ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

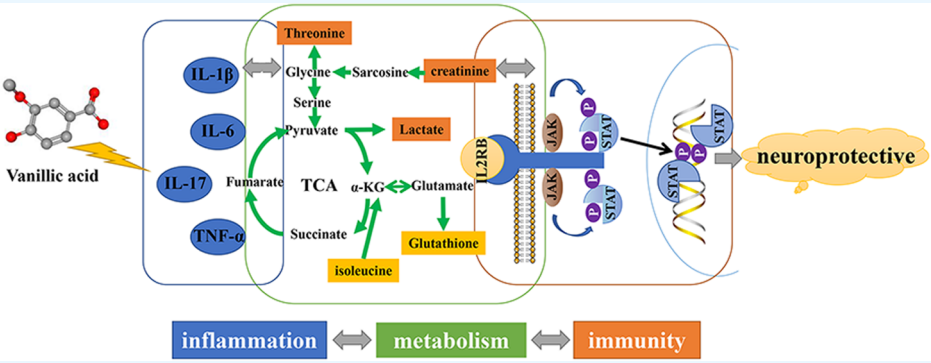
Vanillic Acid Protects PC12 Cells from Corticosterone-Induced Neurotoxicity via Regulating Immune and Metabolic Dysregulation Based on Computational Metabolomics

Tianxing Lv,[#] Daojin Xue,[#] Peng Wang, Wenxia Gong,[*] and Kexin Wang[*]

Cite This: *ACS Omega* 2024, 9, 40456−40467

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Vanillic acid is widely used in the food industry and exhibits an excellent neuroprotective effect. Nevertheless, the mechanisms underlying them are largely unexplored, especially the interactions between the neuroprotection effects of vanillic acid and inflammation−immunity−metabolism. A cell metabolomics-based mathematics algorithm was reported to interpret the potential mechanism of vanillic acid on corticosterone-induced PC12 cells by regulating immune and metabolic dysregulation. Our results showed that vanillic acid markedly inhibited the level of inflammatory factors in corticosterone-induced PC12 cells. Cell metabolomics results suggested that vanillic acid regulated the abnormality of corticosterone-induced PC12 cell metabolic profiles and markedly regulated 11 differential metabolites. Our designed scoring model base entropy weight algorithm showed that the core targets (IL2RB, IFNA13, etc.) and metabolites (lactate, ethanolamine, etc.) regulate the immunity−metabolism of vanillic acid. Furthermore, we demonstrated that vanillic acid inhibited IL2RB expression and modulated the related pathway, JAK1/STAT3 signaling. The JAK inhibitor ABT-494 was further applied to validate the effect of vanillic acid on the JAK/STAT pathway. Results indicate that vanillic acid regulates the abnormal interactions of inflammation−immunity−metabolism by repressing the IL2RB-JAK1-STAT3 pathway. Methodologically, this study contributes to the decoding of vanillic acid's antidepressive effect from the metabolism perspective combined with computer algorithms and mathematics models.

1. INTRODUCTION

With the changes in the lifestyle and social and psychological environment, particularly with COVID-19, the incidence of depression has increased each year worldwide.[1] People with depression are prone to suicide, which may lead to a disability. Currently, the main intervention measures for depression are drugs and psychotherapy. Antidepressants can only partially alleviate depression and cannot completely cure it; therefore, the therapeutic effect is not ideal. It is estimated that approximately 30−50% of patients with depression are insensitive to the existing antidepressants.[2] Therefore, it is particularly important to find other safer and more effective methods to prevent and treat depression, reduce its occurrence, and promote its recovery.

The pathogenesis of depression is complex, including abnormal neuroplasticity, abnormal neuroendocrine, deficient neurotrophic factors, excessive inflammatory response, oxidative stress, etc.[3] Among which the hypothalamic−pituitary−adrenal (HPA) axis in patients with depression is hyperactive, which leads to immoderate secretion of cortisol and damaged hippocampal neurons, leading to depression.[4] PC12 cells are a kind of pheochromocytoma cells with neuroendocrine effects, and their cell membrane has abundant glucocorticoid (GC) receptors.[5] The HPA axis is mainly regulated by GC, where cortisol is the main GC in the human body, while the main GC

Received: March 29, 2024
Revised: August 1, 2024
Accepted: August 2, 2024
Published: September 23, 2024

in rodents is corticosterone. Some studies have showed that the markers of depression BDNF, SOD, and MDA were prominently decreased in corticosterone-induced PC12 cells.[6,7] In addition, some classic antidepressants, such as venlafaxine and fluoxetine, also exhibit protective effects on corticosterone-induced PC12 cells.[8,9] The antidepressant property of berberine was also evaluated in a corticosterone-induced depression mouse model by Qin et al.[10] Research on depression most commonly uses PC12 cells damaged by corticosterone because this corresponds to the mechanism of HPA axis hyperfunction in patients with depression.[11]

Recently, studies have fully confirmed the role of inflammatory disorders in mental diseases such as depression.[12] Among them, abnormal immune regulation is the important foundation of the disorder of inflammatory response in depression, which is closely related to the indexes of neurobiochemical reactions, mental behavior, and physical symptoms related to depression. With the in-depth study of the neuroimmune metabolism interaction, neuroinflammatory reactions and pathological changes driven by abnormal immune metabolism have become important research directions for the pathological mechanism of depression and drug intervention.[13]

Vanillic acid is widely used in the food industry as a spice, food additive, and preservative.[14] Vanillic acid has many pharmacological activities, such as antioxidation, anti-inflammation, neuroprotection, and antiapoptosis, and preclinical research has shown a bevalneficial effect on depression.[15,16] Chuang et al. reported that vanillic acid could markedly reduce behavioral despair in the forced swim test of mice, thus showing an antidepressant effect through the AMPAR−Akt−mTOR signaling transduction pathway.[16] Lin et al. found that vanillic acid could alleviate depression by regulating monoamine oxidases A and B and the estrogen receptor.[17] Vanillic acid exhibited beneficial effects in treating depression by regulating multiple pathways.[18] However, the potential role and mechanism of vanillic acid in inflammation−immunity−metabolism regulation in depression remain unclear.

Cell metabolomics is a technology that takes all metabolites in a single cell as the object to characterize and analyze the state of life activities. It can comprehensively reflect the changes in the types and contents of metabolites of organisms after drug administration, and cell metabolites are considered to be the best indicators of the organism phenotype.[19] Its integrity, objectivity, dynamics, and accuracy make it suitable for studying complex diseases. Systematic biological methods show unique effects in revealing the potential therapeutic mechanism of drugs at the molecular level. System biology relies heavily on mathematical algorithms to meet data dependence requirements. For example, Zhang et al. successfully predicted pathogenic genes using a computer algorithm based on the relation inference algorithm CIPHER.[20] Chen et al. explored the mechanism by which emodin improves myocardial hypertrophy using machine learning.[21] However, there are few studies on the potential mechanism of drug antidepressive effects from a metabolism perspective combined with computer algorithms and mathematical models.

Herein, the corticosterone-induced PC12 cell model was applied to investigate the effects of vanillic acid on inflammatory cytokines. Then, we attested for the first time that vanillic acid regulates the abnormal metabolism of corticosterone-induced PC12 cells, and we applied a mathematical algorithm to find and further validate the changes in proteins involved in vanillic acid-regulated inflammation−immunity−metabolism dysregulation. A methodological reference was provided for determining how vanillic acid works as an antidepressant from an immune and metabolic dysregulation perspective.

2. MATERIALS AND METHODS

2.1. Reagents. Jingzhu Biotechnology Co., Ltd. (Nanjing, China) provided vanillic acid (≥98% purity by HPLC). RPMI-1640 (HyClone; GE). Sangon Biotechnology Co. Ltd. supplied fetal bovine serum (FBS), trypsin, and MTT.

2.2. Cell Culture and Treatment. The PC12 cells were obtained from the Chinese Academy of Sciences Cell Bank (China) and cultured at 37 °C with 5% CO_2 in 1640 medium supplemented with 10% FBS.

The PC12 cells were plated in 96-well plates, 6-well plates, and 100 mm dishes. Incubation of PC12 cells for 24 h at 37 °C with different concentrations of vanillic acid and corticosterone followed. Our previous research[22] has confirmed that 1−50 μM vanillic acid is not toxic and 10−25 μM vanillic acid markedly improved the cell viability and LDH level compared with 400 μM corticosterone, and the above concentrations were selected as the measured concentrations for further research.

2.3. Cell Viability Assay. At 37 °C for 2 h, confluent layers of PC12 cells were treated with different concentrations of vanillic acid; 10 μL of MTT solution was added per well, and incubation at 37 °C for 4 h was performed on the plates. After this, the culture supernatant was detached and the cells were lysed using 100 μL of dimethyl sulfoxide. Absorbance was tested at 570 nm.

