JOURNAL OF PROTEOMICS 99 (2014) 54–67

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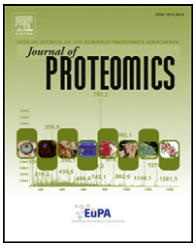

Novel pathophysiological markers are revealed by iTRAQ-based quantitative clinical proteomics approach in vascular dementia

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ARTICLE INFO

Article history:
Received 7 September 2013
Accepted 8 January 2014
Available online 19 January 2014

Keywords:
Vascular dementia
Clinical proteomics
Mass spectrometry
iTRAQ
Vascular dysfunction
Oxidative stress

ABSTRACT

Vascular dementia (VaD) is a leading cause of dementia in the elderly together with Alzheimer's disease with limited treatment options. Poor understanding of the pathophysiology underlying VaD is hindering the development of new therapies. Hence, to unravel its underlying molecular pathology, an iTRAQ-2D-LC–MS/MS strategy was used for quantitative analysis of pooled lysates from Brodmann area 21 of pathologically confirmed cases of VaD and matched non-neurological controls. A total of 144 differentially expressed proteins out of 2281 confidently identified proteins (false discovery rate = 0.3%) were shortlisted for bioinformatics analysis. Western blot analysis of selected proteins using samples from individual patients (n = 10 per group) showed statistically significant increases in the abundance of SOD1 and NCAM and reduced ATP5A in VaD. This suggested a state of hypometabolism and vascular insufficiency along with an inflammatory condition during VaD. Elevation of SOD1 and increasing trend for iron-storage proteins (FTL, FTH1) may be indicative of an oxidative imbalance that is accompanied by an aberrant iron metabolism. The synaptic proteins did not exhibit a generalized decrease in abundance (e.g. syntaxin) in the VaD subjects. This reported proteome offers a reference data set for future basic or translational studies on VaD.

Biological significance
Our study is the first quantitative clinical proteomic study where iTRAQ-2D-LC–MS/MS strategy has been used to identify the differential proteome in the VaD cortex by comparing VaD and matched control subjects. We generate testable hypothesis about the involvement of various proteins in the vascular and parenchymal events during the evolution of VaD that finally leads to malfunction and demise of brain cells. This study also establishes quantitative proteomics as a complementary approach and viable alternative to existing

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http://dx.doi.org/10.1016/j.jprot.2014.01.011

neurochemical, electron microscopic and neuroimaging techniques that are traditionally being used to understand the molecular pathology of VaD. Our study could inspire fellow researchers to initiate similar retrospective studies targeting various ethnicities, age-groups or sub-types of VaD using brain samples available from brain banks across the world. Meta-analysis of these studies in the future may be able to shortlist candidate proteins or pathways for rationale exploration of therapeutic targets or biomarkers for VaD.

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1. Introduction

Vascular dementia (VaD) is the second most common cause of age-related dementia after Alzheimer's disease (AD) [1]. Significantly extended life expectancy coupled with the sedentary lifestyle is predicted to increase the incidence of type 2 diabetes, hypertension and hyperlipidemia, all of which are risk factors for vascular disease and subsequent vascular cognitive impairment. The worldwide number of patients with vascular causes of dementia is expected to rise assuming no effective prevention strategies or curative treatments are developed and implemented [2].

Knowledge of VaD pathophysiology has been lagging behind AD for a number of reasons. The lack of suitable animal models and difficulties in obtaining well-characterized clinical samples has hampered the progress of basic and translational research on VaD. Immunohistochemical, neurochemical and electron microscopic studies using post-mortem clinical samples remained the mainstay of mechanistic research [3–5]. Neuroimaging together with clinical observation in patients has provided vital complementary information that forms the basis for the current understanding of VaD [6]. Multiple clinicopathologic substrates have been implicated in the pathogenesis (e.g. cortical or subcortical microinfarcts, demyelination of white matter, cribriform change of basal ganglia and white matter) that links cerebrovascular changes to cognitive impairment, independent of Alzheimer's type pathology [7]. However, the molecular events that drive these vascular and parenchymal changes in the brain are still poorly understood. Given the complexity and heterogeneity of cerebrovascular disorder and the presence of co-morbidities in VaD patients, it is likely that rather than an individual or small number of proteins, multiple candidate proteins present in networks are perturbed leading to the spectrum of cognitive and behavioral symptoms.

The advent of quantitative proteomic technologies using isobaric labeling strategy has made it possible to quantify several proteins in a single experiment for comparative study of global protein regulation across various biological samples. Isobaric tag for relative and absolute quantification (iTRAQ) is one of the most commonly used in vitro isotopic labeling strategies in different areas of biological science and medicine for simultaneously quantifying 4- or 8-plex samples [8]. Clinical proteomics of dementia have flourished in the recent times with a rapid increase in the number of studies analyzing different parts of the post-mortem brain of AD patients for expression profiling studies [9,10]. Apart from the well-known β-amyloid and tau hypothesis, the involvement of pathological events such as excitotoxicity, oxidative stress and inflammation has been highlighted. Post-translational modifications such as carbonylation and phosphorylation of various key proteins have also been found to contribute to AD.

Despite having immense potential to elucidate disease mechanisms, proteomic studies of VaD using preclinical or clinical samples are lacking. Recently, we have successfully applied an iTRAQ-based shotgun neuroproteomic strategy in the area of ischemic stroke to study validated pre-clinical models [11,12], and ischemic infarcts from autopsied human brain [13]. Here, we apply a similar iTRAQ-two dimensional-liquid chromatography–tandem mass spectrometry (iTRAQ-2D-LC–MS/MS) based quantitative proteomic approach on post-mortem VaD and matched non-demented control specimens from Brodmann area 21(BA21) of the temporal lobe for better understanding of the underlying molecular mechanism of VaD. We focused on the temporal lobe because medial temporal lobe atrophy is a common finding in dementia and our recent study suggested that there is a vascular basis for neurodegeneration [14]. The temporal lobe is also relatively free of large infarcts thus making it ideal to detect the survival response of the demented brain [15]. The iTRAQ experiment identified differentially expressed proteins from the pooled lysates of the two groups. Bioinformatics analysis of the proteomic data set revealed the aberrant regulation of proteins related to multiple cellular or subcellular events associated with VaD such as vascular dysfunction and oxidative stress. Some representative deregulated proteins were further validated by Western Blotting (WB) using individual patients. This study is the first quantitative proteomic investigation to reveal the differential global proteomes between VaD and age-matched control brain that provide a valuable data set for future basic or translational studies with individual proteins to propose potential therapeutic targets or biomarkers of VaD.

2. Materials and methods

2.1. Reagents

Unless indicated, all reagents and assay kits were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Patients and clinical assessments

We obtained frozen brain tissues from 10 non-demented elderly controls and 10 age-matched VaD subjects. The tissues were obtained from the Newcastle Brain Tissue Resource, Institute for Ageing and Health, Newcastle University. Demographic details of the subjects are summarized in Table 1 and can be found for individual subjects in the Supplemental Table 1. For this study, we assessed samples of the grey and white matter from BA21 area of the temporal lobe. VaD was

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