Circulating cell-free microRNA as biomarkers for screening, diagnosis, and monitoring of neurodegenerative diseases and other neurologic pathologies

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INTRODUCTION

There are several basic types of clinical tests: (i) genetic tests that help predict predisposition to a particular disease; (ii) screening tests, which are applied to a large population for early detection of a disease, preferably prior to its clinical manifestation; (iii) diagnostic tests, which are applied when a person has clinical symptoms of a disease or when the pathology has been detected by a screening test; (iv) predictive tests aimed to predict the disease outcome and drug sensitivity; and (v) disease and treatment monitoring tests. Screening tests are most important for the early detection and successful treatment of a disease, especially if its progression leads to significant changes in the nature of underlying pathologic processes. Cancers and neurodegenerative diseases are most common examples of such diseases. Cancer progression leads to tumor invasion into surrounding tissues, metastasis and clonal evolution, which dramatically complicates treatment (Richards, 2009; Caldas, 2012). Similarly, progression of neurodegenerative diseases leads to a switch from metabolic abnormalities to neu-rite and synapse destruction and finally to irreversible neuronal death (Bredesen, 2009; Snyder et al., 2012). Further, disease progression leads to involvement of new brain areas and cell types in the pathology (von Bernhardi and Inestrosa, 2008). The new diagnostic methods based on imaging techniques and analysis of proteins and other components in the cerebrospinal fluid (CSF) have been developed recently (Apostolova et al., 2010; Fagan et al., 2011; Mori et al., 2012); these methods, however, are not suitable for the first line screening due to their invasiveness and relatively high cost.

EARLY DIAGNOSIS OF NEUROLOGIC DISEASES

There are hundreds of brain disorders and many ways to classify them; however, from the viewpoint of diagnostics it is helpful to consider the diseases based on the need for different types of diagnostics, and on the underlying pathologic processes that need to be accurately and specifically detected by successful diagnostic tests. First of all, all neurologic diseases fall into two large groups: acute and chronic pathologies. Obviously, there is a need in diagnostic and monitoring, but not screening tests for acute disorders, such as stroke, brain traumatic injury, and infections (of course, screening tests for predisposition to acute diseases, such as stroke, would be useful). On the other hand, numerous studies have demonstrated that, due to high compensatory potential of the brain, symptoms of chronic neurodegenerative diseases, such as Alzheimer’s (AD), Parkinson (PD), Huntington (HD) diseases, vascular and frontotemporal (FTD) dementias occur 10–20 years after the beginning of the pathology. The massive neuronal death, characteristic of late stages of neurodegenerative diseases, makes any therapeutic treatment late in the course of a disease extremely difficult, as illustrated by recent failures of anti-AD therapies in late stage clinical trials (Sperling et al., 2011; Pillai and Cummings, 2013). This phenomenon stresses the
importance of the development of screening tests capable of detecting such diseases in early asymptomatic stage.

Further, it is instructive to group neurologic diseases according to the nature of the underlying physiological and pathological processes. For example, although molecular mechanisms of various neurodegenerative diseases are different, many processes, e.g., neurite retraction, dysfunction, and destruction of synapses, followed by neuronal death, are characteristic of neurodegeneration in general (Von Bernhardi and Inestrosa, 2008); massive death of neurons and glial cells accompany initial stages of stroke and brain traumatic damage (McIntosh et al., 1998; Zheng et al., 2003); dysfunction of dopaminergic neurons is characteristic of addiction to many drugs (Kreek et al., 2012), etc.

Finally, physical location of pathological processes occurring during different stages of disease development is important for the focused search for specific biomarkers. The neurodegenerative diseases provide a good example of the diversity of physical locations involved: although many underlying processes are common for AD, PD, and FTD, initial pathological events characteristic for different diseases are localized in different brain areas: hippocampus for AD, midbrain for PD, and frontal lobe for FTD. At later stages, each disease spreads to new areas of the brain, which significantly worsens clinical symptoms and narrows treatment options; this expansion can in principle be used for disease monitoring.

A large number of studies focus on the development of minimally invasive molecular tests for early detection of AD, PD, and other brain pathologies. These include reports of some encouraging early data on the development of blood-based assays for AD diagnosis based on analysis of a large number of proteins or antibodies in human blood (Ray et al., 2007; Nagele et al., 2011; aging early data on the development of blood-based assays for this expansion can in principle be used for disease monitoring.