2.4. LDH Leakage Assay. Confluent layers of PC12 cells were treated with 1−20 μM vanillic acid at 37 °C for 2 h. The supernatants and cell lysates were detected by using an LDH diagnostic kit.

2.5. Western Blot Assay. Whole-cell lysates mentioned above were centrifuged to collect the supernatants, and their protein concentrations were determined. A quantity of 50 μg of protein was employed for conducting SDS-PAGE to segregate and was then applied to PVDF membranes. The primary antibodies IL2RB (dilution 1:500, Proteintech, Cat No. 13602-1-AP), JAK1 (1:1000, Proteintech, Cat No. 66466-1-Ig), pJAK1 (1:500, CST, Cat No. 33315), STAT3 (1:1000, Proteintech, Cat No. 10253-2-AP), pSTAT3 (1:500, CST, Cat No. 9145), β-actin (1:1000, Proteintech, Cat No. 81115-1-RR), and GAPDH (1:1000, Proteintech, Cat No. 60004-1-Ig) were used in this analysis.

2.6. Inflammatory Cytokine Assay. The levels of inflammatory cytokines were determined by using an ELISA kit. The levels of interleukin (IL)-1β, IL-6, IL-17, and TNF-α were detected according to the manufacturer's instructions.

2.7. Cell Metabolomics Analysis. PC12 cells treated with vanillic acid were collected and quenched with liquid nitrogen. Intracellular extracts were prepared by repeated freeze−thaw and ultrasonic crushing. Utilizing a water suppression 1D noesygppr1d pulse sequence, the ^{1}H NMR spectra were captured, featuring 64 scans, a spectral width of 12345.7 Hz, 65,536 spectral size points, and a relaxation delay of 1.0 s. Initially, MestReNova software processed the NMR data, eliminating any manually phased, baseline-corrected, and residual water (δ 4.65−5.0). Chemical shifts in the 1H-NMR spectra were aligned with TSP. Subsequently, the data matrix

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

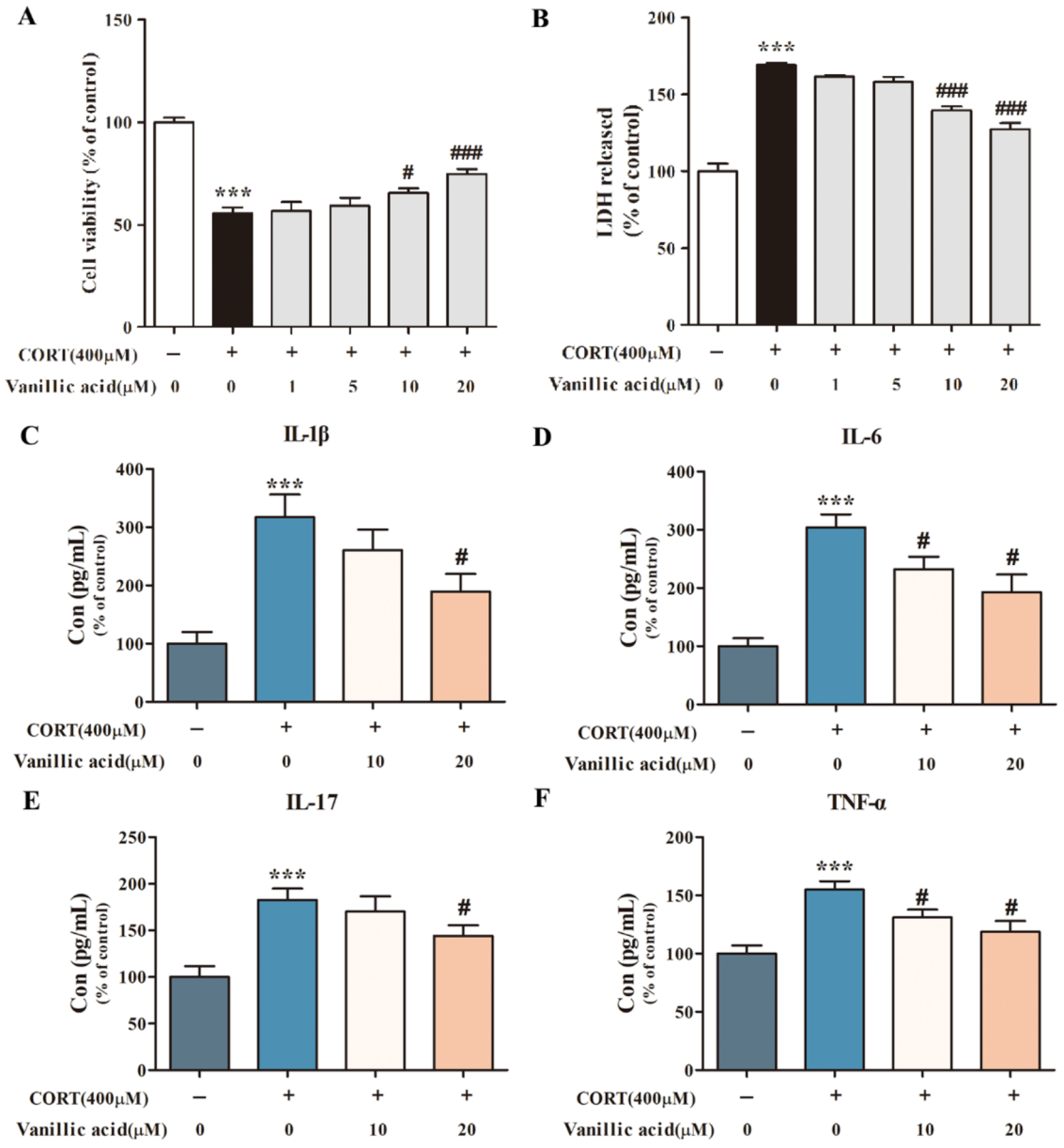


Figure 1. Effect of vanillic acid on viabilities (A) and LDH release (B) of corticosterone-induced PC12 cells. IL-1β (C), IL-6 (D), IL-17 (E), and TNF-α (F) levels in corticosterone-induced PC12 cells. $n = 3$, $\bar{x} \pm$ SEM $^{\#}p < 0.05$, and $^{\#\#\#}p < 0.001$ compared with the corticosterone-induced PC12 cells model group. $^{***}p < 0.001$ compared with the control group.

was collected by dividing it into segments at intervals of 0.01 ppm, spanning δ 0.68−9.00, along with all data points normalized. Subsequently, the text was transferred to Simca-P 13.0 software, compatible with PCA, PLS-DA, and OPLS-DA. Differential metabolites were pinpointed based on the VIP value (>1) and a *t*-test ($p < 0.05$). MetaboAnalyst 6.0 was applied to obtain the metabolic pathway.

2.8. Screening of Targets and Metabolites to Regulate the Immunity−Metabolism of Vanillic Acid. Immunology Database and Analysis Portal (ImmPort) (https://immport.niaid.nih.gov) was applied to predict the immune-related genes. MestReNova software was utilized to process NMR data. All data points are derived after the baseline, phase correction, integration, and normalization. All texts were analyzed by PCA, PLSDA, and OPLSDA. Integration of VIP > 1 and $p < 0.05$ identified differential metabolites. Random forest and correlation analysis were performed by using metaboAnalyst 6.0.

2.9. Construction of the Coexpression Matrix of Vanillic Acid-Regulated Immune-Related Genes and Differential Metabolites. The expression data of immune-related genes were extracted from microarray datasets using the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) database, including GSE98793 and GSE52790 datasets. Gene analysis employs the Sangerbox platform. The GSE98793 datasets contained 128 major depressive disorder whole-blood samples and 64 healthy control samples, and the GSE52790 datasets contained 10 major depressive disorder whole-blood samples and 12 healthy control samples. The creation of the coexpression matrix involved computation of its correlation with immune-related genes and differential metabolites.

