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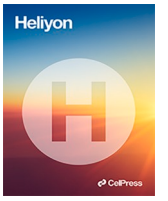

Research article

Pharmacological mechanism of Shaoyao Gancao Decoction in the treatment of depression based on bioinformatics and animal experiment

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ABSTRACT

In this study, the pathogenic genes of depression were calculated and analyzed by bioinformatics method, and then the key genes of Shaoyao Gancao Decoction in the treatment of depression were deduced and predicted through the correlation study with the target of Shaoyao Gancao Decoction. Through the production of LPS depression model mice, drug treatment, behavioral test and hippocampal tissue sample detection, it was found that Shaoyao Gancao Decoction can regulate the levels of IL-10, TNF- α, BDNF, SMAD3, FGFR1 and FGFR2 to improve depression, which can provide a theoretical basis for exploring the efficacy of Shaoyao Gancao Decoction in the treatment of depression.

1. Introduction

Depression is defined by ongoing negative thoughts and emotions that interfere with mood, cognition, motivation, and behavior. It is the top cause of disability globally, impacting more than 280 million individuals [1], and it is currently the second most common cause of disability globally, following cancer, but it is expected to become the most common by 2030 [2]. It is an important factor affecting global healthcare and the economy [3,4]. Depression not only affects an individual's mental and psychological health, but also disrupts other systems, such as the heart, kidneys, nervous system and immune system [5]. The specific manifestations of this condition include depressive symptoms, diminished interest in activities, fatigue, impaired functioning, sleep disturbances, and psychomotor disorders [6,7]. Consequently, these symptoms can significantly impact patients' productivity, self-awareness, and self-esteem while also compromising their overall quality of life and potentially increasing the risk of suicide."At present, depression has been widely studied, but its pathophysiological mechanism is still poorly known. there are many kinds of antidepressants in clinic, such as selective serotonin reuptake inhibitors, serotonin norepinephrine reuptake inhibitors and so on. But, these drugs often take several weeks of treatment to achieve therapeutic effect, and may also be ineffective [8]. Some studies have shown that antidepressants are less effective or even ineffective in 30–40 % of patients with refractory depression [9].

In this scenario, Traditional Chinese medicine (TCM) is gaining popularity among patients looking for more effective treatment, as

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it is regarded as an important component of complementary and alternative medicine [10]. TCM has a clinical history of thousands of years with the treatment concept of low toxicity, high efficiency and integrated treatment. More and more clinical evidence shows that traditional Chinese medicine is effective in the treatment of depression [11–14]. Shaoyao Gancao Decoction, a classic Chinese medicine formula, derived from Treatise on Febrile Diseases and included in the "Catalogue of Ancient Classic Prescriptions (First Batch)" released by the State Administration of Traditional Chinese Medicine of China. This medicinal formulation, comprising Shaoyao and Gancao, is believed to possess promising therapeutic potential for the treatment of depression.

With the deepening of biomedical basic research, such as the development of metabolomics, the amount of medical and pharmaceutical data has become extremely large, and the processing and analysis of these data has become a major challenge. Bioinformatics is a method for comprehensive analysis of big data [15].

In this study, We used bioinformatics methods to explore and study the potential targets of Shaoyao Gancao Decoction in the treatment of depression, and conducted animal experiments to verify the findings(Fig. 1). This work was expected to contribute to exploring the pathogenic mechanism of depression and the therapeutic principle of Shaoyao Gancao Decoction.

2. Materials and methods

2.1. Data collection

Depression gene expression datasets containing GSE98793, GSE81761 and GSE92538 were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Therapeutic target information of Shaoyao Gancao Decoction came from the TCMSID database (https://tcm.scbdd.com/) [16].

2.2. Differentially expressed genes

Initially, data quality checks and log conversion normalization were carried out to remove any outliers. The 'limma' package in R software was utilized to compare DEGs between the depression and control groups [17]. A p-value of less than 0.05 was established as the threshold for determining statistical significance. The volcano plot of DEGs was created using R software.

