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Research report

Quantitative proteomics analysis of the liver reveals immune regulation and lipid metabolism dysregulation in a mouse model of depression

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HIGHLIGHTS

- iTRAQ was used to identify differential proteins between CUMS and CON mice.
- 66 significantly differentiated proteins were further analyzed by IPA.
- 3.Pathways disrupted by CUMS are involved in inflammation response, immune regulation, lipid metabolism, and NFκB signaling network, and our findings provide novel insight (liver–brain axis) into the multilayered mechanisms of MDD.
- Four associated proteins were validated by western blotting.

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ABSTRACT

Major depressive disorder (MDD) is a highly prevalent and debilitating mental illness with substantial impairments in quality of life and functioning. However, the pathophysiology of major depression remains poorly understood. Combining the brain and body should provide a comprehensive understanding of the etiology of MDD. As the largest internal organ of the human body, the liver has an important function, yet no proteomic study has assessed liver protein expression in a preclinical model of depression. Using the chronic unpredictable mild stress (CUMS) mouse model of depression, differential protein expression between CUMS and control (CON) mice was examined in the liver proteome using isobaric tag for relative and absolute quantitation (iTRAQ) coupled with tandem mass spectrometry. More than 4000 proteins were identified and 66 most significantly differentiated proteins were used for further bioinformatic analysis. According to the ingenuity pathway analysis (IPA), we found that proteins related to the inflammation response, immune regulation, lipid metabolism and NFκB signaling network were altered by CUMS. Moreover, four proteins closely associated with these processes, hemopexin, haptoglobin, cytochrome P450 2A4 (CYP2A4) and bile salt sulfotransferase 1 (SULT2A1), were validated by western blotting. In conclusion, we report, for the

Abbreviations: MDD, major depressive disorder; CUMS, chronic unpredictable mild stress; ITRAQ, isobaric tag for relative and absolute quantitation; GO, gene ontology; IPA, ingenuity pathway analysis; SPT, sucrose preference test; BW, body weight; FST, forced swimming test; OFT, open field test; CYP2A4, cytochrome P450 2A4; SULT2A1, bile saltsulfotransferase 1; BCA, bicinchoninic acid; SCX, strong cation exchange; LC–MS/MS, liquid chromatography–tandem mass spectrometry; HPLC, high performance liquid chromatography; FDR, false discovery rate; SEM, standard error of the mean; IDO, indoleamine-2,3-dioxygenase; SERT, serotonin transporter; CRS, chronic restraint stress; ROS, reactive oxygen species; HPA, hypothalamic–pituitary-adrenal; CON, control; NFκB, nuclear factor-kappaB; LPS, lipopolysaccharide; RXR, retinoid X receptor; HCD, higher-energy collisional dissociation.

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first time, the liver protein expression profile in the CUMS mouse model of depression. Our findings provide novel insight (liver–brain axis) into the multifaceted mechanisms of major depressive disorder.

1. Introduction

Major depressive disorder (MDD) is a common and debilitating psychiatric illness with a lifetime prevalence of 21%, and which significantly contributes to financial distress, increased hospitalization, functional impairment and suicide [1–3]. However, the relationship between the depressive phenotype and its underlying pathophysiology remains poorly understood. Therefore, experimental animal models involving chronic unpredictable mild stress (CUMS) have been established to further mechanistic research. In this animal model, CUMS from daily physical and mental irritations were found to mimic the stressors encountered in human society; hence, this model is regarded as one of the most naturalistic and predictive animal models of human depression [4].