Intracellular concentration of miRNA changes in various physiological and pathological processes due to modifications in their transcription, maturation, and stability (Jorio and Croce, 2012). Such changes of miRNA levels in different brain areas are characteristic of many neurodegenerative diseases and other brain pathologies (reviews: Saugstad, 2010; Fiore et al., 2011).

A number of recent papers review the available data on changes in miRNA expression in different brain areas involved in AD development (Fiore et al., 2011). A comprehensive review by Tan et al. (2013) presents an analysis of the published evidence for the involvement of miRNA in the four processes playing critical roles in AD pathogenesis: accumulation of amyloid-β, tau toxicity, inflammation, and neuronal death. While some of the results appear compelling, many more studies are necessary to further elucidate the precise roles of individual miRNA in AD pathogenesis and their involvement in different stages of the disease. Some of the published data appear contradictory, possibly due to differences in methods employed for miRNA measurement and normalization in different studies. For example, both activation and inhibition of the brain-enriched miR-9 expression in hippocampus of AD patients have been reported (review: Jin et al., 2013).

Investigations of the miRNA involvement in PD have focused on analysis of miRNA expression in the midbrain and of miRNA...
role in functioning of dopaminergic neurons and the α-synuclein synthesis. Downregulation of miR-133b in midbrain of PD patients (Kim et al., 2007) as well as in mouse models of PD has been reported in several studies (reviews: Harraz et al., 2011; Filatova et al., 2012; Mouradian, 2012). miR-7 and miR-153 have been found to downregulate synthesis of α-synuclein (Junn et al., 2009; Donakis, 2010); suppression of the expression of these miRNA in midbrain of PD patients is expected but has not been demonstrated yet.

Deregulation of 15 miRNA in the brain of the mouse model of prion-induced neurodegeneration has been demonstrated (Saha et al., 2008). Further, changes in miRNA expression caused by or at least accompanying schizophrenia, autism, cognitive dysfunction, drug addiction, neuroblastoema, and other neurologic disorders have been reported (review: Jin et al., 2013).

For the use of miRNA in diagnostics, it is also important that miRNA secretion varies depending on cellular physiology (Wang et al., 2009; Pigiati et al., 2010, Palma et al., 2012). In addition to miRNA release into extracellular space and subsequent appearance in the bodily fluids due to cell death, miRNA appear in circulation due to bleeding of apoptotic bodies, budding and shedding of microvesicles, active secretion in the form of exosomes and of miRNA complexes with proteins (AGO2, NPM1, and others) and high density lipoproteins (HDL; reviews: Sun et al., 2012; Zandberga et al., 2013). All these forms of cell-free miRNA are highly stable in the bloodstream and other bodily fluids. The secretion of miRNA is selective and can be significantly changed by various pathological processes. For example, changes in the spectrum of miRNA secreted in exosomes from prion-infected neuronal cells, as compared to uninfected cells, have been demonstrated (Bellingham et al., 2012).

IDENTIFICATION OF POTENTIAL miRNA BIOMARKERS IN BODILY FLUIDS

The development of screening and diagnostic tests for various diseases based on analysis of miRNA in bodily fluids—mainly in plasma or serum, but also in urine, saliva, CSF, and milk—is a very active area of research. Below are three approaches commonly used in such studies, including studies in the area of neurodegenerative diseases:

1. Measurement of hundreds miRNA in a bodily fluid from patients with a pathology of interest and from control subjects using miRNA array or next generation sequencing (NGS; Qin et al., 2013). An obvious advantage of this approach is that large numbers of various miRNA can be analyzed. Detection of miRNA sequence variations, which can be informative, e.g., for tumor diagnostics, is another advantage of NGS (Williams et al., 2013). Many potential biomarkers have been found by these techniques. However, the miRNA array-based and sequencing techniques are not sufficiently sensitive to detect many miRNA whose concentration in bodily fluids is relatively low. Usually only 30–40% of plasma or serum miRNA detectable by an individual RT-PCR is measurable by various miRNA arrays. This is also true for the RT-PCR-based array, largely due to significantly lower amounts of plasma analyzed by array compared to individual RT-PCR. In addition, reproducibility is very important for screening tests addressing early disease stages when changes in biomarker concentrations usually are not high. Unfortunately, variability of miRNA array tests is significantly higher than that of the individual miRNA RT-PCR (Lindner et al., 2013). As a consequence, potential biomarkers selected by array analysis have to be confirmed by RT-PCR, and indeed in many cases the array data is not confirmed. Further, even when miRNA array-selected potential biomarkers are validated by RT-PCR, they cannot be automatically considered useful for screening or diagnostic purposes. Most of the miRNA detectable in bodily fluids by arrays are ubiquitous miRNA expressed in all or many tissues, and many of them derive from blood cells (Pritchard et al., 2012; Lindner et al., 2013); the detection of changes in their concentrations in patients with one pathology does not mean that the same miRNA cannot be involved in other diseases of different organs. Many miRNA are associated with a particular pathology type, such as cancer, inflammation, hypoxia, etc., and changes in their concentration in bodily fluids can be associated with diseases of different organs. For example, changes of miR-155 concentrations were found in the bloodstream of patients with breast, esophageal, lung, pancreatic cancers and lymphomas (Blair and Yan, 2012; Xie et al., 2013). Level of miR-21 increases in plasma/serum of patients with osteosarcoma, bladder, esophageal, gastric, lung, breast, colorectal cancers, neck squamous cell carcinoma, and other tumors (Farazi et al., 2011; Blair and Yan, 2012; Xie et al., 2013). It follows that the potential biomarkers found by miRNA arrays should be also tested in other pathologies, not only in healthy control subjects.