2.3. Weighted gene Co-expression network analysis

WGCNA was utilized to construct a co-expression network in DEGs based on the scale-free topology principles [18]. Initially, all DEGs were assessed using the WGCNA package in R software to determine the best soft threshold power. Subsequently, a weighted co-expression network was created to categorize DEGs into separate modules, each labeled with a unique color. Finally, the correlation between each module and DEGs was investigated, and the module demonstrating the strongest correlation with depression was selected for enrichment analysis.

2.4. Functional enrichment analysis

The Metascape database (http://metascape.org) enabled a deeper understanding of the biological significance of Differentially Expressed Genes (DEGs) through Gene Ontology enrichment analysis. Pathway enrichment analysis utilized the Molecular Signatures Database (MSigDB) Hallmark Gene Sets and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway. GSEA analysis was conducted using the GSEA software with a significance criteria of a P-value <0.05.

2.5. Identification of key genes

First, download the target genes of Shaoyao Gancao Decoction from TCMSID database, intersect with the target genes obtained from the previous GSEA analysis, and then use the STRING(https://cn.string-db.org/) to make the Protein-Protein Interaction(PPI) network diagram to identify the key proteins.

2.6. Gene ontology and pathway enrichment analysis

Metascape(San Diego, CA, USA, v3.5) is an effective method to study the potential biological processes and related pathways of transcriptome and genome data [19]. In the key module, Metascape was used for gene ontology (GO) analysis [20] and KEGG pathway analysis [21–23].

2.7. Evaluation of inflammatory

CIBERSORT, a widely used analytical tool, is a deconvolution algorithm. A linear support vector regression method was employed to denoise the bulk gene expression matrix through machine learning techniques [24]. This process was conducted utilizing the CIBERSORT package in R. In cases where CIBERSORT output was less than 0.05, the relationship between candidate diagnostic biomarkers and inflammation was examined using the Spearman correlation coefficient in R with the help of the 'reshape2' and 'ggExtra' functions.

2.8. Animal experiments

2.8.1. Animal

Female C57BL/6J mice were kept in cages measuring 32 cm × 16 cm × 16 cm in a controlled environment with lighting from 7:00 a.m. to 7:00 p.m. and with unrestricted access to food and water. The ambient temperature and relative humidity were kept at 22 °C ± 2 °C and 55 % ± 5 %, respectively. The experiments followed the guidelines of the Administration Office of Laboratory Animals in Beijing, China and were approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University.

2.8.2. Drug treatments and experimental design

24 mice were randomly divided into 3 groups (8 mice in each group): Con group, LPS groups (2 mg/kg/d, ig) and SYGC group (Shaoyao Gancao Decoction,3 g/kg/d, ig). The experimental flow is shown in Fig. 7A.

2.8.3. Behavioral test

After the administration, conduct the behavioral tests, including sucrose preference test(SPT), open field test(OFT) and elevated plus maze test(EPMT). The operation method was as usual [25].

2.8.4. Brain tissue sample collection

Hippocampal tissues from each group (Con, LPS, and SYGC groups) were collected for Western blot and ELISA. Following cervical dislocation, the mice were sacrificed and their entire brains were removed and kept on ice. The hippocampi were subsequently separated from the brains, placed in Eppendorf tubes, and preserved at −80 °C for future use.

2.9. Sample detection

The sample of hippocampus was detected by ELISA and Western blot. IL-10, TNF- α and BDNF were tested by ELISA. Determination of TP53 FGFR1, FGFR2, SMAD3 by Western blot. ELISA kit was from Nanjing Boyan Biotechnology Co., Ltd. The reagent used by Western bolt came from Abways Technology,Inc.

