Alzheimer's & Dementia 11 (2015) 600-607

Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study

Massimo S. Fiandaca[a,1], Dimitrios Kapogiannis[b,1], Mark Mapstone[c,1], Adam Boxer[d], Erez Eitan[b], Janice B. Schwartz[e], Erin L. Abner[f], Ronald C. Petersen[g], Howard J. Federoff[a], Bruce L. Miller[d], Edward J. Goetzl[e,*]

[a]Departments of Neurology and Neuroscience, Georgetown University Medical Center, Washington, DC, USA
[b]Clinical Research Branch, Intramural Research Program, National Institute on Aging, Baltimore, MD, USA
[c]Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA
[d]Department of Neurology, Memory and Aging Center, UCSF Medical Center, San Francisco, CA, USA
[e]Department of Medicine, UCSF Medical Center and the Jewish Home of San Francisco, San Francisco, CA, USA
[f]Department of Neurology, Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA
[g]Department of Neurology, Mayo Clinic, Rochester, MN, USA

Abstract

Background: Proteins pathogenic in Alzheimer's disease (AD) were extracted from neurally derived blood exosomes and quantified to develop biomarkers for the staging of sporadic AD.

Methods: Blood exosomes obtained at one time-point from patients with AD (n = 57) or frontotemporal dementia (FTD) (n = 16), and at two time-points from others (n = 24) when cognitively normal and 1 to 10 years later when diagnosed with AD were enriched for neural sources by immunoabsorption. AD-pathogenic exosomal proteins were extracted and quantified by enzyme-linked immunosorbent assays.

Results: Mean exosomal levels of total tau, P-T181-tau, P-S396-tau, and amyloid β 1–42 (Aβ1–42) for AD and levels of P-T181-tau and Aβ1–42 for FTD were significantly higher than for case-controls. Step-wise discriminant modeling incorporated P-T181-tau, P-S396-tau, and Aβ1–42 in AD, but only P-T181-tau in FTD. Classification of 96.4% of AD patients and 87.5% of FTD patients was correct. In 24 AD patients, exosomal levels of P-S396-tau, P-T181-tau, and Aβ1–42 were significantly higher than for controls both 1 to 10 years before and when diagnosed with AD.

Conclusions: Levels of P-S396-tau, P-T181-tau, and Aβ1–42 in extracts of neurally derived blood exosomes predict the development of AD up to 10 years before clinical onset.

Keywords: Preclinical AD; Neural exosomes; P-Tau; Aβ1–42; Biomarkers

1. Introduction

Roles in the pathogenesis of Alzheimer's disease (AD) have been attributed to altered proteins accumulating inside and on the surface of neurons [1,2]. Increases in brain tissue oligomeric amyloid β (Aβ) peptides and phosphorylated tau (P-tau) detected by central nervous system (CNS) imaging and in cerebrospinal fluid (CSF) levels of soluble Aβ1–42 and P-tau have been documented years before the signs of AD [3–6]. Times for progression from preclinical stages to clinically apparent AD with threshold detectable amyloid deposition and abnormal elevation of CSF P-tau proteins

[1]These three authors contributed equally to the reported research.
*Corresponding author. Tel.: +1-703-254-7529; Fax: +1-415-406-1577.
E-mail address: edward.goetzl@ucsf.edu

1552-5260/$ - see front matter
http://dx.doi.org/10.1016/j.jalz.2014.06.008

are estimated to be up to 17 years [3,5]. The potential prognostic sensitivity of protein biomarkers is supported by the timing of induction of AD-like disease in rodent models after the transgenic overexpression of putatively neuropathogenic proteins [7–9].