2. The second approach is based on analysis of disease-specific miRNA identified by comparison of miRNA isolated from pathologic and normal tissue, organ, or cell type. Here, subsequent to identification of disease-specific miRNA, e.g., by array followed by RT-PCR, their presence in bodily fluids is analyzed. There are obvious advantages to this strategy. First, a limited number of circulating miRNA should be tested, which makes the use of individual RT-PCR appropriate, thus increasing sensitivity and reproducibility of the analysis. Second, an observation of a correlation between changes in miRNA concentrations in a bodily fluid and in an organ involved in pathology directly suggests that the miRNA is a suitable biomarker for screening tests. However, such a correlation does not always exist and sometimes miRNA concentrations in a bodily fluid and in a target organ change in opposite directions (Boxer et al., 2011; Cuk et al., 2013). This phenomenon can be explained by several factors: (i) if pathology is caused by, or associated with, the change in concentration of a ubiquitous miRNA, the effect of the pathology on the concentration of this miRNA in circulation could be very limited, since only a small fraction of the miRNA in circulation comes from the affected organ or tissue; (ii) changes in miRNA concentration due to pathology development can be accompanied by much more prominent opposite changes in miRNA secretion/excretion, which neutralizes or even overcomes the effect of changed miRNA expression. Of course, these limitations do not preclude successful use of the described approach for the discovery of circulating miRNA biomarkers. In the area of neurodegenerative and other neurologic pathologies this approach can be especially productive for the miRNA biomarker discovery in CSF (see below) because:

(i) CSF is not separated from brain by the blood–brain barrier;
and (ii) the amount of miRNA secreted or excreted from other organs to CSF is very limited (Cogswell et al., 2008).

(3) The third approach was proposed recently for searching for biomarkers of mild cognitive impairment (MCI), AD, and other neurodegenerative diseases (Sheinerman et al., 2012; Sheinerman and Umansky, 2013). It is based on analysis of circulating brain-enriched miRNA in bodily fluids, e.g., in plasma. Restricting the analysis to brain-enriched miRNA significantly increases the chances that any observed changes in concentrations of these miRNA in plasma are caused by brain-related processes. In addition, it is proposed to include miRNA enriched or at least present in neurons and synapses, i.e., neuronal compartments, which are involved in neurodegenerative diseases from early stages of pathology development, years before massive death of neurons. It could be also useful to search for miRNA enriched in the brain area, which suffers first during development of a particular disease, e.g., in hippocampus for AD and in midbrain for PD potential biomarkers. As a number of miRNA to be investigated is limited, RT-PCR can be used here, which is critically important since plasma concentration of many brain-enriched neurite/synapse miRNA is too low to be reliably detectable by miRNA arrays. The advantages and disadvantages of the three approaches described above are summarized in Table 1.

**NORMALIZATION OF miRNA CONCENTRATION IN BODILY FLUIDS**

The concentration of miRNA detected in bodily fluids depends on many biological and technical factors. Biological factors include miRNA levels in various tissues, intensity of secretion and excretion into extracellular space, forms of circulating miRNA (mammals and other species, complexes with proteins and lipids) affecting their ability to cross various barriers, e.g., blood–brain, placental, and kidney barriers, and miRNA stability and half-life in the bloodstream. Technical factors include variability during bodily fluid collection and storage, methods used for miRNA extraction, and the presence in bodily fluids of various factors affecting miRNA purification and RT-PCR. As a consequence, the importance of miRNA normalization is currently broadly recognized (Meyer et al., 2010). At the same time no single normalization method is commonly accepted. Several methods of circulating miRNA normalization have been described in literature:

(1) **Normalization per spiked miRNA, which is absent in the investigated species, e.g., C. elegans or plant miRNA absent in mammalian cells** (Sarkar et al., 2009; Kroh et al., 2010; Sanders et al., 2012). Such miRNA is spiked into a bodily fluid after addition of a lysing solution that inhibits nuclease activity and then is used as a normalizer by the ΔCT approach compensating for variability caused by RNA extraction and the possible presence of RT-PCR inhibitors.

