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PROTEOMICS REVEALS ENERGY AND GLUTATHIONE METABOLIC DYSREGULATION IN THE PREFRONTAL CORTEX OF A RAT MODEL OF DEPRESSION

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Abstract—Major depressive disorder (MDD) is a prevalent debilitating psychiatric mood that contributes to increased rates of disability and suicide. However, the pathophysiology underlying MDD remains poorly understood. A growing number of studies have associated dysfunction of the prefrontal cortex (PFC) with depression, but no proteomic study has been conducted to assess PFC protein expression in a preclinical model of depression. Using the chronic unpredictable mild stress (CUMS) rat model of depression, differential protein expression between the PFC proteomes of CUMS and control rats was assessed through two-dimensional electrophoresis followed by matrix-assisted laser desorption ionization-time of flight-tandem mass spectrometry. Differential protein expression was analyzed for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway over-representation. Four differential proteins were selected for Western blotting validation. Twenty-nine differential proteins were identified in the PFC of CUMS rats relative to control rats. Through KEGG analysis, energy and glutathione metabolic pathways were determined to be the most significantly altered biological pathways. Two of the four differential proteins selected for Western blotting validation – glyoxalase 1 and dihydropyrimidinase-related protein 2 – were found to be significantly downregulated in CUMS relative to control rats. In conclusion, proteomic analysis reveals that energy and glutathione metabolism are the most significantly altered biological pathways in the CUMS rat model of depression. Further investigation on these processes and proteins in the PFC is key to a better understanding of the underlying pathophysiology of MDD. **Crown Copyright © 2013 Published by Elsevier Ltd. All rights reserved.**

Key words: depression, chronic unpredictable mild stress, rat, proteomic, prefrontal cortex, energy metabolism.

INTRODUCTION

Major depressive disorder (MDD, major depression) is a debilitating psychiatric mood disorder with a lifetime prevalence of 16% that contributes to increased rates of disability and suicide (Bakish, 2001; Kessler et al., 2003). However, the pathophysiology underlying MDD remains poorly understood.

A mounting number of functional imaging, lesion, and brain stimulation studies have implicated the prefrontal cortex (PFC) in depression (Koenigs and Grafman, 2009). Dysfunction of glutamatergic neurotransmission in the PFC has been implicated in the pathophysiology of depression (Feyissa et al., 2009; Karolewicz et al., 2010). Resting state dorsolateral PFC (DLPFC) hypoactivation observed through fMRI neuroimaging is well-established in depression, and this hypoactivation has been observed to increase toward normal levels with antidepressant treatment (Fales et al., 2009); moreover, high-frequency repetitive transcranial magnetic stimulation (rTMS) of the left DLPFC has been shown to be effective in treating treatment-resistant depression (Schutter, 2009). Based on this evidence, PFC dysfunction is clearly associated with the pathophysiology of MDD and requires further investigation.

Proteomics, the quantitative analysis of protein expression in biosamples (Anderson and Anderson, 2005), has provided insights into the pathophysiology of several disease states (Taurines et al., 2011). Proteomic profiling methods have revealed considerable pathophysiological changes in animal models of depression and human depression (Martins-de-Souza

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Abbreviations: 2-DE, two-dimensional electrophoresis; ALPFC, anterolateral PFC; ANXA3, annexin A3; CUMS, chronic unpredictable mild stress; DLPFC, dorsolateral PFC; DPYSL2, dihydropyrimidinase-related protein 2; ETC, electron transport chain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLO1, lactoylglutathione lyase; GSTM5, glutathione S-transferase Mu 5; IEF, isoelectric focusing; KEGG, Kyoto Encyclopedia of Genes and Genomes; MALDI-TOF-MS/MS, matrix-assisted laser desorption ionization-time of flight-tandem mass spectrometry; MDD, major depressive disorder; MW, molecular weight; OD, optical density; PET, positron emission tomography; PFC, prefrontal cortex; PHGDH, phosphoglycerate dehydrogenase; PI, isoelectric point; PTM, post-translational modification; rTMS, repetitive transcranial magnetic stimulation; SDCBP, syntenin-1; SPSS, Statistical Package of Social Science; TCA, tricarboxylic acid.

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et al., 2010). In addition, our group has successfully established a plasma-based proteomic profiling approach for MDD (Xu et al., 2012). However, no proteomic study has yet been conducted to assess PFC protein expression in a preclinical model of depression.