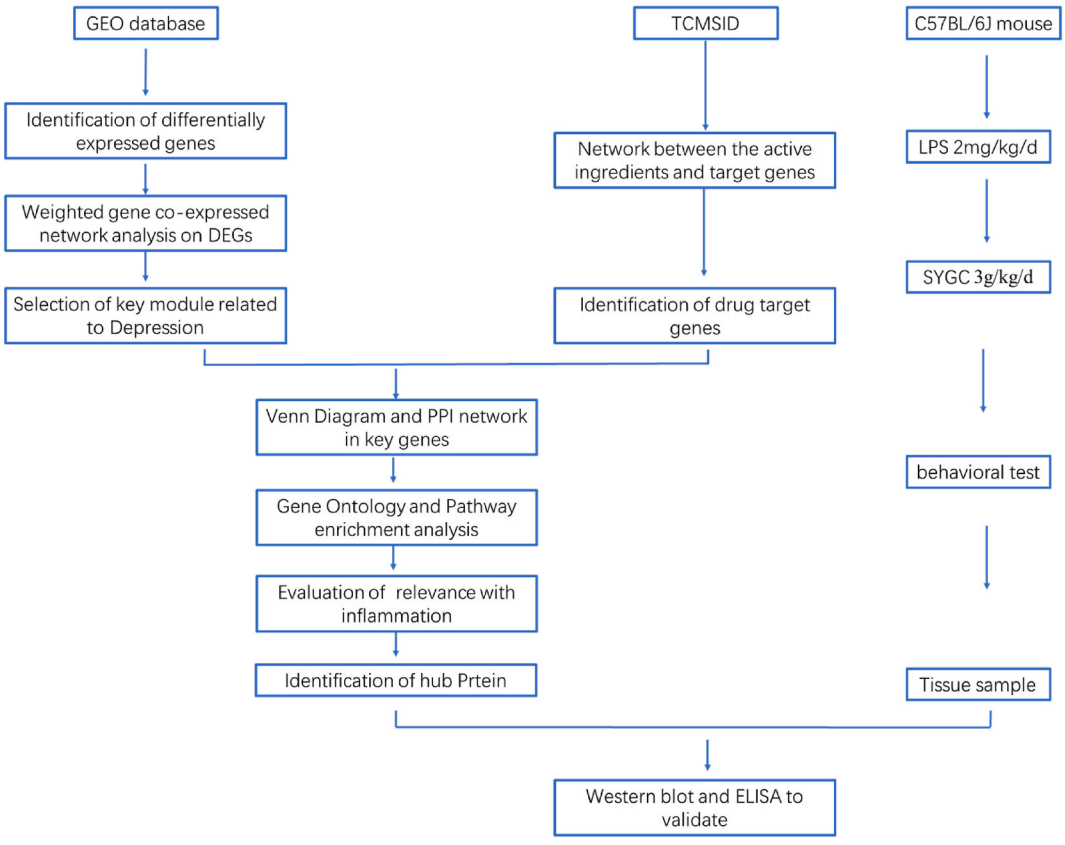

Fig. 1. Workflow chart of the present study.

2.10. Statistical analysis

One-way ANOVA and Tukey's post hoc test were utilized to analyze all results with GraphPad Prism 9 software from GraphPad Software Inc., USA.

3. Results

3.1. Workflow chart

The workflow chart has been shown in Fig. 1.

3.2. Identifications of differentially expressed Genes(DEGs)

We analyzed a total of 10,890 genes from the three GSE datasets and identified 418 differentially expressed genes (DEGs) with an adjusted p-value<0.05 between depression patients and controls. Among these DEGs, 225 genes were upregulated and 193 genes were downregulated in depression. The volcano map of the DEGs is shown in Fig. 2A, while the heatmap for the top 50 DEGs is displayed in Fig. 2B.

3.3. WGCNA analysis

The 418 identified DEGs were further processed with the WGCNA package in R software, and a scale-free co-expression network (scale-free $R^2 > 0.9$) was established using a soft thresholding power of 8 (Fig. 3A) and had a relatively good average connectivity. The DEGs were clustered into 7 modules—blue, brown, green, grey, red, turquoise and yellow—with a minimal module size≥20. The cluster dendrogram of the DEGs is shown in Fig. 3B. The correlation between each module and depression was calculated and plotted (Fig. 3C). The results indicated that yellow(0.31, $p < 0.0001$) were the most modules related to depression, respectively. Herein, the yellow module, including 216 DEGs, was considered as a key module correlated to depression.

3.4. Functional enrichment analysis

GSEA results showed DEGs enriched in "Alanine, aspartate and glutamate metabolism", "Bacterrila invasion of epithelial cells", "ECM-receptor onterraction", "Ferroptosis", "IL-17 signaling pathway", "Nicotine addiction", "Nitrogen metabolism", "Proteasome", "Renin-angiotensin system", "Terpenoid back hone biosynthesis "(Fig. 4A). "Gliogenesis", "myelination", "glial cell differentiation", ensheathment of neurons" etc. were resulted by GO analysis(Fig. 4B). And KEGG analysis meant that "P13K-Akt signaling pathway", "MAPK signaling pathway" etc. were significant(Fig. 4C).