(2) **Normalization per ubiquitous and least variable circulating miRNA** (Peltier and Latham, 2008; Latham, 2010; Lardizábal et al., 2012). This method is used for comparing circulating miRNA concentrations in controls and in one or a relatively small number of pathologies, which are not associated with changes of a normalizer miRNA. For example, miR-16 is widely used as such normalizer (Kroh et al., 2010). One needs to be mindful, however, that miR-16 is involved in regulation of apoptosis and its expression is changed in many pathologic processes, which leads to changes of its concentration in plasma and other bodily fluids (Schaefler et al., 2010; Katsuura et al., 2012; Wang et al., 2012b). Similar considerations apply to other miRNA normalizers of this type.

(3) **Normalization per other (not miRNA) small RNA, mainly small nuclear or small nucleolar RNA.** This approach was often used in earlier studies of circulating miRNA, and is still used although more rarely (Sanders et al., 2012). In addition to problems similar to the ones described in the previous paragraph (changes in the RNA concentration associated with some pathologies), the utility of this approach is limited by the fact that these RNA are larger than miRNA, bound to different proteins, and their stability is different from that of miRNA (Lardizábal et al., 2012).

**Table 1.** Current approaches to analysis of miRNA in plasma. The same approaches can be used for miRNA analysis in different bodily fluids (urine, saliva, and milk).

| Steps | miRNA array/NGS | Disease-specific analysis | Organ/cell-specific analysis |
|-------|----------------|--------------------------|-----------------------------|
| 1. | miRNA isolated from plasma | 1. miRNA-isolated from pathologic and normal tissue | 1. miRNA isolated from plasma |
| 2. | Array analysis | 2. Array analysis | 2. RT-PCR analysis of organ, cell-enriched mRNA |
| 3. | RT-PCR verification | 3. RT-PCR verification | 3. Validation |
| 4. | Validation | 4. Selected miRNA analyzed in plasma | 4. Validation |
|       | 5. Validation |       |       |

### Advantages
- 100s of miRNA tested
- Disease-related miRNA tested
- High sensitivity
- Organ/cell-specific miRNA tested
- High sensitivity

### Disadvantages
- Low sensitivity
- Same miRNA can be involved in pathologies of various organs
- miRNA levels in tissue and plasma do not always correlate
- Selected miRNA can be underdetectable in plasma
- miRNA biomarkers not enriched in target organs or cells can be missed

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Sheinerman and Umansky Circulating microRNA as neurodegeneration biomarkers

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in developing tests based on analysis of circulating miRNA, and as gliomas, glioblastoma and brain metastasis of breast and lung cancers, increase of miR-10b and miR-21 levels in CSF of patients with gliomas, compared to controls with various neurologic pathologies, including patients with primary CNS lymphoma and carcinomatous meningitis, glioblastoma and brain metastasis of breast and lung cancers, compared with various non-neoplastic conditions, such as memory problem, dementia, PD, encephalitis, and others. These two miRNA were also significantly upregulated in glioblastoma, compared to normal brain. The use of CSF levels of seven miRNA – miR-10b, miR-21, miR-125b, miR-141, miR-200a, miR-200b, and miR-200c – permitted the authors to achieve high accuracy in separation between all classes of samples. Again, only miR-125b from this list is a brain-enriched miRNA; all other miRNA are associated with carcinogenesis itself, so their usefulness as potential biomarkers is based on their presence in CSF, which is isolated from tumors located in other organs outside of CNS.

Microparticles containing miRNA, including brain-enriched ones, have been found in CSF and the spectrum of miRNA detectable in CSF is changed after brain injury (Patz et al., 2013). miR-451 was detected in CSF microparticles only after brain injury, which can be explained by more effective secretion of this miRNA from abnormal cells (Pigati et al., 2010).