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

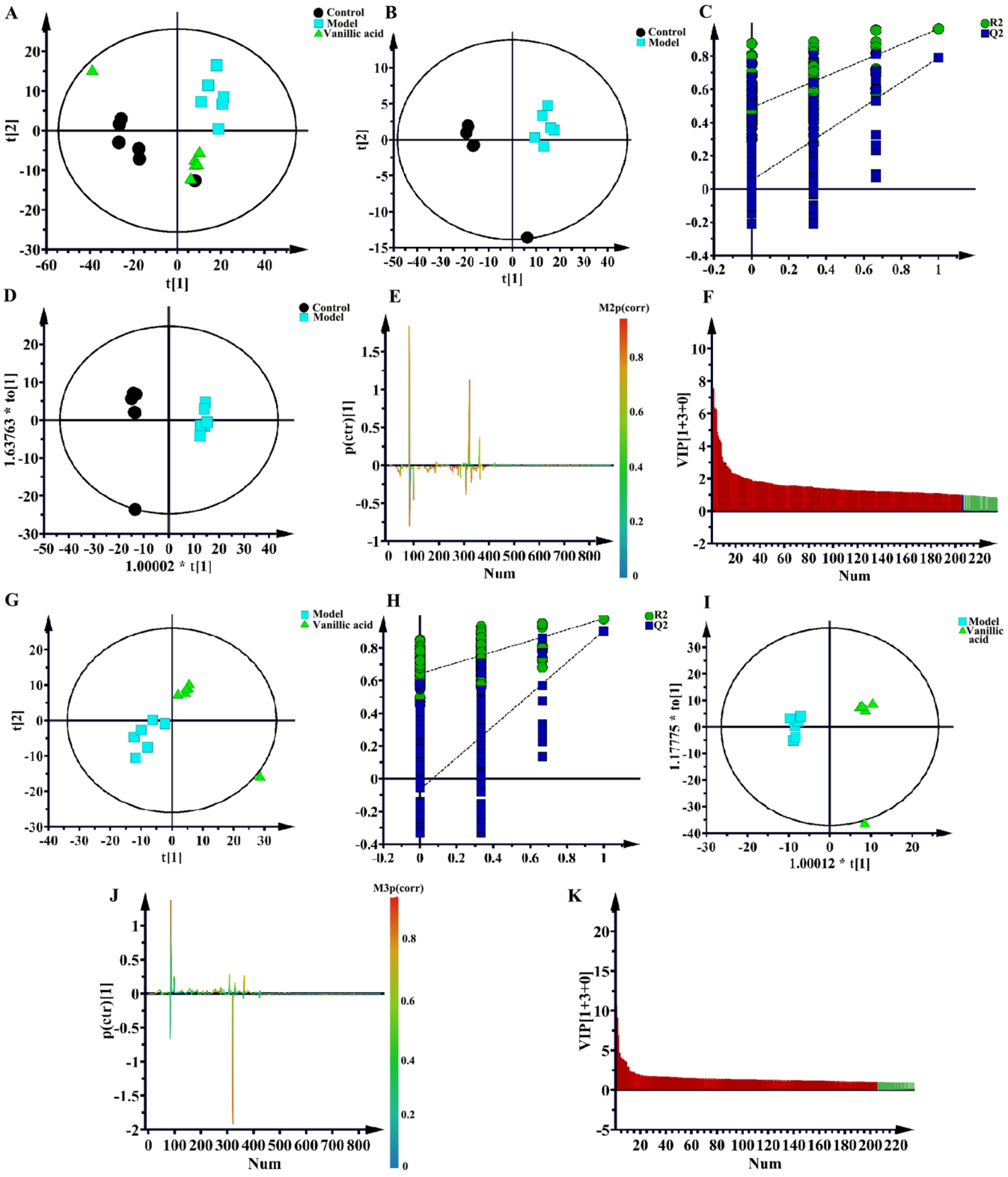


Figure 2. Vanillic acid had a significant effect on corticosterone-induced PC12 cell metabolism. PCA scores plots derived from ^{1}H NMR spectra of the control group, model group (corticosterone-induced PC12 cells), and vanillic acid group (A). PLS-DA scores plots from different groups: control group compared with the model group (B) and model group compared with the vanillic acid-treated group (G). PLS-DA corresponding validation plot of the permutation test for 200 times from different groups: control group compared with the model group (C) and model group compared with the vanillic acid-treated group (H). OPLS-DA score plots from different groups: control group compared with the model group (D) and model group compared with the vanillic acid-treated group (I). The OPLS-DA corresponding S-line derived from ^{1}H NMR spectra of the control group compared with the model group (E) and model group compared with the vanillic acid-treated group (J). Variable importance in projection (VIP) plot from different groups: control group compared with the model group (F) and model group compared with the vanillic acid-treated group (K).

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

2.10. Entropy Weight Algorithm. The Z value was used to evaluate the importance of immune-related genes and differential metabolites regulated by vanillic acid. The specific formula is as follows

$$Y_{ij} = \frac{X_{ij} - \min(X_i)}{\max(X_i) - \min(X_i)} \quad (1)$$

$$p_{ij} = Y_{ij} / \sum_{i=1}^{n} Y_{ij} \quad (2)$$

$$E_j = -\ln(n)^{-1} \sum_{i=1}^{n} p_{ij} \ln p_{ij} \quad (3)$$

$$W_i = \frac{1 - E_i}{k - \sum E_i} \quad (4)$$

$$Z_l = \sum_{i=1}^{3} X_{li} W_i \quad (5)$$

where $X_{(i)}$ is the correlation of each immune-related gene and differential metabolite, Y_{ij} represents the standardized value for each $X_{(i)}$, E_j represents the information entropy of each $X_{(i)}$, W_i is the weight of each E_j, and Z_l represents the importance of immune-related genes and differential metabolites. A larger Z value represents more important immune-related genes and differential metabolites.

2.11. Statistical Analysis. Notable variances were examined through a two-way variance analysis, succeeded by Tukey's subsequent test for numerous comparisons and Student's *t*-test for contrasting two distinct groups. Corrections to the *p*-values were made through the application of the Benjamini–Hochberg false discovery rate. Findings were deemed to hold statistical significance when p was less than 0.05.

3. RESULTS

3.1. Effects of Vanillic Acid on the Viability of Corticosterone-Induced PC12 Cells. This study established a model of PC12 cell injury induced by a high concentration of corticosterone based on the theory of HPA axis activation in depression, which has been widely used in studying the efficacy and mechanism of antidepressants.[11] The viability of PC12 cells was 55.4% after exposure to 400 μM corticosterone after 24 h of incubation (Figure 1A). However, treatment with different concentrations of vanillic acid can improve cell viability to different degrees. Among them, 10 and 20 μM vanillic acid significantly increased the cell viability by 10.2 and 19.2%, respectively (Figure 1A).

3.2. Effect of Vanillic Acid on LDH Release in Corticosterone-Induced PC12 Cells. To further verify the protective effect of different concentrations of vanillic acid on the corticosterone-induced PC12 cell model, the release rate of LDH was determined. LDH is an intracellular enzyme. When the cell permeability changes, the activity of the intercellular LDH increases. Therefore, whether the cell permeability changes, i.e., whether the cell is damaged, can be judged by measuring the release rate of cell LDH. The release rate of LDH in the corticosterone-induced PC12 cell model group was markedly increased compared with the control group. However, 10 and 20 μM vanillic acid could markedly reduce the release rate of LDH (Figure 1B). Therefore, 10 and 20 μM vanillic acid was used in the follow-up study.

3.3. Effects of Vanillic Acid on Inflammatory Cytokine Levels in Corticosterone-Induced PC12 Cells. Inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and various ILs, may be involved in the emergence and progression of depression.[23] The effects of vanillic acid on cell supernatant levels of IL-1β, IL-6, IL-17, and TNF-α were analyzed in corticosterone-induced PC12 cells by using ELISA kits. Once induced by corticosterone, the IL-1β, IL-6, IL-17, and TNF-α levels were markedly increased by 217.5, 204.1, 82.6, and 55.0%, respectively, compared to the control group. However, pretreatment with 10 and 20 μM vanillic acid decreased the IL-1β levels by 57.0 and 128.1%, decreased the IL-6 level by 71.7 and 111.3%, decreased the IL-17 level by 12.5 and 38.4%, and decreased the TNF-α level by 24.0 and 36.2%, respectively (Figure 1C–F). The results indicated that vanillic acid could markedly suppress inflammatory cytokine levels in corticosterone-induced PC12 cells. Therefore, 20 μM vanillic acid was selected for the metabolomics and follow-up experimental study.

3.4. Vanillic Acid Regulates the Abnormality of Metabolic Profiles of Corticosterone-Induced PC12 Cells. The typical proton nuclear magnetic resonance (^{1}H NMR) spectra of corticosterone-induced PC12 cells are shown in Figure S1. According to the information on metabolite peaks and their corresponding chemical shifts, the ^{1}H NMR spectrum was identified using Chenomx NMR suite software (trial version 7.5, Chenomx Inc., Edmonton, AB, Canada), the HMDB database, and the published literature.[24,25] The identification results are listed in Table S1. A total of 39 metabolites were identified in the ^{1}H NMR spectra of corticosterone-induced PC12 cells. Unsupervised principal component analysis was utilized to view the metabolic profiles among the groups. The principal component analysis score chart showed that the samples of the control and corticosterone-induced PC12 cell model groups were significantly separated along the t2 axis, that is, corticosterone had a significant impact on PC12 cell metabolism, which was consistent with the determination results of cell viability, LDH, and inflammatory factors (Figure 2A). The vanillic acid group was close to the control group, indicating that vanillic acid had a callback trend (Figure 2A).

