the potential functions of miRNAs and summarize the current progress regarding using circulating miRNAs as potential biomarkers for the diagnosing and prognosing several CNS diseases. Therefore, this review will provide a comprehensive and wide review of the literatures of biomarker studies in CNS diseases.

## Sources and Potential Functions of Circulating MicroRNAs

MicroRNAs control various important biological processes by regulating gene expression. Recently, a number of miRNAs have been detected in extracellular human body fluids, including plasma or serum, urine, saliva, and other body fluids (Cogswell et al. 2008; Mitchell et al. 2008; Park et al. 2009; Hanke et al. 2010; Zubakov et al. 2010). In 2008, Mitchell et al. demonstrated the existence of endogenous miRNAs in human plasma; since then, the putative biological functions of cell-free miRNAs in the peripheral blood have been studied and have become the focus of the translational research. In particular, the characteristic changes of miRNAs in the serum and plasma are in accordance with changes that have been observed in targeted tissues under certain pathological status, including cancer, cardiovascular disease, injuries, etc. Recently, studies have suggested that the miRNAs in the plasma and serum might be derived from circulating blood cells under healthy conditions, but might be released from pathological tissues during an illness (Chen et al. 2008; Fichtlscherer et al. 2010). The strong correlation between circulating miRNAs and tissue miRNAs indicates that circulating miRNAs might serve as biomarkers for various diseases.

Although miRNAs have been detected in body fluids, the sources of circulating miRNAs are largely unclear. Some hypotheses have proposed that these miRNAs are passively leaked or actively released from cells into the circulatory system. Recent studies have suggested that circulating miRNAs are the by-products of necrotic or apoptotic cells. For example, cardiocyte-specific miR-208a is leaked into the circulating blood from apoptotic cells after an acute myocardial infarction (Corsten et al. 2010). This passive leakage of miRNAs has been observed during tumor metastasis and chronic inflammation. The leaked miRNAs have been found to be combined with argonaute proteins or in apoptotic bodies, which protect miRNAs against RNase degradations (Wang et al. 2010; Arroyo

et al. 2011). However, multiple lines of evidence support the idea that cellular miRNAs can also be actively secreted into the circulating blood. The actively secreted miRNAs are observed to reside in exosomes and microparticles or are bound to lipoproteins (Valadi et al. 2007; Gibbins et al. 2009; Vickers et al. 2011). Similar to hormones and cytokines, the secreted miRNAs might serve as novel signaling molecules of cell-to-cell communication as well (Valadi et al. 2007). Several excellent reviews have discussed this topic in depth.

### Advantages of and Research Strategy for Circulating MiRNA as Biomarkers

To be classified as an ideal biomarker, it is crucial that miRNAs show satisfactory predictability and the ability to be supervised and inspected during disease and prognosis. The ease of obtaining and detecting the biomarker from clinical sample is also important. Recently, neuroimaging and protein-based biochemical markers in CSF or plasma/serum have been identified as attractive biomarker candidates for accurately diagnosing early stage CNS diseases. However, neuroimaging is not a suitable first screening step during the diagnostic process because it requires costly equipment. In addition, another major obstacle in the development of imaging measures as effective biomarkers is the paucity of longitudinal studies. For protein-based biomarkers, the diversity of posttranslational modifications, the sequence variations among various tissues and species, and the difficulties in developing suitable high-affinity and high-sensitivity assay methods complicate the clinical diagnostic process. Circulating miRNAs have become new and promising forms of potential biomarkers in diagnosing CNS diseases because of the limitations of other methods and the following advantages: (1) unlike other nucleic acid molecules that are unable to exist in an extracellular environment without being damaged, circulating miRNAs exhibit an unexpected stability in various body fluids. MiRNAs can remain stable not only in the RNase-rich blood environment, but also in extreme conditions, including boiling temperatures, high or low pH, extended storage, and freeze–thaw cycles (Chen et al. 2008; Mitchell et al. 2008); (2) the sequences of most miRNAs are conserved among various species and have been identified in various tissue and cell types, and miRNAs can easily be detected using quantitative reverse transcription-polymerase chain reaction (qRT-PCR); (3) miRNAs expression is tissue- or cell-specific and is altered during the pathophysiological processes. Most importantly, the changes of miRNAs levels in circulation reflect the changes in diseased tissues; (4) obtaining clinical samples that contain circulating miRNAs is a noninvasive and simple process;

(5) compared with previous studied biomarkers, miRNAs have a lower complexity which facilitates exploration. These advantages indicate that circulating miRNAs have the potential to be useful candidates for diagnosis and other clinical applications in human diseases. However, there are limitations for this potential biomarker. Because one miRNA can target many proteins and one protein can be regulated by many miRNAs, there is an inherent complexity in the relationship between a single miRNA and a pathogenic protein. Therefore, assaying combinations of multiple miRNAs, rather than a single miRNA, may improve the sensitivity and specificity of the diagnosis. In addition, because of the small amount of circulating miRNAs and the large amount of proteins, extracting miRNA from serum or plasma is technically challenging. Because no suitable endogenous controls can be endorsed, data normalization is another critical issue. Consequently, it is imperative to develop standardization methods for quantifying the circulating miRNA. Despite these limitations, the application value of circulating miRNAs in various diseases is gradually being uncovered.