CELL-FREE miRNA IN PLASMA AND SERUM AND CNS DISORDERS

Due to massive damage and death of neurons and glial cells the concentration of circulating cell-enriched miRNA is dramatically increased in the bloodstream after a stroke. For example, in the rat model, the plasma level of miR-124 is up to 150 times higher than in plasma of sham-operated animals (Laterza et al., 2013). However, it seems unlikely that their concentrations in CSF can be strongly affected by non-CNS tumors. This conjecture needs to be proven for these miRNA to be used as glioma biomarkers. Teplyuk et al. (2012) found a significant increase of miR-10b and miR-21 levels in CSF of patients with glioblastoma and brain metastasis of breast and lung cancers, compared with various non-neoplastic conditions, such as memory problem, dementia, PD, encephalitis, and others. These two miRNA were also significantly upregulated in glioblastoma, compared to normal brain. The use of CSF levels of seven miRNA – miR-10b, miR-21, miR-125b, miR-141, miR-200a, miR-200b, and miR-200c – permitted the authors to achieve high accuracy in separation between all classes of samples. Again, only miR-125b from this list is a brain-enriched miRNA; all other miRNA are associated with carcinogenesis itself, so their usefulness as potential biomarkers is based on their presence in CSF, which is isolated from tumors located in other organs outside of CNS.

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could not be calculated. Studies characterizing various neurodegenerative pathologies (synapse ever, indicating that these biomarker miRNA pairs detect processes control. The biomarkers do not distinguish AD from MCI, how-

disease. It is perhaps not surprising then that both the rest of dementia patients are diagnosed with other neurode-
syndrome. On average, 10–15% of MCI patients annually con-

miR-137, miR-181c, miR-9, miR-29a, and miR-29b in serum of MCI and AD patients with their levels in serum of age-matched controls. The choice of miRNA was based on the previous study (Geekiyanage and Chan, 2011) that demonstrated their involvement in AD pathogenesis and downregulation of these miRNA in the brain cortex of sporadic AD patients. Using RT-PCR and normalization per spiked cel-miR-39 and internal miR-22, miR-191, and miR-126 the authors demonstrated that the levels of miR-137, miR-181c, miR-9, miR-29a, and miR-29b are sig-

Finally, two experiments 32 miRNA were analyzed, then 13 most promis-

implant and found that one of these miRNA, miR-34b, is significantly lower in serum of MCI and AD patients, compared to controls. Since each group of patients and controls included only seven subjects, sensitivity and specificity of MCI and AD detection could not be calculated.

Sheinerman and Umansky (2012) investigated the use of circulating miRNA for early detection of MCI. Since neuronal death, a late event in the development of AD and other neurodegenerative diseases, is preceded by metabolic changes, neurite retraction, synaptic dysfunction, and synapse loss, the authors suggested that these processes could cause excessive secretion and excretion of miRNA from brain areas involved in the pathology. Thus, brain-enriched miRNA, including neurite- and synapse-enriched miRNA were measured in plasma of MCI and AD patients and age-matched control subjects. Described above “miRNA pair” approach was used for data normalization. In the preliminary experiments 32 miRNA were analyzed, then 13 most promis-

One factor impeding the progress in the field is the difficulty of comparing the data reported by different groups due to the use of different methods for searching for potential circulating miRNA biomarkers, different techniques for miRNA measure-
ment and data normalization. Broad acceptance of best practices and uniform methods for analysis of circulating miRNA and the development of statistical apparatus for comparing the results obtained by different techniques will be important to overcome the problem.

Further, when miRNA arrays are used for initial miRNA screen-
ing, many tissue-enriched miRNA are not detectable and, as a consequence, ubiquitous miRNA, including miRNA associated with common pathologic processes such as carcinogenesis or inflammation, are often selected as potential biomarkers. These miRNA can successfully differentiate patients with a particular disease from healthy control subjects, but not necessarily from patients with similar pathologies of other organs, since it is highly likely that the plasma or serum levels of these miRNA will also be affected in such patients. From this perspective, searching for miRNA biomarkers in CSF has advantages for detecting CNS dis-

SUMMARY AND PERSPECTIVES

In spite of the rapidly growing number of publications on diag-
nostic applications of circulating cell-free miRNA, their use for screening of CNS diseases is in early stages of development.

One study (Gaughwin et al., 2011) described a search for a plasma miRNA, which could be used for early diagnosis of HD. The authors tested many miRNA regulated by mutant HTT protein and found that one of these miRNA, miR-34b, is significantly elevated in plasma from carriers of mutant HTT prior to sympt-

tom onset. However, it is unclear if miR-34b can be a specific biomarker for HD, since this miRNA is expressed in many tissues, being especially enriched in pulmonary system, Fallopian tubes, and testicles, and is also deregulated in various pathologies.