3.5. Identification of Differential Metabolites Regulated by Vanillic Acid. A multivariate statistical analysis was conducted to examine the difference between endogenous metabolites before and after the administration of vanillic acid in corticosterone-induced PC12 cells. The PLS-DA pattern recognition method was utilized for the analysis (Figure 2B,G). The results showed that the control and corticosterone-induced PC12 cell model groups and corticosterone-induced PC12 cell model and vanillic acid groups could be discriminated. Results from validating the model revealed that every R^2 and Q^2 value on the left side was lower than the original points on the right side, and the point where the regression line of Q^2 intersects with the longitudinal axis was less than zero, signifying the recognized model's substantial dependability (Figure 2C,H). The OPLS-DA and the corresponding S-line load diagram were drawn (Figure 2D,E,I,J) for additional examination of the varied metabolites associated with corticosterone-induced PC12 cells and vanillic acid therapy. The S-line diagram and VIP value (Figure 2F,K) were united to determine the differential metabolites with VIP

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456–40467

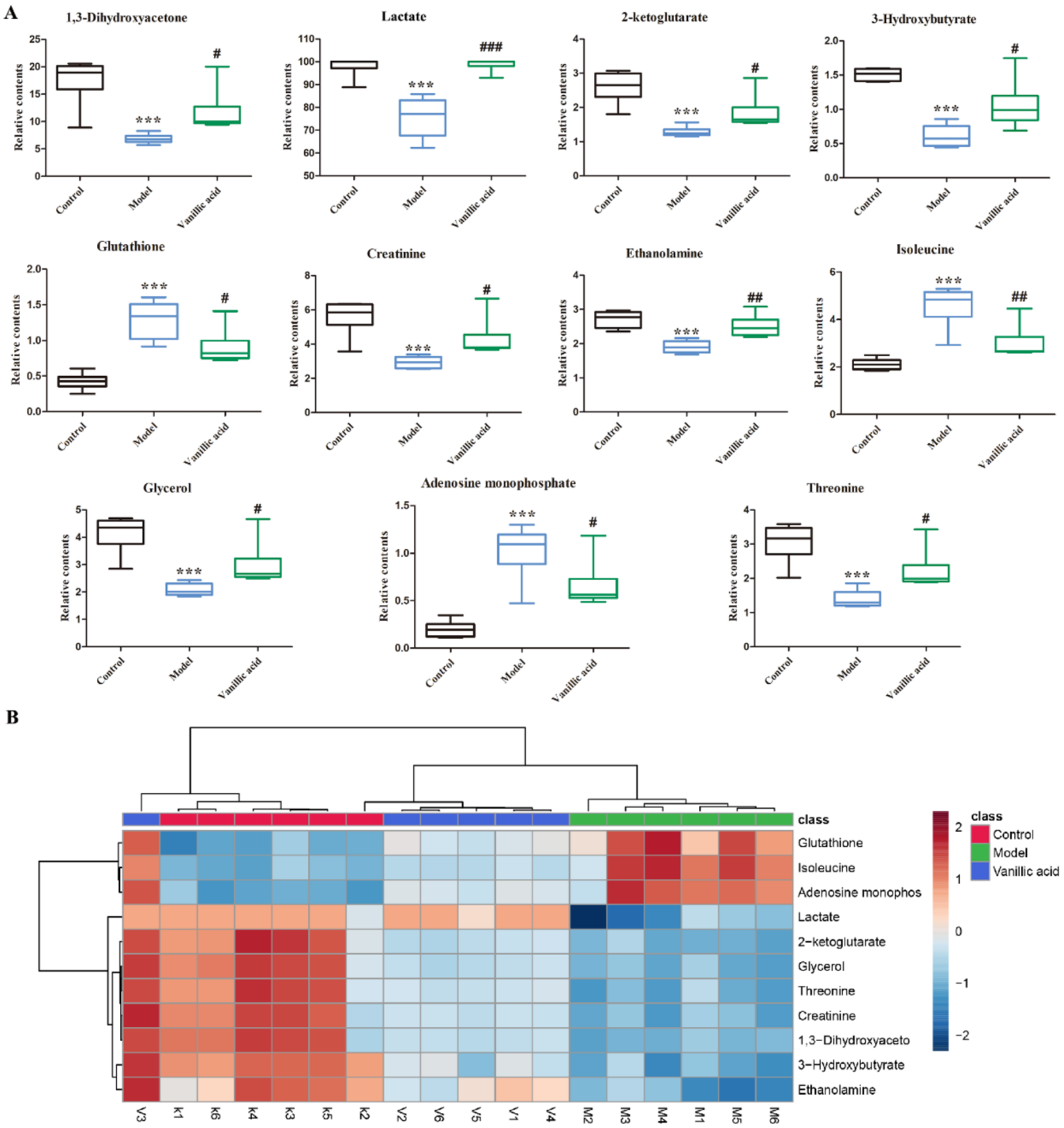


Figure 3. Differential metabolites affected by vanillic acid in corticosterone-induced PC12 cells (A). Heat map of the cluster analysis of differential metabolites (B). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with the corticosterone-induced PC12 cell model group. $^{***}p < 0.001$ compared with the control group. k1−k6 represents the control group, M1−M6 represents the model group, and v1−v6 represents the vanillic acid group.

> 1 and $p < 0.05$. A total of 17 and 15 differential metabolites were identified in control and corticosterone-induced PC12 cell model groups and corticosterone-induced PC12 cell and vanillic acid groups, respectively. Eleven potential differential metabolites were identified in total (Figure 3A).

Compared with those of the control group, the levels of glutathione, isoleucine, and adenosine monophosphate increased, while the levels of 1,3-dihydroxyacetone, lactate, 2-ketoglutarate, 3-hydroxybutyrate, creatinine, ethanolamine, glycerol, and threonine decreased in the corticosterone-induced PC12 cell model group (Figure 3A). Vanillic acid notably regulated the levels of differential metabolites compared to those of the corticosterone-induced PC12 cell model group. Heat map analysis of all differential metabolites among the control, model, and vanillic acid groups confirmed the recovery effect of vanillic acid (Figure 3B).

3.6. Correlation Analysis. Correlation coefficient analysis was performed to investigate the relation between differential metabolites in the control, corticosterone-induced PC12 cell model, and vanillic acid groups. The results suggested a strong

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

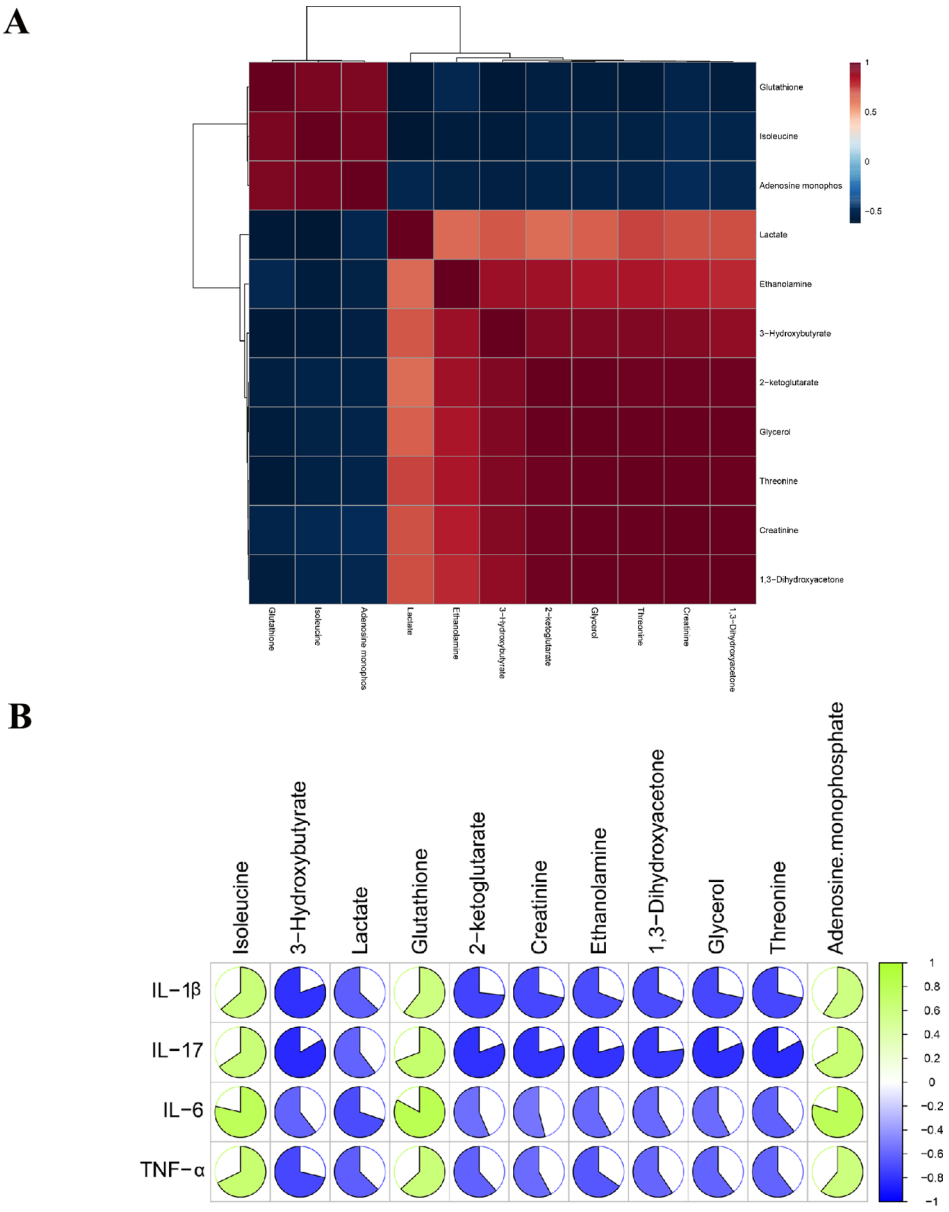


Figure 4. Correlation analysis results of differential metabolites (A) and correlation analysis of inflammatory factors and differential metabolites (B) by using metaboAnalyst 3.0.