At present, the strategy for discovering aberrant expressed circulating miRNAs has taken two forms: one is screening and detecting differential circulating miRNAs from the plasma or serum of patients or pathological animals directly, and the other is distinguishing the differential expression of miRNAs from diseased tissues and then verifying the miRNA targets in the body fluids. qRT-PCR is the most widely used method for quantifying circulating miRNA levels. This method offers the advantages of simplicity and high sensitivity and reproducibility for detecting low levels of abundant circulating miRNAs. Microarray-based expression analysis is another method that is frequently used to screen the differential expression of miRNAs. Compared with qRT-PCR, microarray analysis requires a large amount of RNA and has lower reproducibility. Deep sequencing technology is an excellent method to discover unknown miRNAs and identify disease-specific miRNA fingerprints. However, this method is unsuitable for routine analysis because of the expensive equipment that is required. Regardless of which quantifying method is used, the miRNA extraction from plasma or serum is the greatest challenge because of the small amount of circulating miRNAs and the large amount of proteins. Because the efficiency of RNA extraction can be affected by the variability in protein and lipid contents of plasma or serum among individuals, Kroh et al. used *C. elegans* synthetic miRNAs (without any sequence similarity to known human, rat, and mouse miRNAs) during the extraction process to control for technical variations and normalize the data (Kroh et al. 2010).

Aberrant levels of circulating miRNAs have now been detected in a variety of disorders and have attracted more

attention as candidate biomarkers, particularly in tumor and cardiovascular diseases. Many studies have shown that a certain amount of miRNAs have potential significance for the diagnosis, metastasis, progression, treatment, and prognosis in a variety of malignant tumors (Zhou et al. 2012). Circulating miRNAs have been implicated as a novel biomarker and have been identified as being involved in >12 cancer disorders (Brase et al. 2010; Wittmann and Jack 2010). Cardiovascular disease is the largest health problem worldwide, and research on the interaction between miRNAs and this disorder has become increasingly important. A series of miRNAs associated with heart disorders display interesting features in the tissues and circulation; for example, cardiac-restricted miR-208 showed both high sensitivity and specificity in of the plasma levels in acute myocardial infarction (Ji et al. 2009). Characteristic changes of miRNAs have been observed in many cardiovascular disorders, including coronary artery disease, acute myocardial infarction, heart failure, essential hypertension, and viral myocarditis (Gupta et al. 2010; Xu et al. 2012). Circulating miRNAs have been closely associated with multiple disorders, and their transcendent properties in biomarker studies indicated that they have great potential in the clinical setting. Therefore, circulating miRNAs are regarded not only as a signal for diagnosing and prognosing diseases, but they also may offer the potential for new insights into miRNA-based intervention therapy.

### The Key Role of MiRNA in CNS Functions and Disorders

The CNS receives external incoming signals and responds with related brain activity processes through its sophisticated neural network connectivity and accurate synaptic-based recognition. MiRNAs are abundant and are expressed in a spatially and temporally controlled manner in the CNS, and numerous data indicate that miRNAs mediate neurobiological processes, including neurogenesis, neural differentiation, and synaptic plasticity, as well as neurologic- and psychiatric diseases.

A close association between miRNA and CNS development and differentiation has been revealed. For example, Dicer is pivotal in the biogenesis of miRNAs, and Dicer mutants show severe defects in neural tube morphogenesis. The symptoms of this malformation can be partially reversed by introducing miR-430 into zebrafish. Progressive cell death was observed when Dicer was postnatally inactivated in the cerebellum (Schaefer et al. 2007) or in the dopaminergic neurons in the forebrain (Kim et al. 2007). In addition, miR-9, miR-124, miR-125, and miR-137 have been demonstrated to play crucial roles in

neurogenesis and differentiation (Rajasethupathy et al. 2009; Zhao et al. 2009; Edbauer et al. 2010; Smrt et al. 2010).

