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Early detection of neurodegenerative diseases
Circulating brain-enriched microRNA

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As has been demonstrated in numerous studies, the development of neurodegenerative diseases, such as Alzheimer (AD), Parkinson (PD), Huntington diseases, vascular and frontotemporal (FTD) dementias, begins 10–20 y prior to the clinical manifestation. Although molecular mechanisms behind various neurodegenerative diseases are different, many processes, e.g., neurite retraction, dysfunction and destruction of synapses and ultimately neuronal death,[1] are characteristic of neurodegeneration in general. AD is the most common and, thus, actively investigated neurodegenerative disease, for which diagnostic tools and therapeutic treatments are being developed. Recent failures of anti-AD therapies in late-stage clinical trials (including dimebon of Medivation and Pfizer, solanezumab of Eli Lilly and bapineuzumab of Pfizer and Johnson & Johnson) highlighted two important points associated with the development of effective treatment for AD: first, therapy in late stages of AD is ineffective, most likely due to massive neuronal death, which precedes symptoms of dementia; and second, detailed stratified analysis of clinical data reveals promising results for treatment of AD patients with earlier, mild stages of the disease. Two approaches were demonstrated to be effective for early detection of AD: PET scan for in vivo detection of β-amyloid depositions, and analysis of levels of β-amyloid protein 1–42, total tau protein and phosphorylated tau181P protein in the cerebrospinal fluid.[2,3] It needs to be mentioned, however, that invasiveness and high cost of these approaches hinder their use for primary screening.

Our paper[4] describes a new approach for early detection of neurodegenerative diseases based on quantitative analysis of circulating cell-free miRNA in the bloodstream. Two approaches are commonly used for analysis of miRNA as potential biomarkers: (1) miRNA arrays to analyze huge numbers of circulating miRNA and (2) analysis of tissue miRNA to identify miRNA, whose expression is changed in this tissue as a result of a particular pathology. Many potential biomarkers of various diseases have been found;[5,6] however, these approaches have significant limitations: concentrations of many circulating miRNA are too low to be detected by microarray analysis, and, as a consequence, only about 30% of miRNA are detected in plasma or serum; in many cases there is no correlation between miRNA concentrations in a particular organ and plasma;[7,8] and finally, since many miRNA are associated with a particular pathology type, e.g., cancer or inflammation, changes in concentrations of such miRNA decrease test specificity.[5,6] To address these challenges, we propose a RT-PCR measurement of circulating miRNA that are significantly enriched in a particular organ, tissue or even cell type. Further, in addition to normalization of miRNA concentrations per spiked or ubiquitous miRNA, we used "miRNA pairs" approach,[9,10] i.e., analysis of ratios of all measured miRNA concentrations to select most promising miRNA biomarker pairs. The innovations reported in our paper can be applied to pathologies of various organs; however, their use for the detection of neurodegeneration appears especially productive for the following reasons: (1) certain miRNA are enriched in neurons of distinct brain compartments and can be used for differentiating diseases characterized by pathological processes in hippocampus (AD), midbrain (PD) or frontal lobe (FTD); (2) disease progression gradually affects new brain areas, and changes in the levels of circulating miRNA enriched in these brain areas can be used for disease monitoring; (3) there are miRNA enriched in neurites and synapses that may appear in the extracellular space and ultimately in the blood stream as a result of axon, dendrite and spine pruning and synaptic loss; and (4) neurodegenerative diseases as well as normal aging are often accompanied by changes in the blood-brain barrier permeability; the use of brain-enriched miRNA pairs may compensate for this factor.

In the first proof-of-principle study, patients with mild cognitive impairment (MCI), a syndrome characteristic of early stages of many neurodegenerative diseases, were compared with age matched controls. Two sets of miRNA biomarker pairs capable of differentiating MCI from controls with 82–92% accuracy were identified.[4] These results are especially encouraging since 10–20% of MCI cases are reversible, and further, some of the normal age-matched subjects could be in a pre-symptomatic stage of MCI. A small longitudinal study demonstrated that in 70% of cases, MCI was detectable by the selected miRNA biomarker pairs at a pre-symptomatic stage, 1–5 y prior to the clinical diagnosis. MCI patients and age-matched control subjects had various non-neurological conditions. The ability of the reported miRNA biomarker pairs to differentiate MCI from age-matched control in the presence of other pathologies supports the proposed selection of biomarkers among the brain-enriched miRNA. It is estimated that approximately 50% of MCI patients progress to AD, i.e., the identified miRNA biomarker pairs detect early stages of AD, although they

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cannot distinguish MCI cases preceding AD dementia from other MCI cases. Interestingly, the same miRNA biomarker pairs differentiated age-matched controls (76–86-y-old) from younger subjects (21–59-y-old). The data suggest that the identified miRNA pairs detect processes common for normal aging and neurodegenerative diseases, e.g. synapse destruction, although these processes are less prominent during normal aging. Other miRNA should be tested for prognosis of MCI development, for example, progression to AD dementia. Additional studies focused on promising miRNA candidates are currently underway at DiamiR.

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