Therefore, the chronic unpredictable mild stress (CUMS) rat model, a well-validated model of depression (Willner, 2005), was used to investigate the differential protein expression in the rat PFC following an unpredictable mild stress period. The PFC proteomes of CUMS and control (CON) rats were analyzed by two-dimensional electrophoresis (2-DE). Twenty-nine differential proteins were identified by matrix-assisted laser desorption ionization-time of flight-tandem mass spectrometry (MALDI-TOF-MS/MS) and then analyzed for pathway over-representation using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Four differential proteins were then selected for Western blotting validation. The findings should aid further investigation into the pathophysiology underlying MDD.

EXPERIMENTAL PROCEDURES

CUMS rat model

First, 18 healthy adult male Sprague–Dawley rats (weight: 230–280 g; age: 3–4 months) were purchased from the animal facility at Chongqing Medical University (Chongqing, China). Unless otherwise stated, the rats were kept under standard conditions (12-h light/dark cycle; lights on at 7:00 AM; 22 ± 1 °C ambient temperature; 52 ± 2% relative humidity; food and water *ad libitum*). After a seven-day adaptation to these standard conditions, the animals were randomly segregated into two groups: CUMS group (n = 9) and CON group without stress (n = 9). The time schedule was as previously reported (Fig. 1) (Yang et al., 2011). This study was approved by the Ethics Committee of Chongqing Medical University, and all procedures were in accordance with the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals). Special care was taken to minimize number and suffering of animals.

The CUMS group was isolated with each rat in a single cage and subjected to a variety of mild stressors: cage tilting for 24 h, swimming in 4 °C cold water for 5 min, swimming in 45 °C hot water for 5 min, fasting for 48 h, water deprivation for 24 h, shaking for 10 min, nip tail for 1 min, wet bedding for 24 h, and inversion of the light/dark cycle. Rats received one of these stressors per day, but the same stressor was not applied in two consecutive days. The stress procedure lasted for 4 weeks prior to behavioral testing, and was completed by food deprivation for 24 h as the final stressor. The CON group was kept in groups of four rats per cage and provided standard daily care (Mu et al., 2007).

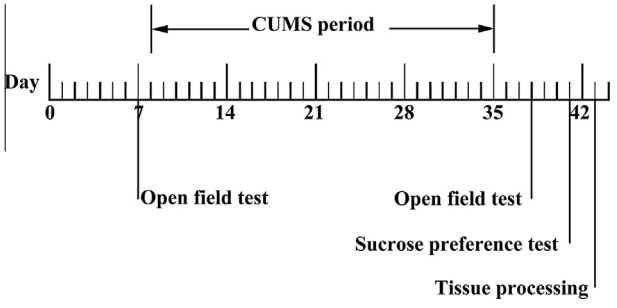

Fig. 1. Time schedule for various procedures. Open-field tests were conducted on days 7 and 38. Sucrose preference test was completed at day 41.

Open-field testing

The open-field test was performed to measure spatial exploration behavior in rodents. Rats were placed in the testing room 30 min before the test start. The test took place in a soundproof room between 8:00 AM and 1:00 PM, unless otherwise stated. Briefly, the apparatus, consisting of a black square cage measuring $100 \times 100 \times 40$ cm^3, was divided into 25×25 cm^2 equal squares on the floor of the arena. A single rat was placed in the center of the cage and after 30 s of adaptation, all behaviors were recorded for 5 min using a Sony DCR-SR45E camera located 190–200 cm above the arena. After the test, rats were returned to their home cages and then to the holding room once every animal was tested. The cage was thoroughly cleaned after each trail. Following this, open-field activity was blindly scored from the video footage as the number of locomotor (defined as locomotion on all four paws) and rearing (defined as upright posture sustained on hindpaws) behaviors. The scores were computed for statistical analysis.

Sucrose preference testing and body weight

Rats were trained to adapt to 1% (w/v) sucrose solution 72 h before testing: two bottles of 1% sucrose solution were placed in each cage, and 24 h later, 1% sucrose in one bottle was replaced with tap water for 24 h. After adaptation, rats were deprived of water and food for 24 h, followed by the sucrose preference test, in which all rats housed in individual cages had free access to two bottles, one containing 1% sucrose and the other tap water. The position of the two bottles (left/right sides of the cages) was randomly varied. All fluid consumption was recorded by weighing the two bottles before testing and after 24 h, and the sucrose preference was calculated as the sucrose preference % = (sucrose intake/total intake) × 100%. Body weights were measured before the CUMS procedure, and then weekly throughout the CUMS period.

Protein sample preparation

The protein extraction was essentially performed as previously reported (Mu et al., 2007). Briefly, six rats (n = 3 per group) were decapitated under deep diethyl ether anesthesia, their PFCs were dissected rapidly from the whole brain, and then individually powderized in liquid nitrogen and suspended in 2 ml of acetone solution containing 0.2% (w/v) DTT and 10% (w/v) TCA.

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