3.5. Identification of key genes

From the TCMSID database, 801 therapeutic targets of Shaoyao Gancao Decoction have been extracted. By intersecting these 801 genes with 216 key depression genes analyzed by WCGNA, 22 common genes were obtained(Fig. 5A,Table 1).Then the PPI network diagram showed TP53, FGFR1, FGFR32 and SMAD3 etc. were the genes with greater weight(Fig. 5B).

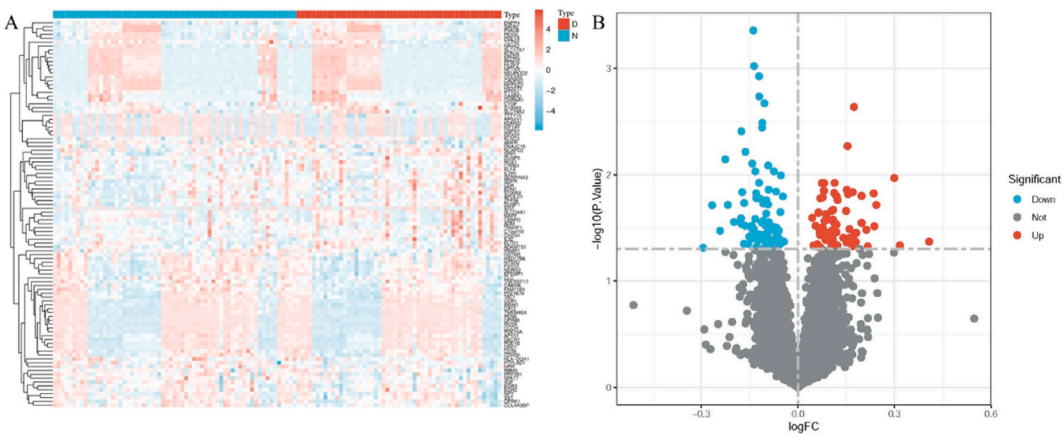

Fig. 2. Expression profile of DEGs. (A) Heatmap of DEGs. (B) Volcano map of DEGs expression levels.

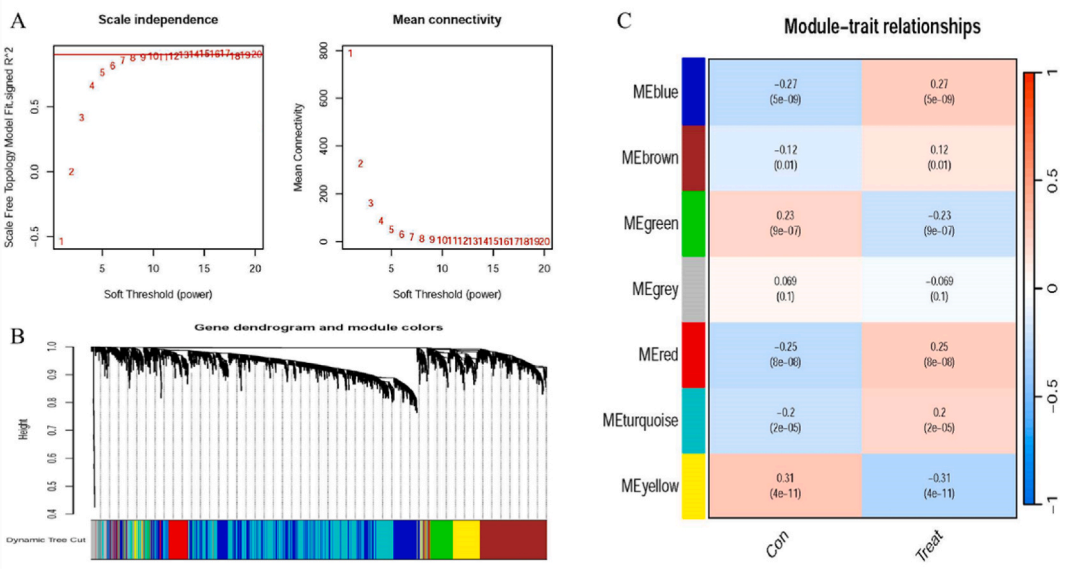

Fig. 3. WGCNA of DEGs. (A) Estimation of the soft thresholding value for a scale-free co-expression network. (B) Cluster dendrogram of all DEGs. (C) Correlation between each module and Control or Treat.