In recent studies, low CSF levels of Aβ1–42 and high CSF levels of P-tau, and positive CNS images of amyloid deposits accurately predicted the development of mild cognitive impairment (MCI) and probable AD [10,11]. However, there was substantial overlap in these biomarkers between patients who subsequently developed AD and those who later manifested other forms of dementia or no signs of dementia, even when concentrations of these CSF proteins were considered together or as ratios. The overlap was even greater when plasma levels of these proteins were used for diagnosis or prediction [12–15]. This high level of prognostic uncertainty combined with the morbidity and the expense of repeated CSF sampling and of neuroimaging procedures emphasizes the importance of developing accurate blood-based tests that predict high risk for AD and distinguish AD from other forms of dementia.

Exosomes are one class of endosome-derived membrane vesicles shed by neural cells, that contain proteins and other constituents of their cellular origin [16]. Exosomes accept amyloid precursor protein from early endosomes, after its cleavage by β-secretase, and the Aβ peptide fragments subsequently generated by γ-secretase are secreted in exosomes [17]. Although this exosome pathway accounts for only a small portion of the total Aβ peptides in neural plaques, it constitutes a prionoid-like mechanism for CNS spread of proteinopathies [18]. The detection of exosome signature proteins in neural amyloid plaques supports the possibility of their role in the generation of AD-associated lesions [17]. Here we use a combination of chemical and immunochemical methods to harvest and enrich neurally derived exosomes from small volumes of plasma or serum in quantities that provide readily detectable amounts of proteins implicated in the pathogenesis of AD.

2. Materials and methods

2.1. Study design, subject characterization, and blood collection

Fifty-seven patients with amnestic MCI (aMCI) or dementia attributable to AD, who had donated blood at one time-point, were identified retrospectively at the Clinical Research Unit of the National Institute on Aging (CRUNIA) in Harbor Hospital, Baltimore, MD, at the Jewish Home of San Francisco (JHSF), San Francisco, CA, and in the neurology clinical services of the University of Rochester (UR), Rochester, NY, the University of California Irvine (UCI), Irvine, CA, and Georgetown University Medical Center, Washington, DC (GUMC) (Table 1). Twenty-four additional patients with AD had provided blood at two time-points in studies at the Mayo Clinic and the University of Kentucky, first when cognitively intact and later when diagnosed with AD. For both groups, the diagnosis of AD had been established according to the revised National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA) criteria [19]. The patients classified as having aMCI had a Clinical Dementia Rating (CDR) global score of 0.5 [20]. Those with AD and mild to moderate dementia had a CDR global score of 1.0. Twenty-eight of the 57 single-time sample AD patients were taking an acetylcholinesterase inhibitor and/or memantine, and 12 were on antidepressant medications; blood was drawn at least 8 hours after their last medication.

Sixteen patients with behavioral variant frontotemporal dementia (bv-FTD) had been evaluated and selected for study at the Memory and Aging Center of the Department of Neurology of the University of California, San Francisco (Table 1). Their diagnosis and assignment to mild dementia or moderate dementia groups (Table 1) was based on standard clinical, mental status, and psychiatric criteria, including discriminant analyses of neuropsychiatric elements, phonological performance, and object understanding that distinguish FTD from AD [21,22]. Seven

Table 1
Characteristics of patients and control subjects

	Total			MCI		Dementia	
Diagnosis	Number	Male/female	Ages, mean ± SD (range)	Number	MMSE scores, mean ± SEM	Number	MMSE scores, mean ± SEM
AD	57	30/27	79.5 ± 6.05 (64–90)	29	27.6 ± 0.30	28	22.9 ± 1.02**
AC	57	30/27	79.6 ± 6.03 (64–90)	0		0	
				Mild dementia		Moderate dementia	
FTD	16	12/4	63.1 ± 8.79 (48–79)	9	26.7 ± 0.73	7	15.0 ± 3.65*
FTC	16	12/4	63.7 ± 7.43 (48–79)	0		0	

Abbreviations: MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; AD, Alzheimer's disease; AC, AD case-controls; FTD, frontotemporal dementia; FTC, FTD case-controls.

NOTE. The significance of differences in values between the MCI/mild dementia and dementia/moderate dementia groups were calculated by an unpaired t test; *$P < .01$ and **$P < .001$.