positive correlation among glutathione, isoleucine, and adenosine monophosphate but a significant negative correlation with lactate, 3-hydroxybutyrate, and threonine, indicating that these metabolites are closely related but the relation between them still needs further explanation (Figure 4A).

For an in-depth examination of the relationship between varying metabolites and inflammation, an analysis was conducted on the Pearson correlation among the concentrations of these metabolites and inflammatory elements in the control, model, and vanillic acid groups. The results showed that glutathione, isoleucine, and adenosine monophosphate strongly correlate with four inflammatory factors, and other differential metabolites have a strong negative correlation with four inflammatory factors (Figure 4B).

3.7. Capturing Key Metabolites of Vanillic Acid Regulation Based on Machine Learning. Random forest, a common method in machine learning, was utilized to capture the key metabolites of vanillic acid regulation. The mean decrease accuracy metric evaluates the significance of differentiating a metabolite within a random forest. Altering a metabolite's value to a random figure results in a mean decrease in the precision of random forest predictions. As the value increases, so does the significance of the metabolite within a random forest. The results suggested that metabolites such as adenosine monophosphate, isoleucine, glutathione, and lactate have great differences among vanillic acid regulation in corticosterone-induced PC12 cell model groups (Figure 5). Martin et al. suggested that lactate can restore adult hippocampal neurogenesis in depression-like behavior model, and the antidepressant effect of lactate needs adult neurogenesis.[26,27] Through metabolomics of 5283 patients with depression, it is found that isoleucine is one of the metabolites closely related to depression.[20]

3.8. Metabolic Pathway Analysis. Analyzing pathways can yield biological insights into similar metabolites, enhancing our comprehension of vanillic acid's healing process.[27] The

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

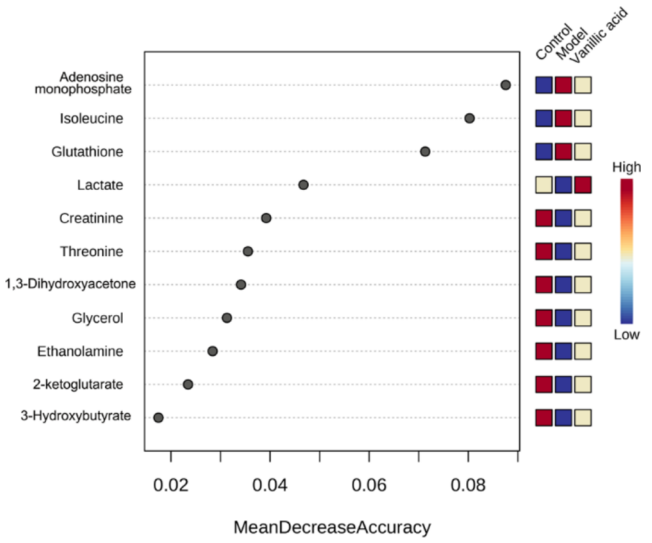


Figure 5. Eleven most important metabolites in random forests. Features were ranked by their contributions to classification accuracy (mean decrease accuracy).

four metabolic pathways most related to vanillic acid-protected corticosterone-induced PC12 cells were identified, including valine, leucine, and isoleucine biosynthesis, butanoate metabolism, glycerolipid metabolism, and synthesis and degradation of ketone bodies (Figure 6A). The metabolite enrichment analysis results further supplemented and supported the results of the metabolomics pathway analysis (Figure 6B).

3.9. Correlation Analysis of Immune-Related Genes and Differential Metabolites. The central peripheral dialogue on the abnormal interaction of "neuroimmune metabolism" is an important basis for the disorder of inflammatory immune mechanisms related to depression. To further explore the mechanism of vanillic acid regulating abnormal immune metabolism in depression, 2498 immune-related genes were obtained from the Immunology Database. The correlation coefficient was calculated between immune-related genes and differential metabolites. The results showed that immune-related genes such as IL2RB, APOBEC3G, and CCL15 have a strong positive correlation with isoleucine, immune-related genes such as IL2RB, APOBEC3G, and TRDV3 have a strong positive correlation with glutathione, and immune-related genes such as ORM1, DEFB112, and DEFB106B have a strong negative correlation with adenosine monophosphate (Table S2).

3.10. Targets and Metabolites for Regulating the Immunity−Metabolism of Vanillic Acid Identification Based on an Entropy Weight Algorithm and Validation. To identify the core targets and metabolites for regulating the immunity−metabolism of acid, a scoring model base entropy weight algorithm was designed, considering the correlation analysis of immune-related genes and differential metabolites. The score of core targets and metabolites for regulating the immunity−metabolism of vanillic acid was calculated (Figure 7A,B). According to the calculation results, the targets such as IL2RB, APOBEC3G, and IFNA13 and the metabolites such as lactate, ethanolamine, and 3-hydroxybutyrate were identified as the core targets and metabolites of vanillic acid regulating the immunity−metabolism of depression.

Furthermore, to validate the implication of the above identified metabolites in the effect of vanillic acid, we observed the effect on inflammatory cytokines by supplementing metabolites. The results suggested that the 3-hydroxybutyrate supplement could inhibit the level of TNF-α and IL-6 in a dose-dependent manner (Figure 7C,D), indicating that the immunity−metabolism regulation effect of vanillic acid was possibly mediated by upregulation of the 3-hydroxybutyrate level.

3.11. Vanillic Acid Modulates the IL2RB-JAK1-STAT3 Signaling Pathway in Corticosterone-Induced PC12 Cells. To clarify the mechanism of vanillic acid regulating the immunity−metabolism of depression, the expression of IL2RB and related signal pathway proteins was assessed. The results showed that corticosterone treatment observably upregulated the expression of IL2RB, and pretreatment with vanillic acid blocked these effects (Figure 7E). IL2RB is a receptor of JAK1/3. The JAK/STAT signaling pathway is a multieffect cell cascade, which may be involved in the pathophysiological process of depression. When PC12 cells were exposed to corticosterone, the expressions of pJAK1 and

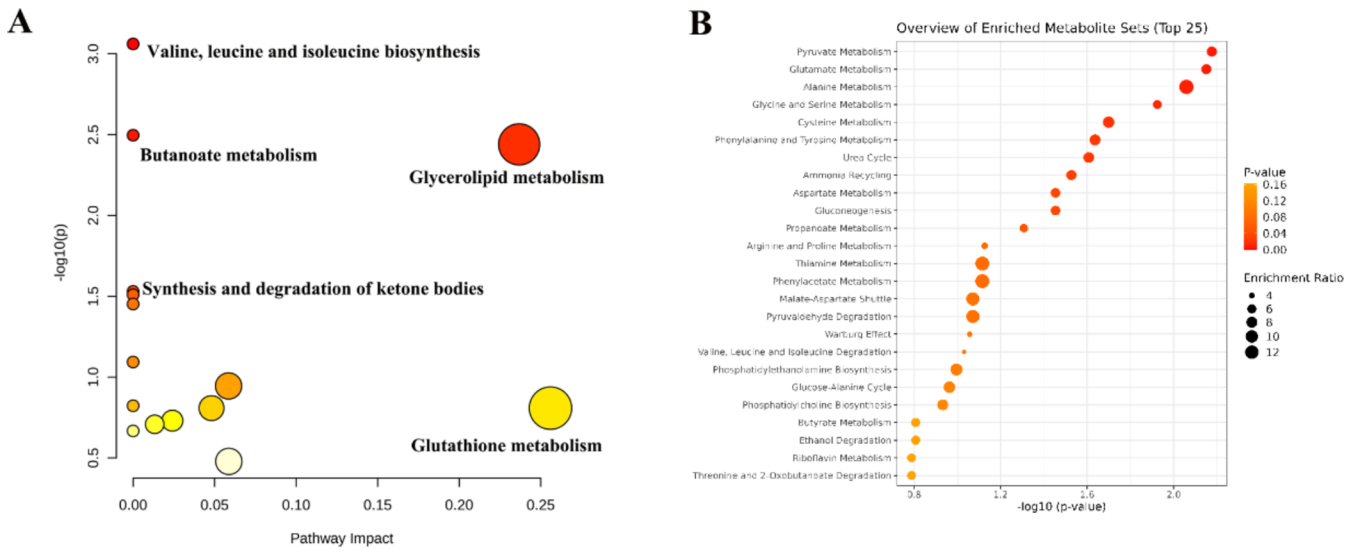


Figure 6. MetPA analysis of the metabolic pathway (A) and the enrichment of the metabolite set (B).