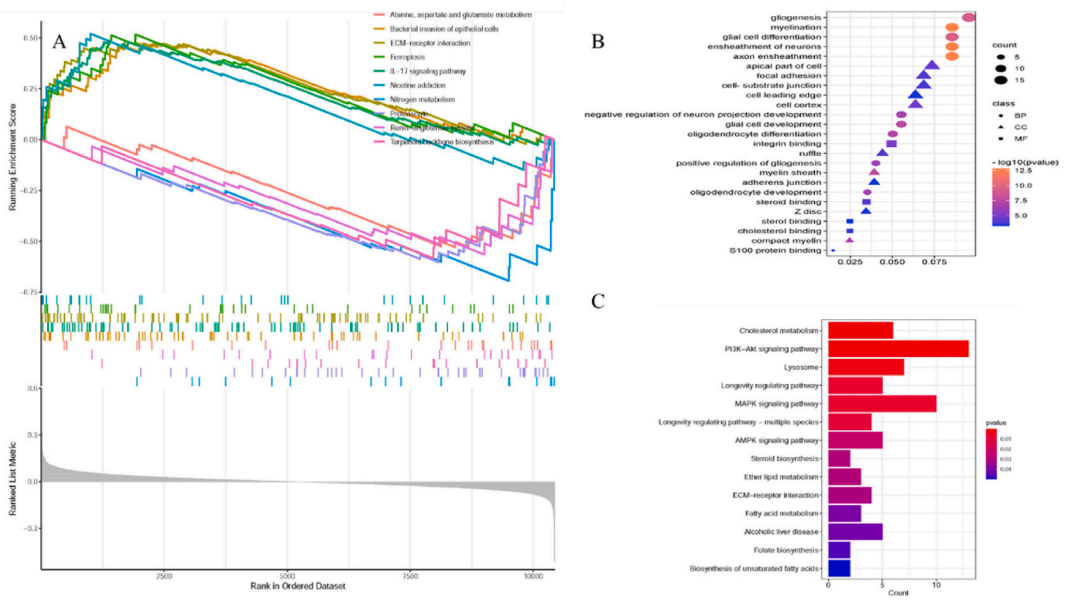

Fig. 4. GSEA enrichment analysis of DEGs between the Control and Treat in merge data cohort. (A) GSEA enrichment analysis results in Control and Treat. (B) GO analysis.(C)KEGG analysis.

3.6. Immune cells are changed significantly between two stages by immune cell infiltration analysis

Immune cell infiltration between the Control and Treat (Fig. 6A and. B). The results showed that the degree of infiltration of plasma cells ($P < 0.001$), T cells CD4 native ($P < 0.001$), T cells follicular helper($P < 0.001$) were notably raised in Treat samples in contrast to Control samples. Going a step further, we analyzed the correlation between 6 key genes which from previous step and immune cells (Fig. 6C).

3.7. Animal experiments

After 7 days of adaptive feeding to mice, LPS group and SYGC group were intraperitoneally injected with LPS (2 mg/kg/d), while

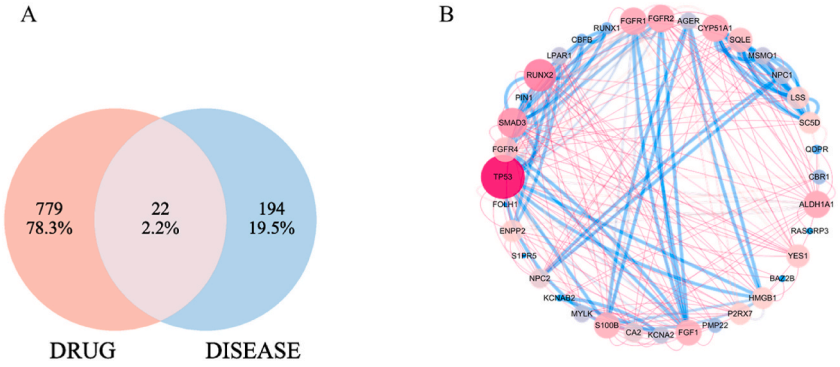

Fig. 5. Identification of key genes. (A) Venn diagram of 22 genes intersected by DRUG genes and DISEASE genes. (B) PPI network.