In the mature CNS, miRNAs are expected to affect the level of mRNAs in dendrites and to regulate local protein synthesis during synaptic plasticity. For example, miR-134 has been identified in mammalian hippocampus neurons and can restrict the dendritic spine size by antagonizing the translation of *limk1* mRNA, which can be prevented by brain-derived neurotrophic factor (BDNF) under synaptic stimulation conditions. In addition, miR-138 has been proposed to negatively regulate the dendritic spine size in rat hippocampal neurons through the local suppression of acyl-protein thioesterase 1 (Siegel et al. 2009), and miR-132 induces activity-dependent dendritic growth by down-regulating p250GAP, which plays an important role in the synaptic structure and function (Klein et al. 2007).

In addition to maintaining the normal physiological functions of the CNS, specific miRNAs are closely related to the presence and development of CNS disorders. Table 1 shows the aberrant expression patterns of miRNAs in certain CNS diseases. For example, among these aberrantly expressed miRNAs, miR-107 is remarkably reduced in early onset in AD patients and has been suggested to accelerate the process of disease by increasing the expression of the  $\beta$ -side amyloid (A $\beta$ ) precursor protein cleaving enzyme 1 (BACE1) (Geekiyana and Chan 2011). Multiple studies have indicated that the Fragile X syndrome pathogenesis may be caused by miRNA-mediated fragile X mental retardation 1 gene transcriptional inactivation (Barbato et al. 2008). In multiple sclerosis (MS) patients, the levels of miR-155 or miR-326 are obviously elevated in regions of brain injury. Knocking-out or silencing one of these two miRNAs in vivo can alleviate the symptoms in an MS animal model (Du et al. 2009; Junker et al. 2009). Because of their potential ability to target regulatory factors (including cyclin D2, PTPN1 and JUN), miR-206 and miR-198 have been correlated with schizophrenia (Hansen et al. 2007). Understanding the roles of miRNAs will provide new insights for diagnosing and treating CNS disorders. Multiple reviews have addressed the role of miRNAs in CNS development and differentiation and in CNS diseases, including AD, PD, and MS, etc. Herein, we discuss this topic briefly.

### Circulating MiRNA as Potential Biomarkers for the Diagnosis and Evaluation of Therapy in CNS Disorders

Given the crucial role of miRNAs in physiological and pathological processes of the CNS, an increasing number of studies have investigated the potential of circulating

**Table 1** Aberrant expression of miRNAs in CNS disorders

Disease	Source	miRNAs	Change	Methods	Species	References <sup>a</sup>
Addiction	Striatum	miR-212	Up	Microarray, qRT-PCR	Mouse	Hollander et al. (2010)
	Ventral tegmental area and nucleus accumbens	miR-181a	Up	In situ hybridization, northern blot, qRT-PCR	Mouse	Chandrasekar and Dreyer (2011)
Alzheimer's disease (AD)	Embryo hippocampus neurons	let-7d, miR-124a	Down	Microarray, qRT-PCR	Zebrafish	Sanchez-Simon et al. (2010)
		miR-133b	Down	qRT-PCR	Mouse	He et al. (2010)
	Brain	let-7	Up	qRT-PCR	Mouse	Zheng et al. (2010)
	Hippocampus neurons	miR-190	Down	Microarray, qRT-PCR	Mouse	Huang and Li (2009)
	PC12 cell	miR-140*	Up	Microarray, northern blot	Mouse	Lippi et al. (2011)
	Prefrontal cortex, hippocampus, midbrain	miR-29a/b	Up	qRT-PCR	Human	Geekyanage et al. (2012)
	Serum	miR-137, -181c, -9, -29a/b	Down	qRT-PCR	Human	Lukiw (2007)
	Hippocampus	miR-9, -128	Up	Northern blot	Human	Cogswell et al. (2008)
	Hippocampus	miR-26a, -27a, -27b, -30e-5p, -34a, -92, -125b, -145, -200c, -381, -422a, -423	Up	qRT-PCR	Human	
	Cerebellum	miR-9, -132, -146b, -212	Down			
Cerebrospinal fluid	Cerebellum	miR-125b, -27a, -27b, -34a, -100, -381, -422a	Up			
		miR-9, -98, -132, -146b, -212, -425	Down			
	Cerebrospinal fluid	Let-7f, miR-105, -125a, -135a, -138, -141, -151, -186, -191, -197, -204, -205, -216, -302b, -30a-5p, -30a-3p, -30b, -30c, -30d, -32, -345, -362, -371, -374, -375, -380-3p, -429, -448, -449, -494, -501, -517, -517b, -518b, -518f, -520a*, -526a	Up			
		miR-10a, -10b, -126, -126*, -127, -142-5p, -143, -146b, -154, -15b, -154, -15b, -181a, -181c, -194, -195, -199a*, -214, -221, -328, -422b, -451, -455, -497, -99a	Down			
	Medial frontal gyrus	miR-27a, -27b, -29a, -29b, -30c, -30e-5p, -34a, -92, -100, -125b, -145, -148a, -381, -422a, -423	Up			
	Temporal lobe cortex	miR-9, -26a, -132, -146b, -200c, -210, -212, -425	Down			
	Temporal cortex	miR-125b, -9, -146a	Up	Microarray, northern blot	Human	Sethi and Lukiw (2009)
		miR-107	Down	In situ hybridization, microarray, northern blot	Human	Wang et al. (2008)
	Hippocampus and superior temporal lobe	miR-146a	Up	Microarray, northern blot	Human	Lukiw et al. (2008); Cui et al. (2010)
	Superior temporal lobe neocortex	Superior temporal lobe neocortex	miR-146a	Up	Microarray, northern blot	Human
miR-197, -511, -320			Up	Microarray, qRT-PCR	Human	Hebert et al. (2008)
Anterior temporal cortex		let-7i, miR-29a/b-1, -9, -15a, -101, -19b, -106b, -22, -26b, -93, -181c, -210, -363	Down			
		miR-106b	Down	qRT-PCR	Human	Hebert et al. (2009)
		miR-15a	Down	qRT-PCR	Human	Hebert et al. (2010)
		miR-124	Down	qRT-PCR	Human	Smith et al. (2011)
Entorhinal cortex and hippocampus		miR-485-5p	Down	qRT-PCR	Human	Faghghi et al. (2010)
Frontal lobe		miR-29a	Down	qRT-PCR	Human	Shioya et al. (2010)
Gray matter		miR-15, -107, -29	Down	Microarray	Human	Wang et al. (2011)