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

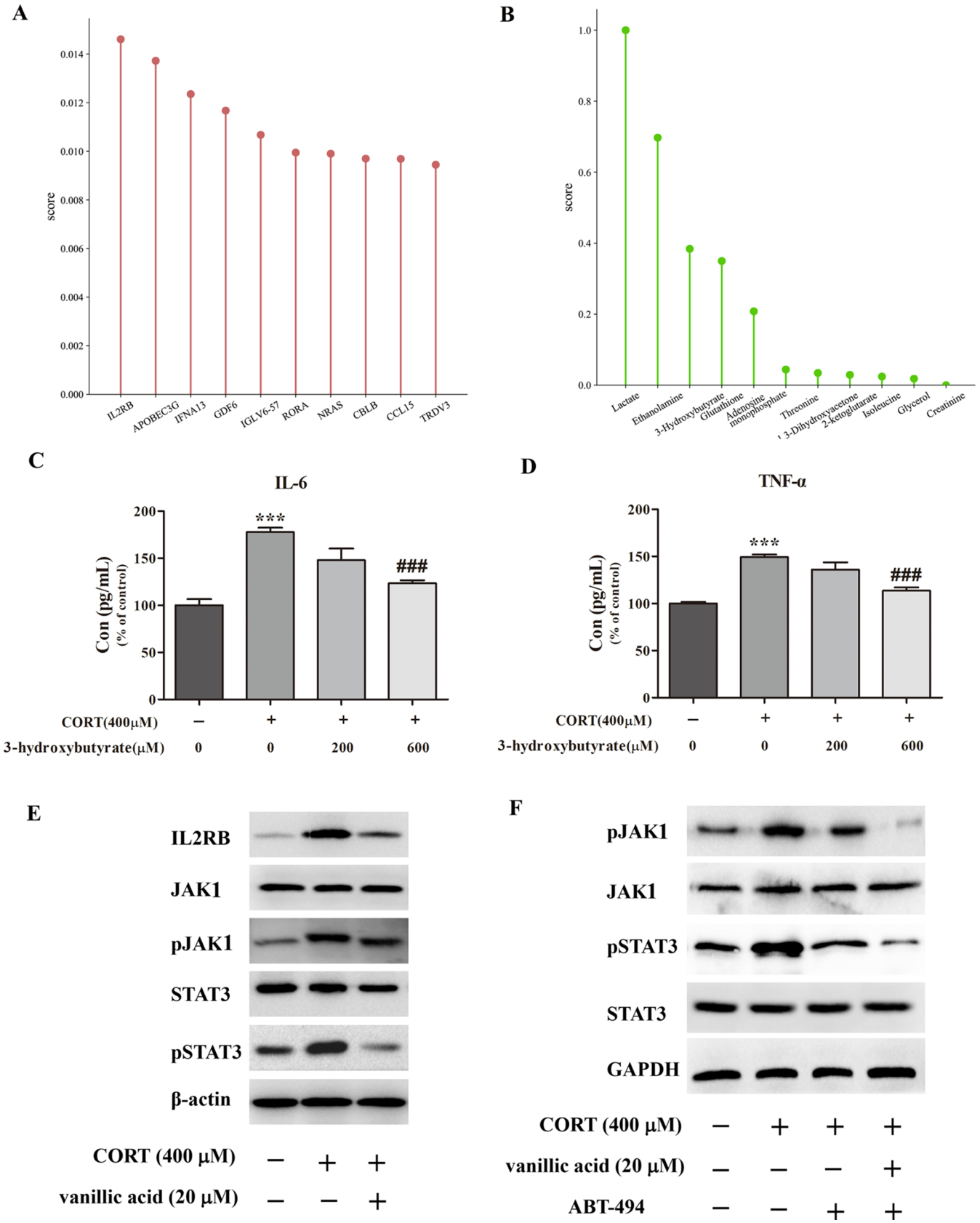


Figure 7. Value distribution of the entropy weight algorithm of each immune-related gene (A) and differential metabolite (B). The effect of 3-hydroxybutyrate on TNF-α (C) and IL-6 (D) levels in corticosterone-induced PC12 cells. Western blot analysis of IL2RB, JAK1, pJAK1, STAT3, and pSTAT3 of corticosterone-induced PC12 cells with or without ABT-494 (20 μM) at 24 h (n = 3) (E, F). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with the corticosterone-induced PC12 cell model group. $^{***}p < 0.001$ compared with the control group.

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

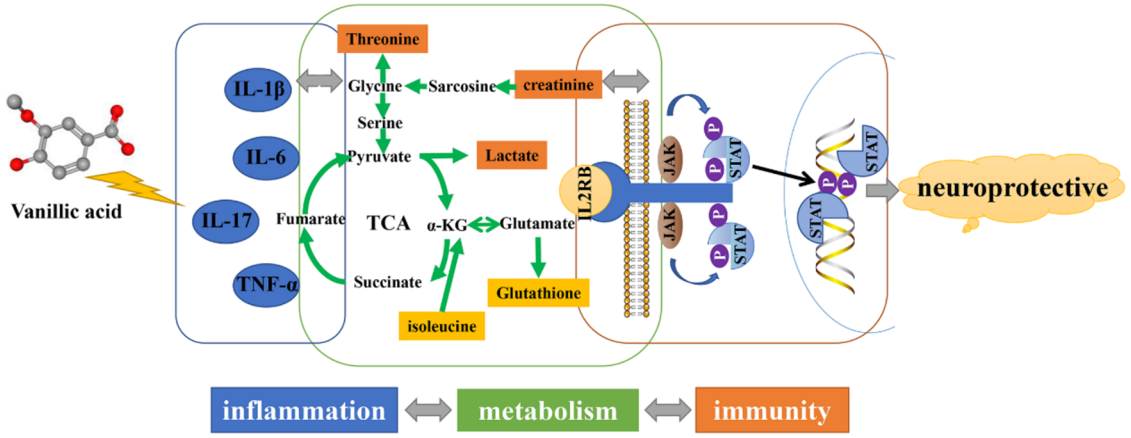


Figure 8. Schematic diagram indicates that vanillic acid exerts antidepressive effects by regulating inflammation−immunity−metabolism.

pSTAT3 were significantly upregulated. However, this upregulation was abolished by treatment with vanillic acid (Figures 7E and S2A−C). To validate the effect of vanillic acid on the JAK/STAT pathway, the JAK inhibitor ABT-494 was applied. Moreover, ABT-494 enhanced the inhibitory effect of vanillic acid on pJAK1 and pSTAT3 (Figures 7F and S2D−E). The aforementioned findings suggest that vanillic acid offers neuroprotection through suppression of the IL2RB-JAK1-STAT3 pathway.

4. DISCUSSION

Depression is one of the main mental diseases affecting people's everyday lives worldwide. Depression symptoms often lead to declining physical activity and daily social and psychological function.[2] It has been reported that food-derived ingredients can regulate the nervous system.[28] For example, the efficacy of vanillic acid in neuroprotection has recently been confirmed in an in vivo study.[29] In our study, cell viability and LDH release results indicated that vanillic acid possessed a neuroprotective effect.

Clinical and experimental studies have shown that depression is related to increased cytokines (such as IL-1β, IL-6, IL-17, and TNF-α) and the overactivity of HPA.[20] The central role of cytokines may also explain the hyperactivity of the HPA axis, which is often observed in depression, because proinflammatory cytokines may lead to the hyperactivity of the HPA axis by disturbing the negative feedback inhibition of circulating corticosteroids.[30] TNF-α is an inflammatory factor closely related to cell necrosis, apoptosis, and regeneration. Immune cells, glial cells, and neurons secrete TNF-α after being stimulated by inflammation. Studies have shown that mice with TNF receptor knockout cannot easily develop depression under stress.[31] IL-1β is a typical multifunctional inflammatory factor and a key factor causing inflammation and the immune response. The levels of IL-1β increased in the peripheral blood of rats with depression model, and the depression performance improved after injection with an IL-1β antagonist.[32] IL-6 is one of the main inflammatory factors that change during nerve injury. Clinical studies have found that the levels of IL-6 in the peripheral blood of patients with depression are significantly higher than those of normal people.[33] Our results indicated that vanillic acid could markedly suppress the inflammatory cytokine levels in corticosterone-induced PC12 cells.

