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Opinion

Plasma long noncoding RNA as a biomarker to differentiate between vascular dementia and Alzheimer's disease

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1. Introduction

With the rapid aging of the global population, neurodegenerative diseases have become a serious problem. Dementia is the most prevalent neurodegenerative disease, with a prevalence rate ranging from 5%-7% among those aged 60 years in most world regions; it is estimated that more than 35.6 million people are afflicted with dementia worldwide in 2010 [1]. In Taiwan, dementia was estimated to affect approximately 1.7-4.3% of the elderly population in 2000 [2], and the prevalence rate has increased to 6.6-8% in 2010-2012 [3]. The World Health Organization has urged that all governments, policy-makers, and other stakeholders address the impact of dementia as an increasing threat and allocate all necessary resources to prepare the health and social care system for the imminent threat of dementia [4]. The most common cause of dementia is Alzheimer's disease (AD), followed by vascular dementia (VaD) and conditions like Parkinson's disease, Lewy body disease, and frontotemporal degeneration [2,5]. Alzheimer's disease (AD) is the predominant cause of dementia in Western countries, comprising 50%70% of the elderly population with dementia, whereas vascular dementia (VaD) makes up only 10-20% of all dementia cases in North America and Europe [6,7]. Interestingly, the relative preponderance of AD and VaD is altered or even reversed in Asian countries. For example, studies in Japan revealed a higher percentage of VaD than AD among patients with elderly dementia (47% vs 35%) and early-onset dementia (43% vs 26%) [8]. The prevalence ratio of VaD and AD in Taiwanese and Chinese population ranges from 1:3 to 1:1,5 [9], which is also much higher than that in Western population. Taken together, these data suggest that VaD contributes considerably to dementia population, particularly in Asian countries. Understanding the pathogenesis of VaD and establishing a reliable tool for early diagnosis of VaD are mandatory to allow early intervention and treatment of VaD. While cerebrovascular incidents such as large artery infarctions, lacunar infarcts and chronic subcortical ischemia are thought to contribute substantively to the development of VaD, not all stroke patients have impaired cognitive function or VaD. On the other hand, patients without a stroke history but with evidence of clinically silent brain infarction on imaging studies have been shown to have higher risk to develop VaD [10]. Population-based studies also revealed conflicting results regarding the risk of dementia and cognitive impairment after stroke[11–13]. Identifying stroke patients who are at risk of developing cognitive impairment and VaD, therefore, is critical for early intervention and preserving cognitive function.

1.1. Trajectories of cognitive function decline after stroke

It has been shown there is a close relationship between a stroke and dementia. Around 10% of stroke survivors develop dementia within 3 months and over 20% of stroke patients have dementia in the subsequent 3 years [14]. In the absence of parenchymal β-amyloid (Aβ) deposition, an ischemic infarct may cause a transient decline in cognitive function, but full or partial recovery is possible without further deterioration of cognitive status. If vascular and inflammatory processes trigger ongoing secondary neurodegeneration, a poststroke cognitive decline occurs. In the presence of amyloid, the ischemic lesion causes a loss of cognitive reserve, preventing cognitive recovery, and a cognitive decline may even be accelerated if secondary degeneration is triggered by inflammatory processes. Hence, the induction of neurodegenerative diseases or dementias is not monogenic but usually results from various factors, including the vascular factors.

1.2. Plasma Long Noncoding RNA as a Biomarker for MCI and VaD in Stroke Patients

Recently, circulating nucleic acids has emerged as a novel biomarker for the diagnosis and management of neurodegenerative diseases and cancer. Among them, long noncoding RNAs (lncRNA) are of particular biological interest. While the expression of messenger RNAs (mRNAs) and microRNAs (miRNAs) account for only 1% of all transcribed species, up to 90% of the mammalian genome is transcribed as lncRNAs, a heterogeneous group of non-coding transcripts longer than 200 nucleotides. LncRNAs have been shown to be functional and involved in specific physiological and pathological processes through epigenetic, transcriptional and post-transcriptional mechanisms.