Table 1 continued

Disease	Source	miRNAs	Change	Methods	Species	References <sup>a</sup>
Amyotrophic lateral sclerosis (ALS)	White matter	miR-424	Up			
	Muscle	miR-212	Down			Williams et al. (2009)
Anxiety	Whole blood	miR-206	Up	Northern blot	Mouse	De Felice et al. (2012)
	Hippocampus	miR-338-3p	Up	Microarray, qRT-PCR	Human	
Autism	Amygdalar	miR-451, -1275, -328-5p, -638, -149, -665	Down	qRT-PCR	Mouse	Shaltiel et al. (2013)
	Cerebellum	miR-34	Up	Microarray, qRT-PCR	Mouse	Haramati et al. (2011)
Bipolar disorder		miR-484, -21, -212, -23a, -598, -95, -129, -431, -15a, -27a, -15b, -148b, -132, -128	Up	Multiplex qRT-PCR	Human	Abu-Elneel et al. (2008)
	Prefrontal cortex	miR-106a/b, -539, -652, -550, -432, -193b, -181b, -146b, miR-140, -381, -320a	Down			
Depression	Plasma	miR-134	Down	qRT-PCR	Human	Rong et al. (2011)
		miR-101, -10a, -130a, -137, -142-3p, -146a, -148b, -155, -190, -20a, -20b, -27a, -301a, -324-5p, -335, -376a*, -489, -494, -497, -660	Down	Multiplex qRT-PCR	Human	Smalheiser et al. (2012)
Down's syndrome	Hippocampus and heart	miR-99a, let-7c, miR-125b-2, miR-155, miR-802	Up	In situ hybridization, microarray, qRT-PCR	Human	Kuhn et al. (2010)
Huntington's disease (HD)	Cortex	miR-132	Up	qRT-PCR	Human	Packer et al. (2008)
	Cortex	miR-9, -9*, -124, -29b	Down			
Multiple sclerosis (MS)	Cortex	miR-29a, -330	Up	qRT-PCR	Human	Johnson et al. (2008)
	Cortex	miR-132	Down			
Huntington's disease (HD)	Cortex	miR-29a, -124a, -132, -135b	Down			
	Cortex	miR-22, -29c, -128, -132, -138, -218, -222, -344, -674*	Down	Microarray, qRT-PCR	Mouse	Lee et al. (2011)
Multiple sclerosis (MS)	Plasma	miR-34b	Up	qRT-PCR	Human	Gaughwin et al. (2011)
	Peripheral blood mononuclear cells (PBMC)	miR-145	Up	Microarray	Human	Keller et al. (2009)
Multiple sclerosis (MS)	Peripheral blood mononuclear cells (PBMC)	let-7g, miR-150	Down	Microarray, qRT-PCR	Human	Martinelli-Boneschi et al. (2012)
	White matter	miR-18b, -493, -599	Up	qRT-PCR	Human	Otaegui et al. (2009)
Multiple sclerosis (MS)	White matter	miR-21, -146a, -146b	Up	qRT-PCR	Human	Fenoglio et al. (2011)
	White matter	miR-155	Up	Microarray, qRT-PCR	Human	Paraboschi et al. (2011)
Multiple sclerosis (MS)	White matter	miR-650, -155, -326, -142-3p, -146a, -34a, -21, -23a, -199a, -27a, -142-5p, -193a, -15a, -200c, -130a, -223, -22, -320, -214	Up	qRT-PCR	Human	Junker et al. (2009)
	White matter	miR-656, -184, -139, -23b, -328, -487b, -181c, -340	Down			
Multiple sclerosis (MS)	Whole blood	miR-17, -20a	Down	qRT-PCR	Human	Cox et al. (2010)
	T regulatory cell	miR-106b, -25	Up	Microarray, qRT-PCR	Human	De Santis et al. (2010)
Multiple sclerosis (MS)	Peripheral blood leukocyte	miR-326	Up	qRT-PCR	Human	Du et al. (2009)
	Plasma	miR-614, -572, -648, -1826, -422a, -22	Up	Microarray	Human	Siegel et al. (2012)
Multiple sclerosis (MS)	Plasma	miR-1979	Down			