In recent years, an increasing number of researchers have started to show an interest in brain–body pathways linking psychological distress (symptoms of depression and anxiety) to physical health [5]. Latest evidence suggests that MDD not only involves brain dysfunction, but is a systemic disease that affects the whole body [6]. The liver is the largest internal organ, and undertakes a metabolic and immunological function [7], playing a crucial role within the human body. Consequently, the novelty of the liver–brain axis [8] becomes more prevalence. Traditional Chinese Medicine relates the harmful effects of rage on liver function, for example, Chaihu-Shugan-San, which is recorded in a medical classic"Jingyue Quanshu", is efficient in treating depression via multitarget effects without side effects. It is used for relieving symptoms caused by liver-qi stagnation which results from repression of anger and distress according to traditional Chinese medicine theory [9,10]. Further study also found evidence of a dose–response relationship between increasing psychological distress and increasing liver disease–related mortality that was not completely explained by health behaviors (such as alcohol consumption), diabetes mellitus, socioeconomic status, and body mass index [11]. Based on this evidence, liver dysfunction is clearly associated with the pathophysiology of MDD and requires further investigation.

Proteins are the executors of physiological function. Accordingly, proteomics (i.e., quantitative analysis of protein expression in biological samples) can improve our understanding of pathophysiological mechanisms and aid in diagnostic tool development [12]. Proteomic profiling methods have identified significant pathophysiological changes in animal models of depression as well as human depression [13]. In addition, using these "-omics" approaches, our research group has performed a series of preclinical investigations in animal models [14–16], focusing on related encephalic regions such as the hippocampus, prefrontal cortex and cerebellum. There are also many clinical investigations [17–19] using clinical specimens such as blood and urine.

Nevertheless, to date, no large-scale proteomic analysis examining the molecular complexes or pathways involved in pathogenesis of the liver–brain axis has been performed. Isobaric tags for relative and absolute quantitation (iTRAQ) is the latest proteomic technology, with high repeatability, accuracy, and precision [20]. Thus, using iTRAQ, we determined comparative proteomic profiles in the liver of CUMS mice and control (CON) mice.

2. Material and methods

2.1. Animals

Forty healthy adult male C57BL/6J mice (8–10 weeks of age) were purchased from the animal facility at Chongqing Medical University (Chongqing, China), and habituated for 1 week. Mice were housed individually, maintained in a temperature- ($23 \pm 1\,^{\circ}C$) and humidity- (40–60%) controlled room under a 12-h light/dark cycle (lights on at 07:00–19:00), with access to food and water ad libitum, except when animals were subjected to light disturbance or deprivation stressors during the CUMS procedure. Animal maintenance and use were 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).

2.2. Chronic unpredictable mild stress paradigm

The CUMS procedure was performed according to previously published studies [21,22], with minor modifications. After 1 week of acclimatization and 1 week of training (for the sucrose preference test), mice were randomly sorted into groups of 20 mice. Next, CUMS mice were subjected to various and repeated unpredictable mild stressors for a period of 1 week (sub-CUMS) and 4 weeks (CUMS). The CUMS protocol was performed according to the procedure described in our previous study [15] (Fig. 1). Weekly stress consisted of the following stressors in a random order: food and water deprivation, wet bedding, paired housing, 45 °Cage tilt, overnight illumination, white noise, odor exposure, and stroboscope. All stressors were applied to animals outside of their housing area in a separate procedure room. On average, two stressors were administered per day.

2.3. Behavioral testing

2.3.1. Sucrose preference test

After 1 week of habituation, mice were exposed to two drinking bottles, one with tap water and the other with 1% sucrose solution in tap water, to accepted sucrose intake training for 1 week. According to the sucrose preference baseline, mice were then divided into two groups with no significant difference, one group was subjected to CUMS, while the other group served as non-stressed controls. Mice were tested for sucrose preference starts at 8:00 on every Sunday morning and end at 8:00 on the next Monday morning. On testing days, mice were housed in individual cages and had free access to both bottles. The 24 h volumes of consumed sucrose solution and water were measured by weight. Sucrose preference was calculated as a ratio of sucrose consumption over total volume intake.

2.3.2. Open-field test

Mice were placed in the testing room 30 min before the test. The test took place in a soundproof room between 8:00 AM and 1:00 PM. Mice were individually placed in the center of an open-field experiment box ($44.5 \times 44.5 \times 45$ cm), and allowed free access to explore the arena for 6 min. Behavior was recorded and analyzed using an automated video-tracking system (SMART, Panlab, Barcelona, Spain). Total distance traveled, time spent in the central area, and