Table 1
Detailed information of 22 genes.

No.	Uniprot ID	Gene symbol	Protein name
1	P16152	CBR1	Carbonyl reductase [NADPH] 1
2	P48449	LSS	Lanosterol synthase
3	P16389	KCNA2	Potassium voltage-gated channel subfamily A member 2
4	Q9UP65	PLA2G4C	Cytosolic phospholipase A2 gamma
5	Q15746	MYLK	Myosin light chain kinase, smooth muscle
6	Q13951	CBFB	Core-binding factor subunit beta
7	Q9H228	S1PR5	Sphingosine 1-phosphate receptor 5
8	P09417	QDPR	Dihydropteridine reductase
9	Q99572	P2RX7	P2X purinoceptor 7
10	P07947	YES1	Tyrosine-protein kinase Yes
11	Q8IV61	RASGRP3	Ras guanyl-releasing protein 3
12	P05230	FGF1	Fibroblast growth factor 1
13	Q04609	FOLH1	Glutamate carboxypeptidase 2
14	O15118	NPC1	NPC intracellular cholesterol transporter 1
15	Q13526	PIN1	Peptidyl-prolyl *cis*-trans isomerase NIMA-interacting 1
16	P04271	S100B	Protein S100–B
17	P00352	ALDH1A1	Aldehyde dehydrogenase 1A1
18	Q01453	PMP22	Peripheral myelin protein 22
19	Q9UIF8	BAZ2B	Bromodomain adjacent to zinc finger domain protein 2B
20	Q92633	LPAR1	Lysophosphatidic acid receptor 1
21	Q13822	ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2
22	P00918	CA2	Carbonic anhydrase 2

Con group was simultaneously given physiological saline. Afterwards, the SYGC group was orally administered with Shaoyao Gancao Decoction(3 g/kg/d), while the other two groups were given corresponding physiological saline, perform this operation continuously for 10 days. Then conduct the behavioral tests-SPT, OFT and EPMT(Fig. 7A). Compared with the Con group, the performance of all tested LPS groups was significantly worse, and after treatment with Shaoyao Gancao Decoction, the behavioral data improved significantly(Fig. 7B–. C, Fig. 7D).

3.8. Sample test

Immune cell infiltration analysis shown immune cells are changed significantly between depression and treat group. So we detected inflammation related characteristic factors in hippocampal tissue, including IL-10, TNF-α by ELISA. Also we detected the content of BDNF at the same time. We seed that, compared to Con group, the content of IL-10 and BDNF descended in the LPS group and rebounded in the SYGC group. What's more, TNF-α gone up in LPS group and subside in SYGC group(Fig. 8A).

From the results of GO and KEGG analysis of the key genes, FGFR2, SMAD3, TP53 and FGFR1 are the most important key proteins. So this study used Western blot to detect the levels of these proteins in hippocampal tissue samples. As show in Fig. 8B, FGFR1 descended in the LPS group and rebounded in the SYGC group. And, FGFR2, SMAD3 and TP53 gone up in LPS group and subside in SYGC group.

4. Discussion

In this study, We screened the genes related to depression by bioinformatics method, analyzed and predicted the possible target protein of Shaoyao Gancao Decoction in the treatment of depression through a series of algorithms, and verified it by animal

