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Differential proteome and phosphoproteome may impact cell signaling in the corpus callosum of schizophrenia patients

Verônica M. Saia-Cereda [a], Juliana S. Cassoli [a], Andrea Schmitt [b,c], Peter Falkai [c], Daniel Martins-de-Souza [a,b,d,*]

[a] Laboratory of Neuroproteomics, Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, SP, Brazil
[b] Laboratório de Neurociências (LIM-27), Instituto de Psiquiatria, Universidade de São Paulo, São Paulo, Brazil
[c] Department of Psychiatry and Psychotherapy, Ludwig Maximilian University (LMU), Munich, Germany
[d] UNICAMP's Neurobiology Center, Campinas, Brazil

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ABSTRACT

Schizophrenia is a multifactorial disease in both clinical and molecular terms. Thus, depicting the molecular aspects of the disease will contribute to the understanding of its biochemical mechanisms and consequently may lead to the development of new treatment strategies. The protein phosphorylation/dephosphorylation switch acts as the main mechanism for regulating cellular signaling. Moreover, approximately onethird of human proteins are phosphorylable. Thus, identifying proteins differentially phosphorylated in schizophrenia postmortem brains may improve our understanding of the molecular basis of brain function in this disease. Hence, we quantified the phosphoproteome of corpus callosum samples collected *post mortem* from schizophrenia patients and healthy controls. We used state-of-the-art, bottom-up shotgun mass spectrometry in a two-dimensional liquid chromatography-tandem mass spectrometry setup in the MSE mode with label-free quantification. We identified 60,634 peptides, belonging to 3283 proteins. Of these, 68 proteins were differentially phosphorylated, and 56 were differentially expressed.

These proteins are mostly involved in signaling pathways, such as ephrin B and ciliary neurotrophic factor signaling. The data presented here are novel because this was the very first phosphoproteome analysis of schizophrenia brains. They support the important role of glial cells, especially astrocytes, in schizophrenia and help to further the understanding of the molecular aspects of this disease. Our findings indicate a need for further studies on cell signaling, which might shape the development of treatment strategies.

1. Introduction

Post-translational modifications (PTMs) play a vital role in regulating the function and localization of proteins (Bremang et al., 2013). Phosphorylation is the most studied and understood PTM because it is the key regulator of intracellular signaling (Morandell et al., 2006; Reinders and Sickmann, 2005). Protein phosphorylation mediates and regulates processes such as energy metabolism, transcription, translation, protein degradation, homeostasis, cell signaling and communication, proliferation, differentiation, and cell survival (Graves and Krebs, 1960; Hunter, 2000). Because they are reversible modifications, changes in protein activity can be precisely controlled by phosphorylation/dephosphorylation in response to cellular or environmental stimuli or both. The phosphorylation sites in eukaryotes proteins are serine, threonine, and tyrosine residues (Thingholm et al., 2009).

The importance of the phosphorylation/dephosphorylation cycle is supported by the number of protein kinases and phosphatases, which constitute about 2% of all human genes (Manning, Whyte, Martinez and Sudarsanam, 2002b; Jaros et al., 2012). Furthermore, over 50% of all proteins are estimated to be phosphorylated during their lifetime (Reinders and Sickmann, 2005), and studies indicate that there may be more than 100 000 phosphorylation sites in the human proteome (Zhang et al., 2002). While some proteins are constitutively phosphorylated, most are only transiently phosphorylated, depending on the cell environment in which the protein is located. Noteworthy is that proteomics bioinformatics has advanced considerably in recent years, which has enabled the identification and quantification of protein phosphorylation in a large-scale quantitative manner, even without enrichment techniques. This progress has allowed the study of brain phosphoproteomics, which may generate useful information about the molecular aspects of the human brain and its disorders (Martins-de-Souza et al., 2011).

The corpus callosum (CC) is the largest white matter structure in the human brain and is enriched in glial cells; it is located at the center of the brain, between the right and left hemispheres, and is responsible mainly for inter-hemispheric communication (Fitsiori et al., 2011). Morphological, electrophysiological, and neurophysiological studies have

* Corresponding author at: Laboratory of Neuroproteomics, Department of Biochemistry and Tissue Biology, University of Campinas (UNICAMP), Rua Monteiro Lobato, 255, 13083-862 Campinas, SP, Brazil.
E-mail address: dmsouza@unicamp.br (D. Martins-de-Souza).

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suggested alterations in the CC of schizophrenia patients (Guo et al., 2000; Innocenti et al., 2003; Rotarska-Jagiela et al., 2008). The findings of these studies indicate that the CC is of pivotal importance in establishing and maintaining schizophrenia, so it is important to increase the understanding of its molecular features.

To better understand the biochemical pathways involved in schizophrenia and the role of glia in the disease, we evaluated the phosphoproteome of the CC. Our aim was to observe whether the deficiencies in cell signaling previously observed in neurons (Curley and Lewis, 2012) are present also in the CC.

