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Research report

Potential antidepressant and resilience mechanism revealed by metabolomic study on peripheral blood mononuclear cells of stress resilient rats

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ABSTRACT

Resilience is an active coping response to stress, which plays a very important role in major depressive disorder study. The molecular mechanisms underlying such resilience are poorly understood. Peripheral blood mononuclear cells (PBMCs) were promising objects in unveiling the underlying pathogenesis of resilience. Hereby we carried out successive study on PBMCs metabolomics in resilient rats of chronic unpredictable mild stress (CUMS) model. A gas chromatography–mass spectrometry (GC–MS) metabolomic approach coupled with principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) was used to detect differential metabolites in PBMCs of resilient rats. Ingenuity Pathways Analysis (IPA) was applied for pathway analysis. A set of differential metabolites including Malic acid, Ornithine, L-Lysine, Stigmasterol, Oleic acid, γ-Tocopherol, Adenosine and *N*-acetyl-D-glucosamine were significantly altered in resilient rats, meanwhile promoting antidepressant research. As revealed by IPA that aberrant energy metabolism, HIFα signaling, neurotransmitter, O-GlcNAcylation and cAMP signaling cascade in peripheral might be evolved in the pathogenesis of coping mechanism. The GC–MS based metabolomics may contribute to better understanding of resilience, as well as shedding light on antidepressant discovery.

1. Introduction

Resilience is defined as "the process of adapting well in the face of adversity, trauma, tragedy, threats or even significant sources of threat" by the American Psychological Association [1]. It is also deemed to the brain's capacity to cope with environmental stress and to achieve stable psychological functioning in response to prolonged stress [2,3]. This phenotype happens to the majority of humans when exposed to chronic stress that avoiding to develop neuropsychiatric disorders such as posttraumatic stress disorder (PTSD) or major depressive disorder (MDD) [4,5]. Current research hotspots focus on active, adaptive coping mechanisms of resilience through multiaspects, including environmental, genetic, epigenetic and neural mechanisms [6,7]. In clinical studies, the main attentions were paid to correlative neuroendocrine markers associated with resilient phenotype, while increasing preclinical evidences arose to complement the human work by identifying the active behavioral, neural, molecular, and hormonal basis of resilience [8].

Although the range of complex mechanisms that lead to resilient phenotype is far from being fully determined, the animal model of resilience has emerged from the study of adaptive stress responses at multiple phenotypic levels [6]. Animal study is critical for biological determinants to count for resilient phenotypes [9,10]. Animal models are widely used to mimic pathogenic process and out-

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comes of depression patients. Comparing to humans, chronic stress leads to the development of depression – or anxiety–like behaviors in only a subset of laboratory animals [8]. Likewise, a portion of stressed rats/mice usually exhibit some deleterious symptoms in response to the stress, but do not exhibit deficits in key behavioral domains. Hereby we summarize that: (I) In chronic social defeat stress mice model of depression, approximately 35% of the stressed mice, considered "resilient", do not exhibit social avoidance [11], though they might still have symptoms of anxiety-like behavior or stress-induced polydipsia [12]; (II) In chronic unpredictable mild stress (CUMS) rat model, rats avoided to display anhedonia–like symptoms(reduced sucrose consumption) were defined as stress resilient rats [13,14]; (III) While in learned helplessness mice model of depression, approximately 70% were still available to escape foot shock with latencies seen in unstressed animals [15,16]. Under such circumstances, though resilient animals exhibit some behavioral adaptations that appear maladaptive, they exhibit clear resistance to many other maladaptive sequelae of the chronic social stress [8].

Peripheral blood mononuclear cells (PBMCs) could serve as an ideal peripheral medium in searching of biomarker, as well as studying mechanism of resilience. PBMCs play critical roles in immune response, metabolism, and communication with other cells and extracellular matrices almost are ubiquitous in the human body, which have been widely used in MDD study [17–20]. A recent report published on Nature that Louveau et al. discovered functional lymphatic vessels lining the dural sinuses [21]. This gives evidence to the possible crosstalk between central nervous system (CNS) and periphery [22]. Since cerebrospinal fluid (CSF) and brain biopsy samples can be clinically impractical to collect for routine screening or diagnostic purposes, PBMCs derived from convenient, low-cost blood sampling display practical advantages as a peripheral clinical sample in diagnostic biomarker development for MDD.