Metabolomics quantitatively delineates how endogenous metabolites react to both internal and external factors, which is capable of directly mirroring the final and phenotypic data of living beings.[23] It has recently played an important role in drug research and development, gene function analysis, metabolic pathways, and regulatory mechanisms. Cell metabolomics results showed that the protective effect of vanillic acid on PC12 cells induced by corticosterone is related to 11 metabolites involving 6 metabolic pathways.

The entropy weight algorithm was utilized to calculate targets and metabolites for regulating the immunity−metabolism of vanillic acid. According to the calculation results, IL2RB is the top immune-related target. Western blotting further validated the protein expression of IL2RB. IL2RB is a receptor of JAK1/3. The JAK/STAT signaling pathway is common for many cytokines and growth factors to transmit cell signals. Recent studies have found that this pathway also plays an important role in nervous system diseases, including neurogenesis,[34] synaptic plasticity,[35] and microglial activation,[36] all related to emotional disorders' pathophysiology. In addition, the antidepressant effect of existing treatments has been proven to be mediated by the JAK/STAT correlation mechanism. Therefore, the JAK/STAT pathway may be involved in the pathophysiology of depression.[37] Phosphorylation of JAK may regulate the activity of STAT3 by phosphorylating tyrosine 705 and serine 727 residues on STAT3. In this study, the expression of JAK1, STAT3, and their phosphorylated proteins was detected by Western blotting. The results showed that p-JAK1 and p-STAT3 increased in the corticosterone-induced PC12 cell model, indicating that the JAK1/STAT3 pathway was activated. However, vanillic acid downregulates the expression of p-JAK1 and p-STAT3. JAK inhibitors further verified the above results.

5. CONCLUSIONS

Vanillic acid regulates metabolic disorders and inhibits the expression of the immune-related target IL2RB by down-regulating the level of inflammatory factors. It inhibits the activation of the JAK1/STAT3 signaling pathway, thus playing an antidepressant role by regulating the central peripheral dialogue with abnormal interactions of inflammation−immunity−metabolism (Figure 8).

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03050.

^{1}H NMR spectra and assignments of major metabolites of PC12 cells, statistical analysis of Western blot assay, and correlation coefficient of immune-related genes and differential metabolites (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Wenxia Gong − *Modern Research Center for Traditional Chinese Medicine, Shanxi University, Taiyuan 030006, China*; Email: gongwenxia@sxu.edu.cn

Kexin Wang − *Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China*; orcid.org/0000-0001-8237-0918; Email: nmgwkx1992@163.com

Authors

Tianxing Lv − *Institute of Infectious Diseases, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou 510440, China*

Daojin Xue − *The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China*

Peng Wang − *School of Pharmacy, Southern Medical University, Guangzhou 510515, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c03050

Author Contributions

#T.X.L. and D.J.X. contributed equally to this research equally. W.X.G., T.X.L., and K.X.W. provided the concept and designed the study. T.X.L., D.J.X., and P.W. conducted the experiments. T.X.L. and K.X.W. participated in data analysis, and T.X.L. wrote the manuscript. K.X.W. and W.X.G. contributed to revising and proof-reading the manuscript. All authors have read and approved the final manuscript.

Notes

Ethics Statements: The research did not include any human subjects and animal experiments.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was financially supported by the Startup Fundation for Doctoral Workstation of Guangzhou Eighth People's Hospital of Guangzhou Medical University (ZY10836), the China Postdoctoral Science Foundation (2022M721534) and the 13th Chaoyang Talent Project of Guangdong Provincial Hospital of Traditional Chinese Medicine (ZY2022YL28). Thanks to Dr. Xin Li, Central Laboratory of Southern Medical University, for providing us with technical guidance for metabolomics testing.

■ REFERENCES

(1) Bilu, Y.; Flaks-Manov, N.; Bivas-Benita, M.; Akiva, P.; Kalkstein, N.; Yehezkelli, Y.; Mizrahi-Reuveni, M.; Ekka-Zohar, A.; David, S. S. B.; Lerner, U.; et al. Data-Driven Assessment of Adolescents' Mental Health During the COVID-19 Pandemic. *J. Am. Acad. Child Adolescent Psychiatry* **2023**, *62* (8), 920−937.

(2) Krishnan, V.; Nestler, E. J. The molecular neurobiology of depression. *Nature* **2008**, *455* (7215), 894−902.

(3) Zhao, Z.; Zhang, L.; Guo, X. D.; Cao, L. L.; Xue, T. F.; Zhao, X. J.; Yang, D. D.; Yang, J.; Ji, J.; Huang, J. Y.; Sun, X. L. Rosiglitazone Exerts an Anti-depressive Effect in Unpredictable Chronic Mild-Stress-Induced Depressive Mice by Maintaining Essential Neuron Autophagy and Inhibiting Excessive Astrocytic Apoptosis. *Front Mol. Neurosci.* **2017**, *10*, 293.

(4) Wichers, M.; Maes, M. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. *Int. J. Neuropsychopharmacol.* **2002**, *5* (4), 375−388.

(5) Tian, J. S.; Liu, S. B.; He, X. Y.; Xiang, H.; Chen, J. L.; Gao, Y.; Zhou, Y. Z.; Qin, X. M. Metabolomics studies on corticosterone-induced PC12 cells: A strategy for evaluating an in vitro depression model and revealing the metabolic regulation mechanism. *Neurotoxicol. Teratol.* **2018**, *69*, 27−38.

(6) Wang, R.; Hu, X.; Liu, S.; Wang, J.; Xiong, F.; Zhang, X.; Ye, W.; Wang, H. Kaempferol-3-O-sophoroside (PCS-1) contributes to modulation of depressive-like behaviour in C57BL/6J mice by activating AMPK. *Br. J. Pharmacol.* **2024**, *181* (8), 1182−1202.

(7) Fu, H.; Liu, L.; Tong, Y.; Li, Y.; Zhang, X.; Gao, X.; Yong, J.; Zhao, J.; Xiao, D.; Wen, K.; Wang, H. The antidepressant effects of hesperidin on chronic unpredictable mild stress-induced mice. *Eur. J. Pharmacol.* **2019**, *853*, 236−246.

(8) Wang, H.; Zhou, X.; Huang, J.; Mu, N.; Guo, Z.; Wen, Q.; Wang, R.; Chen, S.; Feng, Z. P.; Zheng, W. The role of Akt/FoxO3a in the protective effect of venlafaxine against corticosterone-induced cell death in PC12 cells. *Psychopharmacology* **2013**, *228* (1), 129−141.

(9) Zeng, B.; Li, Y.; Niu, B.; Wang, X.; Cheng, Y.; Zhou, Z.; You, T.; Liu, Y.; Wang, H.; Xu, J. Involvement of PI3K/Akt/FoxO3a and PKA/CREB Signaling Pathways in the Protective Effect of Fluoxetine Against Corticosterone-Induced Cytotoxicity in PC12 Cells. *J. Mol. Neurosci.* **2016**, *59* (4), 567−578.

(10) Qin, Z.; Shi, D. D.; Li, W.; Cheng, D.; Zhang, Y. D.; Zhang, S.; Tsoi, B.; Zhao, J.; Wang, Z.; Zhang, Z. J. Berberine ameliorates depression-like behaviors in mice via inhibiting NLRP3 inflammasome-mediated neuroinflammation and preventing neuroplasticity disruption. *J. Neuroinflammation* **2023**, *20* (1), 54.

(11) Li, M.; Teng, H.; Sun, G.; Zhao, J.; Fan, M.; Zhao, Z.; Zhou, J.; Zhao, M. Transcriptome profiles of corticosterone-induced cytotoxicity reveals the involvement of neurite growth-related genes in depression. *Psychiatry Res.* **2019**, *276*, 79−86.

(12) Kovacs, D.; Kovacs, P.; Eszlari, N.; Gonda, X.; Juhasz, G. Psychological side effects of immune therapies: symptoms and pathomechanism. *Curr. Opin Pharmacol.* **2016**, *29*, 97−103.

(13) Zheng, X.; Wang, G. J.; Hao, H. P. Update on immune and metabolic dysregulation in major depressive disorder and the implications for drug intervention. *Yao Xue Xue Bao.* **2017**, *52* (1), 19−25.

