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RESEARCH HIGHLIGHT

MicroRNAs circulate around Alzheimer's disease

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Abstract

A select group of microRNAs identified in blood samples can differentiate between Alzheimer's disease, other neurological disorders and age-matched healthy controls with high accuracy.

Millions of people suffer from Alzheimer's disease (AD) every year, and the number of afflicted individuals is expected to increase drastically worldwide as the elderly population expands. AD is the most common form of dementia and often onsets after age 65. Currently, analysis of cerebrospinal fluid proteins, such as Aβ42, tau and p-tau, can only truly indicate a confirmation of AD diagnosis based on a clinical suspicion, or as a differential diagnosis from other neurological diseases that cause dementia-like symptoms. Thus, the search to find early-stage biomarkers of this disease is of utmost importance.

Recently, blood-based biomarkers for AD have been proposed as attractive diagnostic tools, as they are non-invasive, highly specific and sensitive, and can be isolated with standard laboratory equipment. A number of studies have identified such blood-based AD biomarkers and demonstrated promising results. For example, one study reported a panel of three blood markers (cortisol, von Willebrand factor and oxidized low-density lipoprotein antibodies) that distinguished AD patients from healthy controls [1], while another group identified 18 proteins in blood plasma that could differentiate AD patients from controls with clinically relevant accuracy, sensitivity and specificity [2].

Circulating microRNAs enter the diagnostics scene

In addition to measuring protein levels in circulation, microRNAs (miRNAs) have also been shown to be effective blood-based biomarkers for a wide array of diseases. The field of circulating miRNAs has been rapidly gaining momentum over approximately the last 5 years, beginning with the discovery that miRNAs themselves can be easily detected and quantified in multiple body fluids, including blood. These circulating miRNAs are resistant to RNases and are in fact very stable in an extracellular environment, in which they can be packaged in microvesicles, exosomes or apoptotic bodies. Circulating miRNAs can also be found in ultrastable protein complexes with Argonaute2, which is the key factor involved in miRNA-mediated repression of target genes [3]. It appears that these miRNAs found in exosomes or in protein complexes may be the remnants of dead cells that have already been cleared. Circulating miRNAs have been profiled from peripheral blood mononuclear cells (PBMCs), plasma, serum and whole blood, although there has been some disagreement as to whether there are distinct or reproducible populations of miRNAs detected from each of these sources.

Nevertheless, quantification of miRNAs found in blood samples has yielded fruitful results for potential early-stage diagnosis of numerous cancers and other pathologies, making them an attractive new class of disease biomarkers. In a collaborative study, miRNome analysis of 454 blood samples from individuals with different cancers or other diseases yielded strongly dysregulated miRNA profiles for all tested diseases. Further, there appeared to be significant correlations between these dysregulated miRNAs and the genomic location of disease-associated genetic variants [4].

A novel miRNA biomarker signature of Alzheimer's disease

Profiling of circulating miRNAs has now been identified as a useful biomarker tool for AD as well. In this issue of *Genome Biology*, Petra Leidinger and colleagues report their identification of a novel miRNA signature for detecting AD from blood samples [5]. They performed high-throughput Illumina sequencing of all human miRNAs from whole-blood samples of 48 patients afflicted with AD and 22 age-matched healthy controls, and saw 82 upregulated and 58 downregulated mature miRNAs in AD patients. It appeared that at least some of these 140 dysregulated miRNAs cluster together within a localized genome region, implicating a possible shared regulatory mechanism of miRNA expression.

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The most upregulated and downregulated miRNAs had already been described in relation to various diseases and thus did not appear to be specific for AD. To differentiate miRNA expression in AD and control samples, the researchers used standard machine learning to yield a 250-miRNA signature with high accuracy, sensitivity and specificity. However, as many of these miRNAs were strongly correlative with each other, the researchers selected a smaller panel of miRNAs with limited cross-correlation that were also dysregulated in AD patients based on the Illumina sequencing results, but not in many other diseases published in the literature.

The next-generation sequencing also detected lowly expressed novel miRNA candidates, which the authors termed brain-miRs. They identified 15 brain-miRs, all of which were upregulated in AD compared with healthy controls. To represent this new class of miRNAs, the researchers selected two brain-miRs to include in their smaller panel. The 12-miRNA panel consists of seven upregulated miRNAs (brain-miR-112, brain-miR-161, hsa-let-7d-3p, hsa-miR-5010-3p, hsa-miR-26a-5p, hsa-miR-1285-5p and hsa-miR-151a-3p) and five downregulated miRNAs (hsa-miR-103a-3p, hsa-miR-107, hsa-miR-532-5p, hsa-miR-26b-5p and hsa-let-7f-5p). While this miRNA signature represents a unique group of miRNAs that has not been identified thus far in its relationship to AD, two of these miRNAs (miR-103 and miR-107) have previously been implicated in the disease, thus increasing confidence in the selection process of this miRNA signature. In a recent study, miR-103 and miR-107 were both shown to reduce expression of their target, ADAM10, which controls the processing of amyloid precursor protein and the formation of amyloid plaques [6].