Recently, metabolomics has been regarded as the latest-omics strategy. It develops rapidly with multiplex advanced approaches of gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS) and nuclear magnetic resonance spectroscopy (NMR) techniques, which offer powerful tools for describing metabolic fingerprinting in biological samples [23,24]. In this way, Metabolomics has the potential to reveal perturbations in metabolic pathways, as well as to provide potential diagnostic biomarkers and putative treatment target. Hereby, among aforementioned three widely applied methods, GC–MS was selected because of its high sensitivity, peak resolution, and reproducibility [25–27]. In this study, we aim to capture PBMC-based metabolic patterns in stress resilient rats of a well-validated CUMS model. This work could furnish valuable insights into PBMC-based metabolic changes that may aid in finding out authentic and easily detected biomarkers for resilience, which possibly paved way for antidepressant discovery.

2. Materials and methods

2.1. Animals

This study was performed strictly according to the recommendations of *Guide for the Care and Use of Laboratory Animals* [28] and approved by the Committee of Chongqing Medical University (CQMU). Male Sprague-Dawley rats were obtained from CQMU's animal center.

2.2. Chronic unpredictable mild stress procedure

The CUMS procedure and sucrose preference test (SPT) were performed as our previous work described [25,29,30]. Following exposure to CUMS, rats not decreasing their SPT significantly were defined resilient [31,32]. The rats' weights were measured before and after the CUMS treatment.

2.3. PBMC collection and pretreatment

PBMC was collected and prepared as previously described [25]. Rats were sacrificed under anesthesia by an overdose of chloral hydrate and blood samples (approximately 8 ml) were quickly collected via heart puncture in EDTA-coated tubes (Becton, Dickinson and Company, Plymouth, UK). Plasma was separated by blood centrifugation at 1500*g* for 15 min within 1 h after blood collection. The remainder was diluted with phosphate buffer saline (PBS) to the original blood sample volume. Subsequently, plasma was overlaid onto lymphoprep (Lymphoprep-density, 1.077 ± 0.001 g/ml; GE Healthcare Bio-Sciences AB, Sweden) and centrifuged at 2000 rpm for 20 min at room temperature. The supernatant was carefully removed. After washing three times with PBS, PBMC pellets were stored at −80 °C until later GC–MS analysis.

2.4. GC–MS sample preparation and derivatization

Preparation and derivatization of PBMC for GC–MS analysis were performed as previously described [25]. Each frozen PBMC sample was added to 1 ml chromatographic grade methanol and 10 μl leucine-13C6 (0.5 mg/ml) as an internal standard and stored at 0 °C overnight. The mixture was sonicated for 30 min and subsequently centrifuged at 14000*g* at 4 °C for 15 min. An 800 μl volume of supernatant was transferred into a new 1.5 ml EP tube, mixed with 400 μl extracting solution, and then evaporated to dryness under a stream of nitrogen gas. The dried residue was mixed with 30 μl methoxamine hydrochloride (20 mg/ml pyridine) and incubated at 37 °C for 90 min with continuous shaking. Subsequently, the solution was derivatized with 30 μl BSTFA (1% TMCS) at 70 °C for 60 min and then placed at room temperature for 30 min before GC–MS analysis.

2.5. GC–MS analysis

GC–MS analysis was performed as described previously [25]. GC–MS analysis was performed using the Agilent 7890A/5975C GC–MS System (Agilent Technologies Inc., USA). Chromatography was performed on a HP-5 MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, USA). The temperatures of the injector, EI iron source, and quadrupole rods were set at 280 °C, 230 °C, and 150 °C, respectively. High purity helium carrier gas flowed at a rate of 1 ml/min. A total of 1 μl for each sample was applied for metabolite separation. The column temperature was initially maintained at 80 °C for 2 min and then raised to 320 °C by 10 °C/min, maintained for 6 min. MS detection was conducted with electron impact ionization mode in the full scan mode (*m*/*z*, 50–600). To avoid the influence induced by instrument signal fluctuations, a random order of continuous sample analysis was adopted.

2.6. Metabolomic data analysis and identification of metabolic signatures

Eight stress resilient rats' PBMC samples were comparatively analyzed against eight control samples. Meanwhile seven resilient rats' PBMC samples were comparatively analyzed vs. seven susceptible rats' PBMC samples. Visual inspections of total ion current chromatogram (TIC) chromatograms were applied to all samples. All displayed strong signals for analysis as well as large peak capacity and good reproducibility in retention time (Fig. 1A and B). A total of 292 individual peaks were detected, which existed in over