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Transcriptional profiling of Alzheimer blood mononuclear cells by microarray

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Abstract

We evaluated pathomechanisms and systemic manifestations of Alzheimer disease (AD), an aging-related dementing neurodegenerative disorder, by expression profiling. Blood mononuclear cell (BMC) transcriptomes of sporadic AD subjects and aged-matched normal elderly controls (NEC) were compared using the human NIA microarray.

Relative to the NEC samples, the Alzheimer BMC exhibited a significant decline in the expression of genes concerned with cytoskeletal maintenance, cellular trafficking, cellular stress response, redox homeostasis, transcription and DNA repair. We observed decreased expression of several genes which may impact amyloid-beta production and the processing of the microtubule-associated protein tau. The microarray results were validated by quantitative real time PCR and revealed gender differences in the levels of altered gene expression.

Our findings attest to the systemic nature of gene dys-regulation in sporadic AD, implicate disruption of cytoskeletal integrity, DNA repair mechanisms and cellular defenses in this condition, and suggest novel pathways of β-amyloid deposition in this disease. BMC are highly accessible and may reflect molecular events germane to the neuropathophysiology of AD.

Keywords: Aging; Alzheimer; Amyloid-beta; Blood mononuclear cell; Cytoskeleton; DNA repair; Microarray; Microtubule-associated protein tau; Neurodegeneration; Oxidative stress

1. Introduction

Alzheimer disease (AD) is a dementing illness characterized by progressive neuronal degeneration, gliosis, and the accumulation of hyper-phosphorylated tau protein (neurofibrillary tangles) and extracellular deposits of β-amyloid (senile plaques) in discrete regions of the basal forebrain, hippocampus, and association cortices [52]. Although the pathogenesis of AD remains incompletely understood, there is evidence that the deposition of β-amyloid1–42 (Aβ1–42) derived from the amyloid precursor protein (APP) contributes to the oxidative stress [45], tau pathology [41], mitochondrial

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insufficiency [49], and synaptic failure [20] characteristic of AD brain which, in turn, may stimulate compensatory immune [44], inflammatory [59] and cellular stress responses [51]. Expression profiling of AD-affected neural tissues has also disclosed dysfunction of pathways subserving signal transduction, lipid metabolism, synaptic vesicle trafficking and cell survival in this condition [3,12,61].

The advent of effective therapies for the management of AD presupposes a thorough appreciation of the salient pathophysiological pathways inherent to this condition. Analysis of aberrant gene expression profiles in the CNS of these patients cannot be accomplished during life, and the possibility of post-mortem RNA degradation and protein modification may confound the interpretation of data derived from neuropathological materials. This conundrum may be circumvented by analysis of AD peripheral tissues in light of mounting evidence of systemic derangements in key cellular functions in affected individuals, including alterations in APP metabolism, antioxidant defenses, acute phase reactants and post-translational protein modifications [13,50,63]. In this regard, expression profiling of fresh blood mononuclear cells (BMC) may offer advantages in deciphering aberrant patterns of gene regulation in AD because (a) these cells are readily procured by simple venipuncture, (b) the CNS communicates with the immune system through multiple molecular, hormonal and neurotransmitter mechanisms [19], (c) abnormal APP expression, altered levels of antioxidant enzymes, oxidative damage to DNA, RNA and protein, deregulated cytokine secretion and augmented rates of apoptosis are features shared by AD brain and lymphocytes [1,31,36,40,50] and (d) BMC have been previously employed in the diagnosis and prognosis of other neurological diseases [28,53,57]. The concept of BMC as a "window" into the CNS has been proposed by Percy et al. in their comprehensive review of the peripheral manifestations of AD [44].

In the present study, we compared the expression patterns of over 6000 genes in BMC derived from patients with rigorously-ascertained mild sporadic AD and cognitively-intact normal elderly controls (NEC) carefully matched for the major risk factors of the disease. The data presented herein provide novel insight into the pathophysiology of AD and support the use of BMC as a potential 'barometer' of neural gene dys-regulation in this common neurodegenerative disorder.

2. Methods

2.1. Subjects

This study was approved by the Research Ethics Committee of the Sir Mortimer B. Davis Jewish General Hospital (JGH). Written informed consent was obtained from all subjects or their primary caregivers. Recruited patients with sporadic AD were assessed by a neurologist or geriatrician at the JGH-McGill University Memory Clinic, a tertiary care facility for the evaluation of memory loss in Montreal. All AD subjects underwent formal neuropsychological testing as previously described [10]. AD was diagnosed according to National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association criteria [35]. Normal elderly controls (NEC) were recruited as volunteers or from Family Practice Clinics at the JGH. The latter scored within 1 S.D. of age- and education-standardized normal values on a series of memory and attention tests. The Mini-Mental State Examination (MMSE) [17] was administered to all subjects. In addition to detailed medical information available in patients' hospital charts, all subjects or their caregivers completed a questionnaire that addressed personal and family history of dementia and other neurological and medical conditions, nutritional status and intake of vitamins and medications. Subjects with chronic metabolic and inflammatory conditions (diabetes, rheumatoid arthritis, chronic active hepatitis) and those with possible familial AD were excluded from the study. Student unpaired *T*-test was performed to assess statistical differences in age, years of formal education and MMSE scores between groups.

2.2. Blood samples and extractions

Whole blood was collected by phlebotomy in EDTA vacutainers (6 mL K2EDTA, Becton Dickinson, USA) and processed within 2 h of procurement. On average, 32 mL of blood was processed at room temperature (RT) according to the procedure of Lacelle et al. [29]. Briefly, blood was centrifuged at $500 \times g$ for 10 min, and the upper layer of plasma removed without disturbing the surface buffy coat. The remaining blood cells were immediately diluted two-fold with phosphate-buffered saline (PBS 1×) and layered on Ficoll-Paque Plus (Amersham Biosciences, Canada) in 15 mL conical tubes. After centrifugation ($400 \times g$; 4 °C; ×30 min), the interphase layer containing BMC was carefully removed, washed in PBS (1×) followed by centrifugation ($500 \times g$; ×10 min). The cell pellet was lysed in Trizol (Invitrogen, Canada) and immediately stored at 80 °C until further processing. For every 30 mL whole blood processed, 4 mL Trizol were added to the cell pellets.

Extractions of RNA was performed as described by Lacelle et al. [29]. Trizol samples were thawed at room temperature and maintained on ice. For 1 mL of Trizol, 0.2 mL of chloroform was added and mixed for 15 s. After 3 min incubation at room temperature, the samples were centrifuged at $12{,}000 \times g$ (4 °C) for 15 min. The upper aqueous layer containing RNA was carefully removed and transferred to another microcentrifuge tube for RNA extraction. Total RNA was solubilized in DEPC-treated water and purified using RNeasy columns (Qiagen, Canada) according to manufacturer instructions. Nucleic acid concentrations were determined at 260 nm by spectrophotometry. RNA quality was determined by the RNA 6000 Pico LabChip kit using the Agilent 2100 bioanalyzer (Agilent Technologies Inc., USA).