Multiple lncRNAs have been shown to play critical roles in brain development, physiological function and diseases. A recent chromatin-immunoprecipitation sequencing study identified a brain-specific cluster of intergenic lncRNAs that are implicated in brain ageing, hippocampal development, oligodendrocyte myelination and signaling pathways involved in neuronal function [15]. BACE1 anti-sense transcript (BACE1-NAT), a lncRNA conserved in mammalian brain, is elevated in neurons exposed to Aβ; increased BACE1-NAT then stabilizes BACE1 (β-secretase), which promotes Aβ production through a positive feedback loop [16]. Another amyloid-responsive lncRNA is the neuronal NAT-Rad18, which is upregulated in cortical neurons after exposure to Aβ; increased NAT-Rad18 leads to post-transcriptional downregulation of the DNA repair protein Rad18 [17], which may contribute to the increased neuronal cell death observed in AD. In addition, the anti-sense lncRNA of ApoE (ApoE-AS) is upregulated in response to CNS injury, which may be involved in the pathogenesis of post-injury neurondegenerative disorders [18]. LncRNAs have also been implicated in neurodegenerative diseases including Huntington's disease [19], Parkinson's disease [20,21], frontotemporal lobar degeneration and amyotrophic lateral sclerosis [22,23]. Exploiting next-generation sequencing technology, we have recently demonstrated that lncRNAs are dynamically regulated in human cardiovascular diseases and could serve as a sensitive biomarker to reflect the disease states [24]. Several groups have demonstrated distinct plasma lncRNA expression signature human patients with cancer [25], cardiac [26] or kidney diseases [27]. With the exquisite sensitivity of lncRNAs, we hypothesized that circulating lncRNA expression signature will reflect the status of cognitive function in patients with stroke and serve as a sensitive biomarker to detect the presence of VaD.

2. Results

2.1. Circulating lncRNA Expression profiles distinguish stroke patients with and without VaD

We have developed the protocols for RNA isolation from human plasma samples and have demonstrated that the quality and quantity of the RNA samples are adequate for downstream RNA-Sequencing application. We have also constructed RNASeq libraries using the plasma RNA

isolated from human subjects. As shown in Figure 1, bioanalyzer analysis of the RNASeq libraries revealed that the constructed cDNA library consists of products derived from lncRNAs (>200 bp), as well as from miRNAs (20-35 bp). Following sequencing analysis, we revealed that there are more than 5000 lncRNAs circulating in human plasma. Figure 2 demonstrated the top 10 most abundant lncRNAs in human plasma, including LIPCAR (n385729), which has been shown to predict major adverse cardiac events in patients with myocardial infarction.

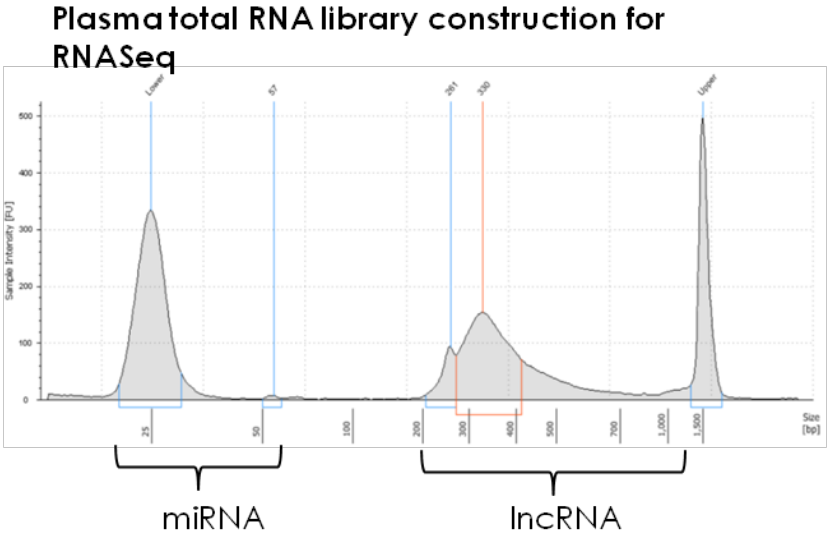

Figure 1. RNA Seq library construction using human plasma RNA. Illustration of the size (x-axis) and abundance (y-axis) distribution of the RNASeq library constructed from human plasma RNA assayed by the Angilent bioanalyzer. The result demonstrated that the protocol preserves lncRNAs (size>200 bp) as well as miRNAs (size 20-35 bp) that are able to be sequenced and analyzed for downstream application.