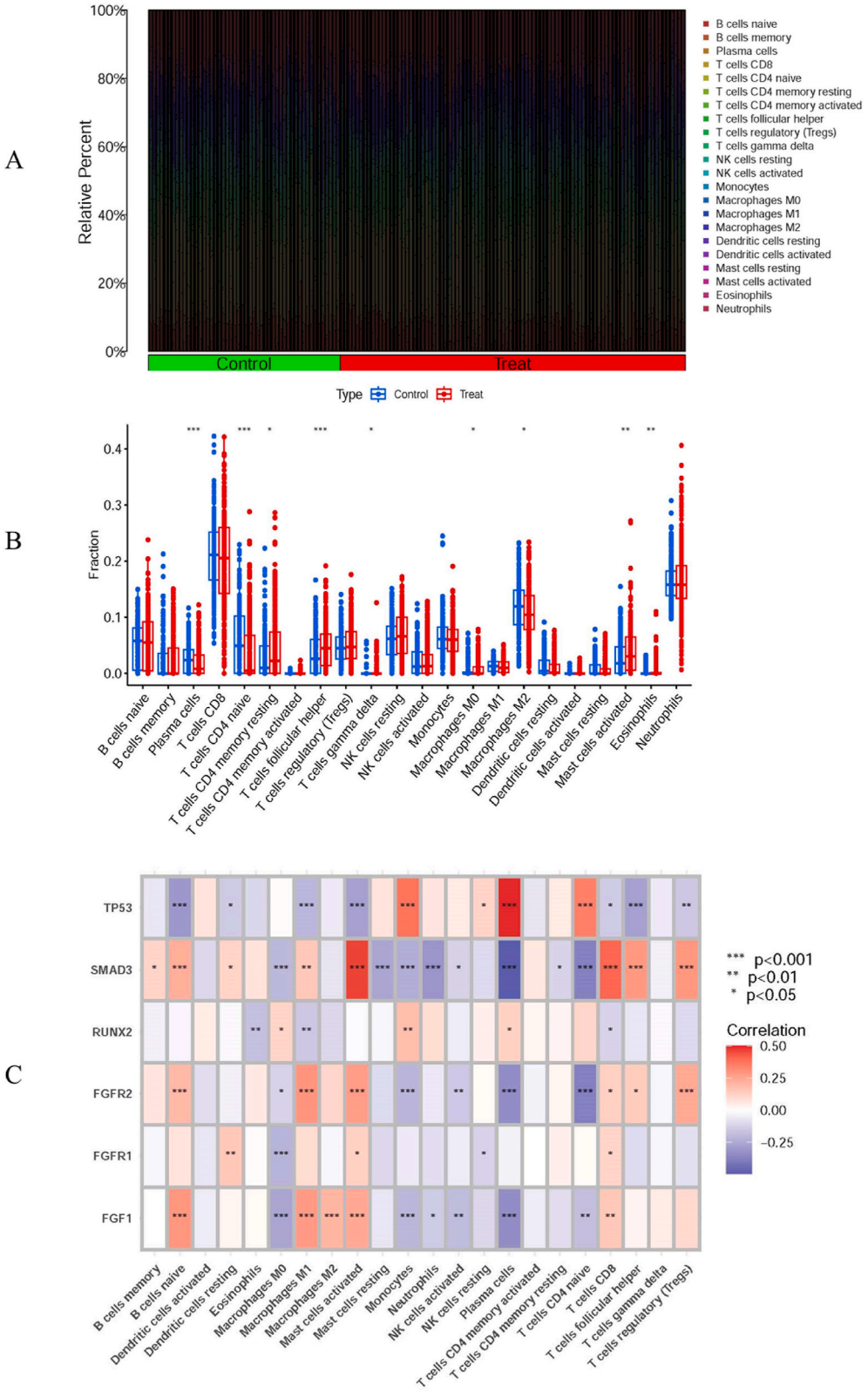

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Fig. 6. Evaluation and visualization of immune cell infiltration between the Control and Treat in merge data cohort. (A) The proportion of infiltrating immune cells in the Control and Treat samples. (B) Boxplot of differential expression between the Control and Treat in merge data cohort. *P < 0.05, **P < 0.01, **P < 0.001.(Blue in the boxes represents the Control and red represents the Treat. (C) Correlation between 6 key genes and immune cells. The darker the color, the stronger the correlation.