2. Materials and methods

2.1. Human samples

The CC was collected postmortem from 5 chronic schizophrenia patients and 5 healthy controls (Table 1). Patient samples were provided by the Psychiatric Center Nordbaden, Wiesloch, Germany, whilst control samples were provided by the Institute of Neuropathology, Heidelberg University, Heidelberg, Germany. The controls had not had any kind of brain disorder or somatic disease and had not taken any antidepressant or antipsychotic medications. More information about the patients and controls can be found in Saia-Cereda et al. (Saia-Cereda et al., 2015). All assessments, postmortem evaluations, and procedures were approved by the ethics committee of the Faculty of Medicine of Heidelberg University, Heidelberg, Germany. Before their death, both patients and controls had given written consent for their brains to be used for research purposes.

2.2. Sample preparation

Twenty milligrams of each CC sample was homogenized with a sample grinding kit (GE Healthcare Life Sciences, Little Chalfont, UK) in 250 μL of buffer containing 7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 70 mM dithiothreitol (DTT), 2% Halt™ Phosphatase Inhibitor Cocktail, and 2% Halt™ Protease Inhibitor Cocktail EDTA-Free (Thermo Fisher Scientific, Waltham, MA, USA) and centrifuged at 14 000 rpm for 10 min at 4 °C. The supernatant was collected and subjected to quantification by a Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and 50 μg of each sample was subjected to reduction, alkylation, and digestion. First, 0.2% Rapigest was added to the samples, which were then incubated at 80 °C for 15 min. As the next step, the samples were reduced with 100 mM DTT at 60 °C for 30 min and alkylated with 200 mM iodoacetamide (IAA) for 30 min at room temperature in the dark. Next, the samples were subjected to digestion in a 1:50 trypsin:total protein ratio solution at 37 °C overnight. This procedure was stopped with the addition of 5% TFA at 37 °C for 90 min. Then, the sample was centrifuged for 30 min at 4 °C and 14 000 rpm. The supernatant was collected, its pH was adjusted with 1 N NH4OH, and i90-t was frozen until the mass spectrometric analyses.

2.3. Liquid chromatography-mass spectrometry

Qualitative and quantitative proteomic and phosphoproteomic analyses were performed in a bidimensional nanoUPLC tandem nanoESI-HDMSE platform by multiplexed data-independent acquisitions (DIA) experiments. The peptides (1 μg) were injected into a 2D-RP/RP Acquity UPLC M-Class System (Waters Corporation, Milford, MA) coupled to a Synapt G2-Si mass spectrometer (Waters Corporation, Milford, MA). The samples were fractionated in first dimension chromatography with an XBridge Peptide BEH C18 NanoEase Column (130 Å, 3.5 μm, 300 μm × 50 mm, Waters Corporation, Milford, MA). Peptide elutions were performed by using discontinuous steps of acetonitrile (11%, 14%, 17%, 20%, and 50% acetonitrile) for 10 min at a flow rate of 2000 nL/min. After each step, peptide loads were carried to second

Table 1
Clinical data of schizophrenia patients and healthy controls.

Case	Age (years)	Gender	PMI (hours)	pH values	Duration of disease (years)	Duration of medication (years)	atyptyp	CPE last dose	CPE last ten years	Cause of death	DSM IV	Age at onset	Last medication	Cigarettes	Alcohol	Hosp	ECT
SCZ	73	M	20	6.6	43	40	1	507.4	1.7	Heart infarction	295.6	30	Perphenazine 32 mg, promethazine 150 mg	30/day	No	33	No
SCZ	43	M	18	6.9	22	20	2	464	2.6	Heart infarction	295.6	20	Zuclopethixol 40 mg, valproate 1200 mg, tiapride 300 mg	0	No	13	No
SCZ	63	F	31	6.8	40	30	3	75	1.8	Heart infarction	295.6	24	Olanzapine 15 mg	30/day	No	30	Yes
SCZ	71	M	28	6.4	40	35	1	782.4	10	Heart infarction	295.6	30	Haloperidol 32 mg, pipamperone 40 mg	40/day	No	12	No
SCZ	81	M	4	6.7	62	50	1	92.8	1.4	Heart insufficiency	295.6	19	Haloperidol 40 mg, prothypendyl 80 mg	20	No	48	No
Control	41	M	7	6.5						Heart infarction				0	No		
Control	57	M	24	6.9						Heart infarction				0	No		
Control	53	M	18	7						Heart infarction				0	No		
Control	66	M	16	6.8						Heart infarction				0	No		
Control	79	M	24	6.4						Heart infarction				0	No		

Atyptyp: relation between duration of atypical treatment and duration of treatment with typical neuroleptics during lifetime; CPE: medication calculated in chlorpromazine equivalents(mg); CPE last 10 years: the sum of medications during the last 10 years in kg; Hosp: hospitalization time in years; ECT: electroconvulsive therapy; PMI: postmortem interval.