**Table 1** continued

Disease	Source	miRNAs	Change	Methods	Species	References <sup>a</sup>
Parkinson's disease (PD)	Midbrain dopaminergic neurons	miR-133b	Down	Northern blot	Human	Kim et al. (2007)
	Amygdala, frontal cortex, substantia nigra and cerebellum	miR-34b/c	Down	Microarray, qRT-PCR	Human	Minones-Moyano et al. (2011)
Schizophrenia	Frontal cortex	miR-205	Down	qRT-PCR	Human	Cho et al. (2013)
	Whole blood	miR-1, -22*, -29	Down	qRT-PCR	Human	Margis and Rieder (2011)
	Plasma	miR-331-5p	Up	Up	Human	Cardo et al. (2013)
	Serum	let-7g, miR-181b, -219-2-3p, -1308, -92a	Up	qRT-PCR	Human	Shi et al. (2012)
		miR-195, -17, -346	Down			
	Frontal cortex	miR-30e, -195	Down	qRT-PCR	Human	Mellios et al. (2009)
	Cerebral cortex	miR-30b	Down	qRT-PCR	Human	Mellios et al. (2012)
	Superior temporal gyrus	miR-181b	Up	Microarray, northern blot, qRT-PCR	Human	Beveridge et al. (2008)
	Dorsolateral prefrontal cortex	miR-346	Down	qRT-PCR	Human	Zhu et al. (2009)
		miR-132, -132*, -154*, -212, -34a, -544, -7	Down	qRT-PCR	Human	Kim et al. (2010)
	miR-106b, -7	Up	Microarray, qRT-PCR	Human	Perkins et al. (2007)	
	miR-26b, -30b, -29b, -195, -92, -30a, -30d, -20b, -29c, -29a, -212, -24, -30e, -9*	Down				
	miR-328, -17-5p, -134, -652, -382, -107	Up	Microarray, qRT-PCR	Human	Santarelli et al. (2011)	

<sup>a</sup> For the references of Table 1, please see the supplemental materials

miRNAs toward clinical diagnosis and therapy assessments in various CNS disorders, particularly AD, Huntington's disease (HD), MS, schizophrenia, and BD.