Several groups (Haghikia et al., 2012; Siegel et al., 2012; Gandhi et al., 2013; Raddi et al., 2013) reported promising results regarding potential use of circulating miRNA in CSF or bloodstream for diagnosis of multiple sclerosis (MS). Since MS is an autoim-
mune neurodegenerative disease, one can expect that concentra-
tion of both brain-enriched miRNA and of inflammation and immune system-associated miRNA could be changed in CSF and plasma/serum. Currently, the majority of the discovered potential biomarkers belong to the latter group: inflammation and immune system associated miRNA.

A limited number of studies report analyses of circulating miRNA in plasma or serum of patients with various neurolog-

ical and psychological disorders, such as depression (Li et al.,

2013), bipolar disorder (Rong et al., 2011), and schizophrenia (Shi et al., 2012), suggesting that the study of circulating miRNA in plasma/serum for diagnosis of neurological disorders represents a promising direction for further more detailed investigations.
An analysis of data in literature suggests a number of optimization steps to increase the chances of success for such efforts. First, the use of brain-enriched miRNA for CNS disorders should increase biomarker specificity. This thesis is supported by data obtained for other organs. For example, in plasma/serum concentration of liver-enriched miR-122 is very common in patients with pathologies of this organ (Zhang et al., 2010), and increase in plasma/serum concentration of heart-enriched miR-1, miR-135a, miR-135b, or miR-499-5p is characteristic of acute myocardial infarction and some other cardiac pathologies (Yiessen et al., 2012). Second, combination of several circulating miRNA biomarkers can increase test specificity, since even expression of ubiquitous miRNA varies from organ to organ and one can expect that spectra of concentrations of several miRNA biomarkers will be different for various pathologies of different organs. For example, Li et al. (2011) made an interesting observation of the age-dependent increase of miR-34a concentration in brain, peripheral blood mononuclear cells, and plasma. This miRNA, however, cannot be used as a sole biomarker of aging, since its concentration in plasma is changed in patients with tumors of various locations and with other pathologies. At the same time, in combination with miR-132 and miR-134 biomarker families described above, this miRNA could be a helpful additional biomarker. Finally, it can be useful to combine tissue-enriched miRNA with miRNA associated with a common pathology type, e.g., cancerogenesis.

The data reviewed herein suggests strongly that the analysis of cell-free miRNA in bodily fluids is a highly promising approach for developing minimally invasive screening tests for CNS disorders. A different question is whether circulating miRNA represent a promising class of molecules for the prognosis of neurological disease outcomes and for disease and treatment monitoring. Naturally, diagnostic biomarkers can be useful for disease monitoring if some pathologic processes underlie the development of the disease during different disease stages. The use of diagnostic biomarkers for disease monitoring is more problematic, however, if disease progression is caused by involvement of new pathologic processes. AD is a good example of such a disease: (i) the pathology is initiated by not fully understood metabolic abnormalities, (ii) these are followed by morphological changes in particular brain areas, such as formation of amyloid plaques and tau protein tangles, neurite retraction, dysfunction, and destruction of synapses, (iii) finally, neurons are dying, and pathology expands to new brain areas and cell types. Thus, it is unlikely that different stages of AD can be differentiated by the same biomarkers. The situation is also complicated by the fact that some processes, e.g., synapse destruction, are common for different pathologies, including normal brain aging and other neurodegenerative diseases. The detection of such common processes is clearly useful for monitoring of normal brain aging and diagnosis of MCI, which is a syndrome characteristic of early stage of various neurodegenerative diseases. However, such a test will not predict MCI outcome. This goal could be accomplished by other tests, such as CSF protein analysis and/or imaging techniques, or by different miRNA biomarkers specific for various AD stages. For example, AD expansion to new brain areas and cell types can be monitored by circulating miRNA enriched in those brain areas and cell types. The switch from synapse destruction to neuronal death can potentially be detected by changes of ratios in plasma of cell-free synapse-enriched and neuronal body miRNA, and so on. Of course, all such proposals need to be tested, preferably in longitudinal studies.

We hope the advances in the analyses of circulating miRNA reviewed here will lead to more efforts toward using miRNA as biomarkers of neurodegeneration, of other neurologic pathologies, and of normal brain aging, and will facilitate the development of screening, predicative, and monitoring tests for these diseases.

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