JOURNAL OF PROTEOMICS 127 (2015) 395–405

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

New insights into the human brain proteome: Protein expression profiling of deep brain stimulation target areas☆

Joaquín Fernández-Irigoyen[a,1], *María Victoria Zelaya*[a,b,1], *Estela Perez-Valderrama*[a], *Enrique Santamaría*[a,*]

[a]*ProteoRed-ISCIII, Proteomics Unit, Clinical Neuroproteomics Group, Navarrabiomed, Fundación Miguel Servet, Instituto de Investigación Sanitaria de Navarra (IDISNA), 31008 Pamplona, Spain*
[b]*Neurological Tissue Bank, Navarrabiomed, Fundación Miguel Servet, 31008 Pamplona, Spain*

ARTICLE INFO

Available online 4 April 2015

Keywords:
Nucleus basalis
Substantia nigra
Proteomics
HPP
Human Proteome Project

ABSTRACT

Deep brain stimulation (DBS) is a neurosurgical procedure that provides therapeutic benefits for movement and affective disorders. The nucleus basalis of Meynert (NBM) and substantia nigra (SN) are considered target areas to apply DBS. Even though the degeneration of NBM and SN underlies the cognitive decline observed in neurological diseases, the protein knowledge derived from both areas is scarce. We have characterized the proteome present in both subcortical brain areas using the Triple TOF 5600 mass spectrometer, identifying 2775 and 3469 proteoforms in NBM and SN respectively. Data mining of MS-generated proteomic data have revealed that: i) 675 proteins tend to localize to synaptic ending, ii) 61% of the global dataset is also present in human CSF and/or plasma, and iii) 894 proteins have not been previously identified in healthy brain by MS. The correlation of NBM and SN proteomic expression profiles with human brain transcriptome data available at Allen Brain Atlas has revealed protein evidence for 250 genes considered with brain-wide expression and 112 genes with region-specific expression in human brain. In addition, protein datasets have been classified according to their chromosomal origin, increasing the current proteome coverage in healthy human brain.

Biological significance
The *nucleus basalis of Meynert* and *substantia nigra* are brain areas of clinical interest to apply the deep brain stimulation (DBS) technology in neurosurgery. Our proteomic characterization has revealed 675 proteins involved in the regulation of synaptic transmission, electrical machinery, and neurotransmitter release in both DBS target areas. Moreover, 2599 identified proteins present capacity to be secreted to the CSF and plasma. Our data contribute to a further step towards the characterization of the

Abbreviations: DBS, deep brain stimulation; NBM, nucleus basalis of Meynert; SN, substantia nigra.

☆ This article is part of a Special Issue entitled: HUPO 2014.

* *Corresponding author at:* Proteomics Unit, Clinical Neuroproteomics Group, Navarrabiomed, Fundación Miguel Servet, Irunlarrea Street, 31008 Pamplona, Spain. Tel.: +34 848 428 540.
E-mail address: esantamma@navarra.es (E. Santamaría).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jprot.2015.03.032

anatomical atlas of the human brain proteome, detecting 652 proteins that are common between different basal ganglia structures.
This article is part of a Special Issue entitled: HUPO 2014.

1. Introduction

Deep brain stimulation (DBS) is a surgical procedure that involves placing a neurostimulator in the brain, which sends out electrical impulses to specific brain regions to block abnormal signals that can cause a number of different neurological disorders [1]. The modulation of brain activity using direct electrical stimulation has become the basis of highly successful therapies in patients with Parkinson's disease, tremor, dystonia, chronic pain, and Alzheimer's disease [2–6]. The DBS technology is usually focused on the stimulation of subcortical structures such as subthalamic nucleus, globus pallidus, amygdala, substantia nigra (SN), and nucleus basalis of Meynert (NBM) [7–11].