Alzheimer's disease is an age-related neurodegenerative disorder that is characterized by progressive memory loss and deteriorated higher cognitive functions (Heese and Akatsu 2006). The A $\beta$  peptide and tau protein are thought to play principal roles in AD development, which may lead to neurodegeneration, as well as synaptic and neuronal loss (Alves et al. 2012). Geekiyanage and Chan (2011) reported that the levels of ceramides were increased concomitantly with an increase in serine palmitoyltransferase long chain-1 (SPTLC1) and SPTLC2 in the brain cortex in a subgroup of sporadic AD patients. Furthermore, the SPTLC1/2 mRNA levels in these AD patients did not differ significantly from those of the control samples, but the negative correlations between the expression of miR-137/-181c and SPTLC1, as well as between miR-9/-29a/b and SPTLC2 protein, were detected in autopsy brain samples. In addition, gain- and loss-of-function experiments confirmed the inhibitory effect of these special miRNAs on SPTLC1/2 and revealed their ability to repress A $\beta$  protein expression. Consequently, the levels of miR-137, -181c, -9, -29a, and -29b in the serum of patients with probable AD and mild cognitive impairment (MCI)/probable early AD were all down-regulated compared with healthy controls (Geekiyanage et al. 2012). Other groups have also reported that miR-9 was decreased in both blood and brain tissue, including cortex and hippocampus, of AD patients (Chan and Kocerha 2012). The consistency of the aberrant expression of miRNAs in the brain and serum indicates that the circulating miRNAs are released from the brain and reflect the pathological processes. Members of the miR-9 and miR-29 family have also been shown to directly regulate BACE1. The down-regulation of miR-9 and miR-29 in AD patients can increase BACE1 expression, thereby inducing an accumulation of A $\beta$  (Hebert et al. 2008). Moreover, members of the miR-29 family were proved to be down-regulated in the gray matter during early AD progression and associated with the density of diffuse amyloid plaques (Wang et al. 2011), supporting the argument that this miRNA may be one of the neuropathologic hallmarks of AD. Consequently, the aberrant expression patterns of circulating miRNAs may be closely associated with the corresponding miRNAs and the causative proteins that they regulate in the brain, which offers strong evidence to recommend them as candidate biomarkers. In addition to miRNAs, neuroimaging and some specific proteins in the CSF might offer other promising potential biomarkers in AD. Positron emission tomography (PET) imaging of  $\beta$ -amyloid protein deposition and magnetic resonance imaging (MRI) of the hippocampal volumes have reportedly detected the conversion of MCI to AD (Barber 2010).

However, these imaging methods can only be used in the validation step and not a first screen step of the diagnostic process. CSF A $\beta$ 42 and tau protein, particularly the ratios of tau/A $\beta$ 42 and p-tau/A $\beta$ 42, are also useful to predict the risk of progression from MCI/very mild dementia to AD (Holtzman 2011; Fagan and Perrin 2012). Unfortunately, the changes in these candidate biomarkers in the plasma do not well match those in the CSF. Therefore, developing a plasma-based screening test remains a major challenge in increasing the efficiency and accuracy of AD diagnoses.

Huntington's disease is an incurable neurodegenerative disease caused by abnormal CAG expansion in the gene encoding the protein Huntington (HTT) (The Huntington's Disease Collaborative Research Group 1993). This dominantly inherited disorder is characterized by widespread mRNA dysregulation in striatal and cortical neurons. As a hereditary disease, HD patients can be identified using predictive genetic testing for the CAG expansion within the HTT gene before they develop symptoms. However, specific biomarkers are needed to track the disease progression and to assess the efficacy of therapeutic interventions. Structural imaging (striatal atrophy measurement and cortical volume loss) and functional and metabolic imaging (abnormal cortical activation and progressive dopamine D2 receptor loss) biomarkers have been studied. Brain-specific compounds in the CSF, such as neurofilament proteins, 24S-hydroxy cholesterol, and homovanillic acid, as well as stress-related hormones and immuno-related cytokines in the plasma, represent other appealing candidates (Weir et al. 2011). Recently, assays of circulating miRNAs have been investigated in HD as a novel approach to identify new biomarkers. The expression of several miRNAs in neurons has changed in pathological conditions of HD, and these changes have been correlated with related factors at the molecular level. In two transgenic mouse models of HD (YAC128 and R6/2), there were some commonly dysregulated miRNAs. Especially at the age when the mice present HD phenotypes, YAC128 mice showed reduced Dicer expression, and R6/2 mice showed reduced Drosha expression, suggesting altered miRNA biogenesis in these mice (Lee et al. 2011). Brain-specific miR-9/9\*, miR-124a, and other miRNAs, including miR-29b and miR-132, are significantly misregulated in the cortices of HD patients. In particular, miR-9/9\* is down-regulated in HD and has been determined to regulate RE1-silencing transcription factor (REST), the abnormal expression of which is considered to be one of the major mechanisms of HD (Packer et al. 2008). In addition, other types of transcriptional dysregulation may also contribute to occurrence of this disease. For example, the function of p53 has been identified as being important in HD and is targeted by miR-34 (He et al. 2007). Importantly, circulating miR-34b has been evaluated in the plasma of HD patients before the onset of



symptom; this circulating miRNA has been suggested to be a novel biomarker for the neurodegenerative disorder. Moreover, miR-34b can be induced using mutant Huntington in neuronally differentiated human cells and in human plasma (Gaughwin et al. 2011). Therefore, comprehensive analysis of miRNAs in the brain and circulatory system may offer new sight into the etiological pathways and preliminary diagnosis.