(14) Sreedhar, R. V.; Roohie, K.; Venkatachalam, L.; Narayan, M. S.; Bhagyalakshmi, N. Specific pretreatments reduce curing period of vanilla (Vanilla planifolia) beans. *J. Agric. Food Chem.* **2007**, *55* (8), 2947−2955.

(15) Wang, Y. S.; Shen, C. Y.; Jiang, J. G. Antidepressant active ingredients from herbs and nutraceuticals used in TCM: pharmacological mechanisms and prospects for drug discovery. *Pharmacol. Res.* **2019**, *150*, No. 104520.

(16) Chuang, H. W.; Wei, I. H.; Lin, F. Y.; Li, C. T.; Chen, K. T.; Tsai, M. H.; Huang, C. C. Roles of Akt and ERK in mTOR-Dependent Antidepressant Effects of Vanillic Acid. *ACS Omega* **2020**, *5* (7), 3709−3716.

(17) Lin, H. Y.; Tsai, J. C.; Wu, L. Y.; Peng, W. H. Reveals of New Candidate Active Components in Hemerocallis Radix and Its Anti-Depression Action of Mechanism Based on Network Pharmacology Approach. *Int. J. Mol. Sci.* **2020**, *21* (5), 1868.

(18) Singh, J. C. H.; Kakalij, R. M.; Kshirsagar, R. P.; Kumar, B. H.; Komakula, S. S.; Diwan, P. V. Cognitive effects of vanillic acid against streptozotocin-induced neurodegeneration in mice. *Pharmaceutical Biol.* **2015**, *53* (5), 630−636.

(19) Gao, L.; Wang, K. X.; Zhang, N. N.; Li, J. Q.; Qin, X. M.; Wang, X. L. 1H Nuclear Magnetic Resonance Based Metabolomics Approach Reveals the Metabolic Mechanism of (−)-5-Hydroxy-equol against Hepatocellular Carcinoma Cells in Vitro. *J. Proteome Res.* **2018**, *17* (5), 1833−1843.
(20) Zhang, P.; Yang, M.; Zhang, Y.; Xiao, S.; Lai, X.; Tan, A.; Du, S.; Li, S. Dissecting the Single-Cell Transcriptome Network Underlying Gastric Premalignant Lesions and Early Gastric Cancer. *Cell Rep.* **2019**, *27* (6), 1934−1947.
(21) Gao, J.; Zhang, K.; Wang, Y.; Guo, R.; Liu, H.; Jia, C.; Sun, X.; Wu, C.; Wang, W.; Du, J.; Chen, J. A machine learning-driven study indicates emodin improves cardiac hypertrophy by modulation of mitochondrial SIRT3 signaling. *Pharmacol. Res.* **2020**, *155*, No. 104739.
(22) Wang, K.; Li, K.; Chen, Y.; Wei, G.; Yu, H.; Li, Y.; Meng, W.; Wang, H.; Gao, L.; Lu, A.; Peng, J.; Guan, D. Computational Network Pharmacology-Based Strategy to Capture Key Functional Components and Decode the Mechanism of Chai-Hu-Shu-Gan-San in Treating Depression. *Front Pharmacol.* **2021**, *12*, No. 782060.
(23) Hochman, E.; Taler, M.; Flug, R.; Gur, S.; Dar, S.; Bormant, G.; Blattberg, D.; Nitzan, U.; Krivoy, A.; Weizman, A. Serum claudin-5 levels among patients with unipolar and bipolar depression in relation to the pro-inflammatory cytokine tumor necrosis factor-alpha levels. *Brain Behav. Immun.* **2023**, *109*, 162−167.
(24) Peng, Y.; Zhang, Z.; He, L.; Li, C.; Liu, M. NMR spectroscopy for metabolomics in the living system: recent progress and future challenges. *Anal. Bioanal. Chem.* **2024**, *416* (9), 2319−2334.
(25) Gouveia, G. J.; Head, T.; Cheng, L. L.; Clendinen, C. S.; Cort, J. R.; Du, X.; Edison, A. S.; Fleischer, C. C.; Hoch, J.; Mercaldo, N.; Pathmasiri, W.; Raftery, D.; Schock, T. B.; Sumner, L. W.; Takis, P. G.; Copié, V.; Eghbalnia, H. R.; Powers, R. Perspective: use and reuse of NMR-based metabolomics data: what works and what remains challenging. *Metabolomics* **2024**, *20* (2), 41.
(26) Carrard, A.; Cassé, F.; Carron, C.; Burlet-Godinot, S.; Toni, N.; Magistretti, P. J.; Martin, J. L. Role of adult hippocampal neurogenesis in the antidepressant actions of lactate. *Molecular Psychiatry* **2021**, *26* (11), 6723−6735.
(27) Bot, M.; Milaneschi, Y.; Al-Shehri, T.; Amin, N.; Garmaeva, S.; Onderwater, G. L. J.; Pool, R.; et al. ... BBMRI-NL Metabolomics Consortium. Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls. *Biological Psychiatry* **2020**, *87* (5), 409−418.
(28) Li, X.; Qin, X.; Tian, J.; Gao, X.; Wu, X.; Du, G.; Zhou, Y. Liquiritin protects PC12 cells from corticosterone-induced neurotoxicity via regulation of metabolic disorders, attenuation ERK1/2-NF-κB pathway, activation Nrf2-Keap1 pathway, and inhibition mitochondrial apoptosis pathway. *Food Chem. Toxicol.* **2020**, *146*, No. 111801.
(29) Xiao, H. H.; Gao, Q. G.; Zhang, Y.; Wong, K. C.; Dai, Y.; Yao, X. S.; Wong, M. S. Vanillic acid exerts oestrogen-like activities in osteoblast-like UMR 106 cells through MAP kinase (MEK/ERK)-mediated ER signaling pathway. *J. Steroid Biochem Mol. Biol.* **2014**, *144*, 382−391.
(30) Horowitz, M. A.; Cattaneo, A.; Cattane, N.; Lopizzo, N.; Tojo, L.; Bakunina, N.; Musaelyan, K.; Borsini, A.; Zunszain, P. A.; Pariante, C. M. Glucocorticoids prime the inflammatory response of human hippocampal cells through up-regulation of inflammatory pathways. *Brain Behav. Immun.* **2020**, *87*, 777−794.
(31) Jiang, N.; Lv, J.; Wang, H.; Huang, H.; Wang, Q.; Lu, C.; Zeng, G.; Liu, X. M. Ginsenoside Rg1 ameliorates chronic social defeat stress-induced depressive-like behaviors and hippocampal neuroinflammation. *Life Sci.* **2020**, *252*, No. 117669.
(32) Won, W.; Choi, H. J.; Yoo, J. Y.; Kim, D.; Kim, T. Y.; Ju, Y.; Park, K. D.; Lee, H.; Jung, S. Y.; Lee, C. J. Inhibiting peripheral and central MAO-B ameliorates joint inflammation and cognitive impairment in rheumatoid arthritis. *Exp. Mol. Med.* **2022**, *54* (8), 1188−1200.
(33) Lu, J.; Jin, K.; Jiao, J.; Liu, R.; Mou, T.; Chen, B.; Zhang, Z.; Jiang, C.; Zhao, H.; Wang, Z.; et al. (YY1) Yin-Yang 1, a transcription factor regulating systemic inflammation, is involved in cognitive impairment of depression. *Psychiatry Clin. Neurosci.* **2023**, *77* (3), 149−159.
(34) Kanski, R.; van Strien, M. E.; van Tijn, P.; Hol, E. M. A star is born: new insights into the mechanism of astrogenesis. *Cell. Mol. Life Sci.* **2014**, *71* (3), 433−447.
(35) Nicolas, C. S.; Peineau, S.; Amici, M.; Csaba, Z.; Fafouri, A.; Javalet, C.; Collett, V. J.; Hildebrandt, L.; Seaton, G.; Choi, S. L.; et al. The Jak/STAT pathway is involved in synaptic plasticity. *Neuron* **2012**, *73* (2), 374−390.
(36) Henrik Heiland, D.; Ravi, V. M.; Behringer, S. P.; Frenking, J. H.; Wurm, J.; Joseph, K.; Garrelfs, N. W. C.; Strähle, J.; Heynckes, S.; Grauvogel, J.; et al. Tumor-associated reactive astrocytes aid the evolution of immunosuppressive environment in glioblastoma. *Nat. Commun.* **2019**, *10* (1), No. 2541.
(37) Kim, H.; Chen, L.; Lim, G.; Sung, B.; Wang, S.; McCabe, M. F.; Rusanescu, G.; Yang, L.; Tian, Y.; Mao, J. Brain indoleamine 2,3-dioxygenase contributes to the comorbidity of pain and depression. *J. Clin. Invest.* **2012**, *122* (8), 2940−2954.

https://doi.org/10.1021/acsomega.4c03050
ACS Omega 2024, 9, 40456−40467