The SN is composed by the more dorsal SN pars compacta (SNc) with primarily dopamine neurons and the more ventral SN pars reticulata (SNr) with primarily GABA neurons [12]. SNr GABA neurons are medium-to-large-sized and have mostly ovoid or fusiform somata that emit two to six primary dendrites, which branch frequently [13,14]. Dopaminergic neurons located in the SNc are characterized by the presence neuromelanin, cholecystokinin, and calretinin and can be subdivided in different clusters of cell groups [15,16]. Specifically, Parkinson's disease is caused by progressive degeneration of dopamine neurons in the SNc, resulting in the deficiency of dopamine in the striatum. Thus, symptoms are developed, such as akinesia, rigidity and tremor [17]. Promising results have been recently published combining SNr-DBS and subthalamic nucleus-DBS where freezing of gait was improved in Parkinson's disease [18].

The nucleus basalis of Meynert (NBM) lies in the substantia innominata, an area that is located within the sublenticular region. It is composed by a group of deeply pigmented cholinergic cells of 20–30 μm in size that release the neurotransmitter acetylcholine [19,20]. It is known that these cells send their axons to the entire cortex, olfactory bulb and amygdala [21,22]. Interest in this area follows the general suggestion that the NBM atrophy produced by a decline in the number, size or function of the NBM cholinergic cells may be responsible for the cognitive impairments associated to Alzheimer's and Parkinson's diseases [23,24]. In fact, NBM has been recently postulated as a promising target structure to apply the DBS in dementia with the aim of activating neuroprotective mechanisms [11,24–26].

In the last years, different proteomic strategies have been used to understand the molecular organization and complexity of different regions of the brain [27]. Specifically, proteomic technologies have been applied in the analysis of DBS target areas such as amygdala and globus pallidus derived from healthy brain [28,29] and substantia nigra derived from parkinsonian subjects [30–33]. Considering that the identification of standard NBM and SN proteomes involved in normal physiology will contribute to the delineation of neurodegenerative diseases mechanisms, we present the qualitative proteome content of both DBS target areas using shotgun proteomics. Using anatomical, protein, and peptide fractionation strategies coupled to nanoLC-MS/MS, we report the identification of 2775 and 3469 proteoforms in NBM and SN respectively derived from 3 healthy patients with no known neuropsychiatric or neurological history. Integrated in-silico studies, have revealed that: i) 894 proteins have not been previously reported in the Human Brain Proteome database [34], ii) 11.5% of the global protein set tends to localize to synaptic terminal, iii) 652 common proteins identified in NBM and SN have been previously detected in functionally related basal ganglia structures such as striatum and globus pallidus, and iv) 2599 proteins (61% of the global dataset) have been previously reported in human CSF and/or plasma. In order to expand our knowledge about the global system biology of human brain, we have also correlated our NBM and SN proteomic expression profiles with the anatomical map of the human brain transcriptome stored in the Allen Brain Atlas [35] in order to analyze the interrelationship of expressed genes and proteins at different levels of organization [36]. As a contribution to the Chromosome-centric Human Proteome Project (C-HPP) initiative, we have also classified the NBM and SN proteome datasets according to their chromosomal origin.

2. Materials and methods

2.1. Sample collection

According to the Spanish Law 14/2007 of Biomedical Research, informed written consent form of the Neurological Tissue Bank of Navarrabiomed was obtained for research purposes from relatives of patients included in this study. The 3 patients with no known neuropsychiatric or neurological history were male and ages ranged from 56 to 79 years (control cases) (supporting Fig. 1). According to standard practices in place at the neurological tissue banks, the right cerebral hemisphere was progressively frozen and stored at −80 °C (*post-mortem*-interval: 5–10 h). The diagnosis was carried out on the left cerebral hemisphere. Therefore, the NBM and SN assessed in this study were the right ones. Following fixation in 10% formaldehyde for approximately three weeks, the brains were sectioned according to the recommendation guide proposed by BrainNet Europe [37]. After a macroscopic study, immunohistochemistry analysis was performed in different brain regions using specific antibodies against Tau protein, β amyloid, TDP-43, PrP, α-synuclein, ubiquitin and α–β crystalline. These brains did not show significant pathology and were considered to be healthy. In particular, the immunohistochemical study of the NBM and SN showed normal tissue without appreciable abnormalities.