Multiple sclerosis is a chronic inflammatory autoimmune demyelinating disease that is found among young adults. As an autoimmune disease of the CNS, MS might be closely linked to the aberrant alterations of the miRNAs that regulate immune cell functions. Recently, many studies have reported that certain miRNAs that highly expressed in immune cells mediate MS. MiR-22 was detected to be increased 2.6-fold in the regulatory T cell of MS patients (De Santis et al. 2010). Moreover, miR-22 may influence the susceptibility to MS because of its inverted tendency to be associated with B cell translocation gene-1 in B cell during the morbid period (Mandel et al. 2004). Correspondingly, an up-regulation of circulating miR-22 was observed in the plasma of MS patients. In addition, miR-422a has been correlated with inflammatory demyelinating diseases by modulating cholesterol 7 $\alpha$ -hydroxylase and has been found to be increased in both the plasma and peripheral blood cells of MS patients (Song et al. 2010). In addition to miR-22 and miR-422a, there were five additional miRNAs (miR-616, miR-648, miR-572, miR-1826, and miR-1979) have been reported to be altered in the plasma of MS patients. MiR-614 affected neuronal differentiation and immune function by modulating relative transcription factor, and miR-648 targets myelin-associated oligodendrocyte basic protein (MOBP), a relatively abundant CNS-specific myelin protein, to stabilize the myelin sheath in the CNS (Kaushansky et al. 2010; Banerjee 2011). Both of these circulating miRNAs, together with miR-572 and miR-1826, are increased in the plasma of patients, whereas miR-1979 is decreased (Siegel et al. 2012). Furthermore, the up-regulation of both miR-18b and miR-599 in peripheral blood mononuclear cells indicated which MS patients were in relapse status, whereas a tendency toward up-regulation of miR-96 indicated that the patients were in remission (Otaegui et al. 2009). Although no evidence has shown that these three miRNAs can reflect prognosis, these miRNAs are good candidates in future biomarker studies in MS, and at least miR-18b and miR-599 have the potential to be good markers to characterize the relapse status. In addition to the miRNAs in immune cells or plasma mentioned above, a series of miRNAs in white matter, the main damaged region in MS patients, have been observed to change, which might reflect the presence of CNS lesions (Junker et al. 2009). Although several differentially expressed

circulating miRNAs have been detected, further studies are needed to explore their values as potential biomarkers in MS. As in AD and HD, several other promising biomarker candidates, including structural and functional neuroimaging (the upper cervical cord area atrophy, whole brain atrophy, black holes, and retinal nerve fiber layer thickness), neurophysiology (visual or motor evoked potentials and multimodal evoked potential scores), inflammatory and neurodegenerative-based CSF markers (chemokine CXCL13, multiple interleukins, interferon gamma, tumor necrosis factor alpha, MOBP, neurofilament, S100B acidic calcium-binding protein, glial fibrillary acidic protein, etc.), and serum autoantibodies against myelin (e.g., anti-MOBP, anti-myelin oligodendrocyte glycoprotein and anti-N-glycosylated peptide) have also been developed and evaluated (Ziemann et al. 2011). Although no biomarker is currently established, these candidates, including miRNAs, provide a perspective that may change the diagnosis of this formidable neurodegenerative disorder from the clinical end-point to sensitive and reliable biomarkers.

Schizophrenia is a severe psychiatric disorder and its typical clinical symptoms include hallucinations, delusions, anhedonia, and social withdrawal (Oertel-Knochel et al. 2011). Environmental influences and related genetic vulnerability are widely considered to be the critical factors in the pathogenesis of this mental illness (van Os et al. 2008). Several altered expression patterns of miRNAs have been detected in schizophrenia patients compared with healthy controls; these expressions may arise from the pathophysiology of the disease. After analyzing the data from the literatures and gene database using bioinformatic methods, Shi et al. quantified a number of circulating miRNAs in the serum of patients suffering from schizophrenia and that of healthy individuals. The up-regulation of circulating miR-181b, miR-219-2-3p, miR-1308, and let-7g and the down-regulation of miR-195 were observed (Shi et al. 2012). The authors concluded that these changes in the serum level of miR-181b, miR-219-2-3p, miR-1308, and let-7g and miR-195 can reflect schizophrenia status and may be used as candidate biomarkers for diagnosing schizophrenia. MiR-181b and miR-195 are relatively highly expressed in the brain. Similar to serum miR-195, brain miR-195 is down-regulated in the prefrontal cortex of schizophrenia patients according to study that employed a postmortem case-control design (Perkins et al. 2007; Mellios et al. 2009). It also has been reported that miR-195 could regulate BDNF and alter the expressions of neuropeptide Y and somatostatin in the gamma-aminobutyric acid (GABA)ergic neurons of the prefrontal cortex in schizophrenia (Mellios et al. 2009). The up-regulation of miRNA-181b in the temporal cortex of schizophrenia patients has also been observed, and this up-regulation inhibits the expression of  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptor

subunit 2 (GRIA2) (Beveridge et al. 2008). MiR-219 shows specific expression in the human brain and in the cerebral cortex of mouse (Coyle 2009; Beveridge et al. 2010). It may regulate *N*-methyl-D-aspartate (NMDA) receptor-related function by controlling the translation of a calcium/calmodulin-dependent protein kinase II subunit, whereas the expression of miR-219 in the mouse prefrontal cortex is reduced by applying an NMDA receptor antagonist. In addition, miR-219 has been observed to be relevant to behavioral deficits in schizophrenia and miR-219-mediated NMDA receptor dysfunction may affect the occurrence of schizophrenia (Kocerha et al. 2009). In addition to circulating miRNAs, molecular genetics, CSF-based biochemical candidates (VGF [nerve growth factor inducible]-derived peptide), transthyretin, fatty acid binding protein 7, apolipoprotein A1, etc.), structural neuroimaging (cortical thickness and ventricle size), and functional neuroimaging (prefrontal hyperactivation and imaging based on altered striatal and cortical dopaminergic neurotransmissions) markers offer other avenues for schizophrenia biomarker research (Oertel-Knochel et al. 2011). Because schizophrenia is one of the most complex mental disorders, the combination of heterogeneous, multifaceted, and multifactorial markers is a promising strategy to capture the subtle and intricate pathological characteristics of the disease.

Bipolar disorder is characterized by repeated episodes of mania and depression. Growing evidence shows that BD might arise from abnormalities of the synaptic plasticity; miRNA-134 localizes in the dendrites of rat hippocampal neurons and controls the size of dendritic spines and modulates the excitatory synaptic transmission by inhibiting lim-domain containing protein kinase 1 (LIMK1) translation. BDNF was able to relieve miR-134-induced inhibition of LIMK1 translation. This miR-134-induced signal regulation plays a critical role in the synaptic development, maturation, and plasticity (Schratt et al. 2006). Rong et al. observed the plasma miRNA levels obtained from BD patients who were presenting manic symptoms (Rong et al. 2011). They found that circulating miR-134 in drug-free patients with bipolar mania was significantly lower than that in normal groups. After extended treatment, the plasma miR-134 level increases gradually. Moreover, a marked negative relationship between severity of manic symptoms (BRMS scores) and the plasma miR-134 level is observed in BD patients before and after various durations of drug treatments. The correlation between miRNA level and the typical symptoms of this disease implies that miR-134 has potential value in diagnosing BD patients and evaluating their therapy. Besides miRNAs, neuroimaging markers (decreased activation and diminution of gray matter and increased activation in ventral limbic brain regions, including the parahippocampal gyrus extending to the amygdala, the

thalamus, and the caudate nucleus) and some peripheral biochemical compounds (such as BDNF, oxidative stress-related compounds, cytokines, etc.) are other appealing candidates (Berk et al. 2009; Houenou et al. 2011). In particular, the BDNF level has been observed to be decreased in both mania and depression when compared to controls, and an increase in BDNF levels following the treatment for acute mania (Fernandes et al. 2011). Because of the interaction between BDNF and miR-134, peripheral BDNF and miR-134 could be used as a biomarker of mood states and disease progression for BD.

Although considerable miRNAs with aberrant expression patterns in the brain have been reported in many CNS diseases, and the mechanisms have been explored gradually, the data regarding the circulating miRNAs in these diseases remain limited. At present, it is still difficult to establish the correlation between certain circulating miRNAs and diseases diagnoses. Therefore, further studies are required. In addition, although these three types of novel potential biomarkers: neuroimaging, miRNA- and protein-based biochemical markers each has respective advantages and limitations, we think that panels of various biomarkers should be combined and used in succession to improve the efficacy and accuracy of the early detection of CNS disorders. For example, blood-based biochemical markers, such as particular miRNAs and proteins, should be used to screen possible patients and then neuroimaging or CNS-based biomarkers can be used to validate the disease.

The discovery of circulating miRNAs opens a new field for diagnosis and therapy assessment in various diseases. In recent years, increasing numbers of aberrant expression patterns of circulating miRNAs have been detected and appear to have potential as noninvasive biomarkers for neuropsychiatric disorders. However, the research on circulating miRNA is in its infancy, and numerous technical problems must be solved, such as establishing standardization procedures for sample preparation, developing unified methodologies for detecting and quantifying the circulating miRNAs and optimizing the results interpretation. With the development of new techniques and further research investigations, circulating miRNAs undoubtedly exhibit a promising perspective in diagnosing CNS disorders and assessing related therapies.

**Acknowledgments** This study was supported by Project of National Science and Technology Support Program in China (2012BAI01B07).

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