Distinguishing Alzheimer's patients from controls using the miRNA panel

To validate these differentially expressed miRNAs in a larger cohort, the researchers performed quantitative RT-PCR (qRT-PCR) analysis of 12 miRNAs in 202 samples from AD patients, healthy controls, and patients afflicted with other neurodegenerative diseases or psychiatric disorders. Ten out of the 12 miRNAs were dysregulated in the same direction when comparing next-generation sequencing and qRT-PCR results, showing that the findings from the screen and validation cohort were concordant. Support vector machine classification on the qRT-PCR data reached 93.3% accuracy, as well as high sensitivity and specificity in distinguishing between AD patients and controls.

The expression profile of this 12-miRNA panel was also able to separate AD patients and patients with other neurological disorders, albeit with lower accuracy. As patients with other neurodegenerative diseases or psychiatric disorders can sometimes exhibit AD-related symptoms, the authors were curious to see if these patients showed comparable dysregulation in the expression of the miRNA signature. Schizophrenia patients showed a pattern most similar to AD, in which there were six upregulated and six downregulated miRNAs. Patients with other disorders also showed either significant overall upregulation or downregulation of all the 12 miRNAs. Lastly, the authors noted that the AD miRNA signature can distinguish other neurological diseases from the control group using support vector machine classification; surprisingly, the group of psychological disorders was more accurate than AD or the group of neurodegenerative diseases in separation from the control group.

Insights into potential miRNA targets

Leidinger *et al.* have shown that identifying dysregulation of miRNA expression in peripheral blood can be a useful biomarker for diagnosis of AD and other neurological disorders [5]. Many studies have investigated the role of miRNAs in neurodegenerative diseases; until this work from Leidinger and colleagues, however, very few reports have analyzed blood-borne miRNA expression profiles in AD patients. Schipper *et al.* [7] profiled miRNA expression in PBMCs of 16 AD patients and 16 controls, in order to identify miRNAs that may be responsible for targeting mRNAs also shown to be downregulated in AD patients. However, the study found only a very slight increase in expression of nine miRNAs in these patients compared with controls.

The identification of targets of significantly dysregulated miRNAs in AD patients remains an important factor for understanding the potential regulatory mechanisms of miRNAs in this disease. Leidinger *et al.* identified 2,354 mRNAs predicted to be targeted by the 10 known miRNAs in the 12-miRNA signature. The authors performed over-representation analysis of these predicted targets and found an enrichment of miRNA targets in Gene Ontology categories of neuron projection development, morphogenesis and other related categories. In fact, many of these predicted targets have already been related to AD or other neurological diseases, including DRD1 (dopamine receptor D1), BDNF (brain-derived neurotrophic factor), IGF1R (insulin growth factor 1 receptor) and DISC1 (disrupted in schizophrenia 1). DISC1 was also a predicted target of a novel miRNA (brain-miR-112) identified from high-throughput sequencing.

It should be noted that these genes are only predicted to be targeted by the miRNA panel using bioinformatics; however, a recent study has shed light on analysis of miRNA-mRNA relationships in AD patients. In an effort to simultaneously examine miRNA and mRNA

expression in AD patients, Nunez-Iglesias *et al*. [8] have provided the first joint profile analysis of miRNAs and mRNAs in brain cortex from AD and age-matched controls. By identifying numerous miRNA-mRNA pairs that are altered in AD versus control patients, the data provide a unique resource for studying the relationships between miRNA and mRNA expression in normal and AD brains [8].

Future directions

Leidinger and colleagues acknowledge that, while this 12-miRNA signature demonstrates impressive accuracy in identifying blood samples from AD patients, this may still not be the most effective diagnostic approach. The authors conclude that a combination of AD-specific miRNA profiles, along with other standard diagnostic tools, may be the best method for diagnosing AD at early stages [5]. Additionally, any direct relationship between AD diagnosis from brain tissue samples or from blood samples remains to be validated, as tissue and blood samples from the same patients were not available for this study. It will be important to show in the future that miRNA profiling from blood or tissue samples will achieve the same AD diagnosis of a patient.

This study profiled miRNAs from whole blood; as mentioned earlier, there has been some debate over whether whole blood or isolated plasma or serum fractions should be utilized when performing blood-based biomarker studies. Nevertheless, a serum profiling of AD patients was, in fact, the first study to show that profiling of circulating miRNAs can be a useful AD biomarker, by identifying four downregulated miRNAs in the serum of probable AD patients and risk factor models [9]. Future work could simultaneously compare miRNA profiles in serum, plasma and whole blood in AD patients and controls to identify the most consistent approach for circulating miRNA biomarker studies.

Leidinger and colleagues also used their miRNA signature to differentiate between blood samples from patients with mild cognitive impairment (MCI) versus healthy controls. A recent report has identified two miRNA classes that could also differentiate patients with MCI from controls [10]. Moreover, these miRNA biomarkers successfully detected MCI in a majority of patients in an asymptomatic stage, 1 to 5 years before a clinical diagnosis [10]. The ability to identify MCI in patients could very well delay the onset of AD, as early stages of many neurodegenerative diseases, including development of AD, are associated with MCI.

Abbreviations

AD, Alzheimer's disease; DISC1, disrupted in schizophrenia 1; MCI, mild cognitive impairment; miRNA, microRNA; PBMC, peripheral blood mononuclear cell; qRT-PCR, quantitative RT-PCR.

Competing interests

The authors declare that they have no competing interests.

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