NEUROSCIENCE

Molecular beacon–based detection of circulating microRNA-containing extracellular vesicle as an -synucleinopathy biomarker

Zhenwei Yu[1,2]†, Yuanchu Zheng[2,3]†, Huihui Cai[2,3], Siming Li[2,3], Genliang Liu[2,3], Wenyi Kou[2,3], Chen Yang[2,3], Shuangshuang Cao[4], Lei Chen[5], Xuedong Liu[6], Zhirong Wan[7], Ning Zhang[8], Xiaohong Li[9], Guiyun Cui[10], Ying Chang[11], Yue Huang[2,12,13], Hong Lv[14], Tao Feng[2,3]*

Copyright ' 2024 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC).

Early and precise diagnosis of -synucleinopathies is challenging but critical. In this study, we developed a molecular beacon–based assay to evaluate microRNA-containing extracellular vesicles (EVs) in plasma. We recruited 1203 participants including healthy controls (HCs) and patients with isolated REM sleep behavior disorder (iRBD), - synucleinopathies, or non– -synucleinopathies from eight centers across China. Plasma miR-4438–containing EV levels were signi cantly increased in- synucleinopathies, including those in the prodromal stage (e.g., iRBD), compared to both non– -synucleinopathy patients and HCs. However, there are no signi cant di erences between Parkinson's disease (PD) and multiple system atrophy. The miR-4438–containing EV levels negatively correlated with age and the Hoehn and Yahr stage of PD patients, suggesting a potential association with disease progression. Furthermore, a longitudinal analysis over 16.3 months demonstrated a signi cant decline in miR-4438–containing EV levels in patients with PD. These results highlight the potential of plasma miR-4438–containing EV as a biomarker for early detection and progress monitoring of -synucleinopathies.

INTRODUCTION

Parkinson's disease (PD), as well as other -synucleinopathies such as multiple system atrophy (MSA), is characterized by -synuclein (- syn) accumulation in both the brain and the peripheral nervous system (1). e precise and early diagnosis of these diseases can be di cult, considering the complexity and heterogeneity of the symptoms (2, 3). ere is an urgent need for an easy and reliable assay that can diagnose -synucleinopathies precisely and early (4).

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression and modify metabolic pathways. They can act as modulators and biomarkers for various diseases, including -synucleinopathies, which are caused by the abnormal accumulation of - syn in the brain (5). Several studies and reviews have explored the role of miRNAs in the diagnosis and pathological pathways of -synucleinopathies (6, 7). We recently reported that the concentration of miR-44438, a recently found miRNA, is increased in the neuron-derived extracellular vesicles (EVs) from the plasma of patients with PD compared to those from healthy controls (HCs). Furthermore, this miRNA is implicated in the exosome generation and -syn secretion in dopaminergic neurons (8).

e blood- brain barrier serves as a protective shield against peripheral macromolecules, yet it poses challenges in monitoring the brain via peripheral bio uids. Recent insights suggested that certain pathological molecules, like amyloid, tau, and -syn, may traverse the blood-brain barrier when encapsulated within EVs (9). We have also shown that oligodendrocyte-derived (CNPase-positive) EVs can be detected in peripheral blood using uorescence nanoparticle tracking analysis (NTA) (10). However, this approach requires highly stable uorescent conjugates, such as quantum dots, to label-the target protein on EVs, limiting its utility. A recent technology that has emerged is nanoscale ow cytometry, which can identify and quantify EVs carrying speci c proteins in cerebrospinal uid and blood. is technology allows the classi cation of EVs derived from di erent cell types; for instance, NCAM, L1CAM, and NMDAR2A distinguish neuron-derived EVs (11), and CD62P is indicative of EVs likely originating from platelets (12). While numerous miRNAs have been implicated in the pathogenesis of neurodegenerative diseases like PD, (13,14), the characterization of miRNA-containing EVs remains unexplored.

Molecular beacons (MBs) are oligonucleotide probes that have a stem-loop hairpin structure and emit uorescence when they hybridize with target DNA or RNA sequences (15). However, these probes su er from cross-quenching when they detect miRNAs in a con ned space, such as exosomes, which suppresses the uorescent signal and detection sensitivity. Mao et al. (16) recently developed a nano-cage MB (NCMB) that encloses the quencher and the uorophore in a DNA cube. When the target miRNA binds to the NCMB, the uorophore is released from the cube, while the quencher remains inside. This assay minimizes the cross-quenching effect and enables the in situ analysis of target miRNAs in bio uids and exosomes.

[1]Department of Pathophysiology, Beijing Neurosurgical Institute, Capital Medical University, Beijing, China. [2]Center for Movement Disorders, Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China. [3]China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing, China. [4]Department of Neurology, Yidu Central Hospital of Weifang, Shandong, China. [5]Department of Neurology, Tianjin Huanhu Hospital, Tianjin, China. [6]Department of Neurology, Fourth Military Medical University, Xi'an, China. [7]Department of Neurology, Aerospace Center Hospital, Beijing, China. [8]Department of Neuropsychiatry and Behavioral Neurology and Clinical Psychology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China. [9]Department of Neurology, Dalian Friendship Hospital, Dalian, China. [10]Department of Neurology, A f liated Hospital of Xuzhou Medical University, Xuzhou, China. [11]Department of Neurology, China-Japan Union Hospital of Jilin University, Changchun, China. [12]Human Brain and Tissue Bank, China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing, China. [13]Department of Pharmacology, School of Biomedical Sciences, Faculty of Medicine and Health, UNSW Sydney, Sydney, NSW, Australia. [14]Department of Clinical Diagnosis, Beijing Tiantan Hospital, Capital Medical University, Beijing, China.
*Corresponding author. Email: bxbkyjs@sina.com
†These authors contributed equally to this work.

Downloaded from https://www.science.org on 11/22/24