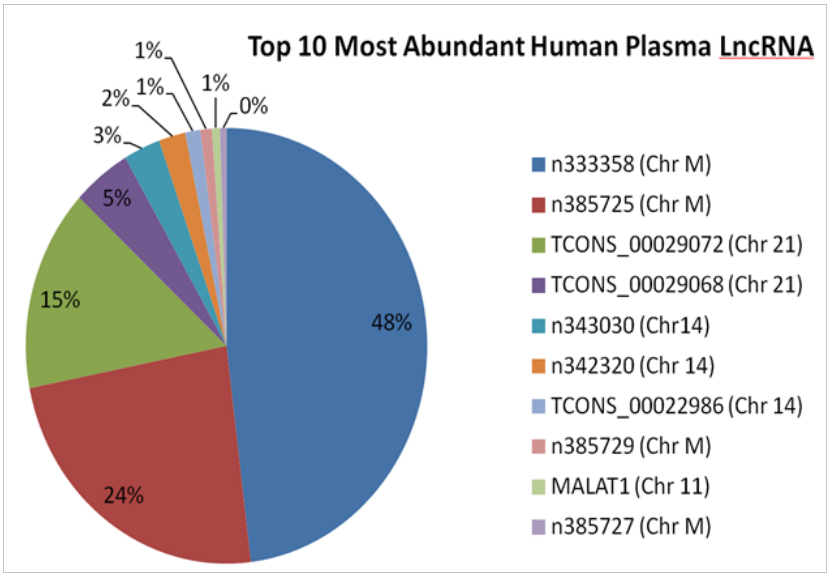

Figure 2. Plasma RNASeq revealed abundant circulating lncRNAs of mitochondrial origin. RNASeq of human plasma RNA demonstrated that 4 out of the 10 most abundant circulating lncRNAs are encoded from mitochondrial genome, which is consistent with our recent data, as well as with the report from microarray-based plasma transcriptome analyses. These mitochondrial lncRNAs include LIPCAR (n385729), which has been shown to predict major adverse cardiac events in patients with myocardial infarction.

2.2. Circulating lncRNA Expression profiles distinguish stroke patients with and without VaD

Using the established experimental and analytical pipeline to isolate and sequence plasma RNA, we went on to conduct RNASeq using plasma samples from stroke patients with (n=30) and without (n=30) VaD. We showed that there are 100 lncRNAs differentially expressed in the plasma from stroke patients with and without VaD. Unsupervised hierarchical clustering demonstrates that these lncRNAs are able to discriminate stroke patients with VaD from those without, suggesting that circulating lncRNA expression signature could serve as a sensitive and specific biomarker to detect cognitive impairment and VaD in stroke patients. Among the plasma lncRNAs that were significantly differentially expressed between stroke patients with v.s. without VaD, two lncRNAs showed robust discriminative power to distinguish between healthy control and stroke patients with v.s. without VaD. lnc-NCF1-1 (AUC 0.78, sensitivity 74.4%, specificity 70%) appeared to be potential useful predictors of the occurrence of VaD in stroke patients.

Taken together, our experimental data strongly suggest that circulating lncRNA expression signature can be a good biomarker to accurately identify stroke patients with impaired cognitive function and VaD, which could provide important insights into the pathogenesis of VaD and serve as a useful tool to identify stroke patients at risk to develop VaD and to guide clinical management to prevent cognitive function deterioration in these patients.

Conflicts of Interest:
The authors declare no conflict of interest.

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