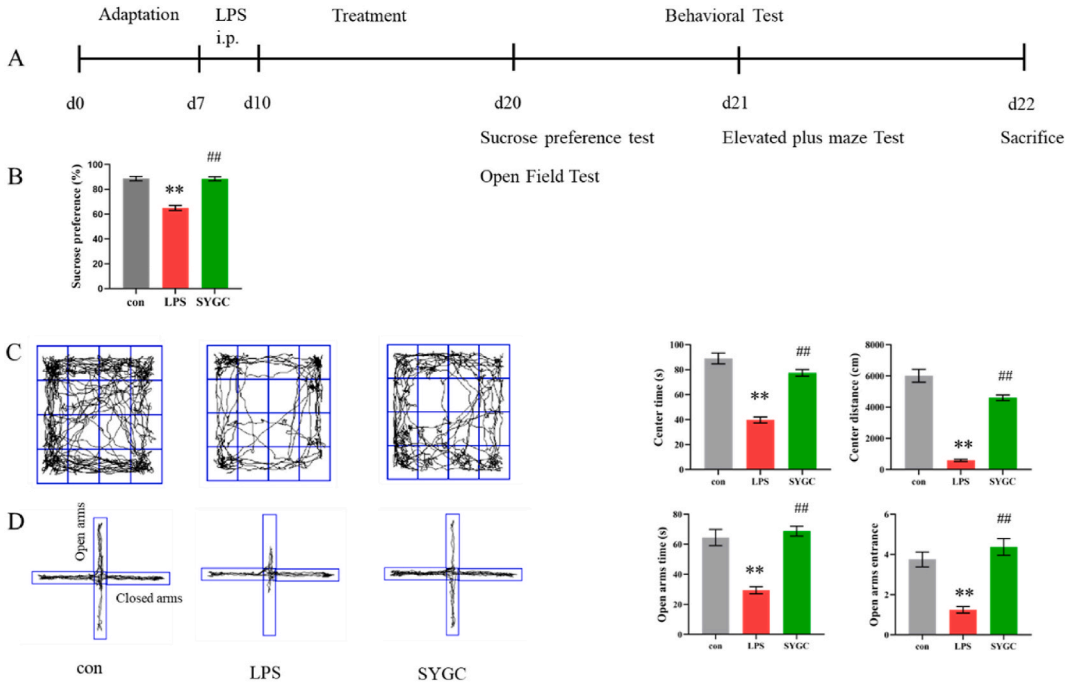

Fig. 7. LPS led mice to express depression-like behaviors and Shaoyao Gancao Decoction can improve. (A)Timeline for the animal experiments. (B) The result of SPT (C) OFT and center time, center distance in the OFT (D) EPMT diagram and open arms time, open arms entrance in the EMPT.The data are presented as mean ± SEM. n = 8 mice per group. One-way ANOVA; **P < 0.01 vs Con. ##P < 0.01 vs LPS. Experimenters were blinded to the treatment.

experiments. These works indicated that the pathogenic genes of depression were closely related to inflammation and some other pathways, and that the therapeutic target of Shaoyao Gancao Decoction was most likely to be FGFR2, SMAD3, TP53 and FGFR1.

From the GES database, we retrieved three gene sets and analyzed a total of 10890 genes. Through bioinformatics data analysis methods such as WCGDA, GSEA, KEGG, GO, etc., we confirmed 216 key genes closely related to depression. By intersecting with drug target data obtained from the TCMSID database, we obtained 22 possible target genes for the treatment of depression with Shaoyao Gancao Decoction, Finally, we validated the role of the top 4 genes in the therapeutic effect of Shaoyao Gancao Decoction on depression through animal experiments.

The role of the brain's innate immune system in the development of neuropsychiatric diseases has been the focus of research in this field [26–28], this study also pointed to this point. As predicted using bioinformatics methods, inflammatory factors such as IL-10 and TNF- α in the hippocampal tissue of LPS model mice were observed that there have been changes, and these indicators have returned to normal levels after drug treatment. Interestingly, the Western blotting results deviated from the expected outcomes. There was no discernible difference in TP53 expression among the three groups, and FGFR1 and Smad3 did not exhibit a return to normal levels in the SYGC group. However, further investigation is required to determine the underlying reasons for these discrepancies, with one potential factor being limitations associated with bioinformatics data analysis. Bioinformatics methods rely on extensive datasets (such as genomic databases) and employ a series of model simulations to generate predictive outcomes [29]. However, due to the intricate nature of human physiology, these results may not always be entirely accurate. In our study, we also observed that while bioinformatics predictions were generally reliable, they were not infallible. This highlights the need for ongoing refinement of our bioinformatics methodologies to enhance their accuracy further. Consequently, it is evident that there are still significant gaps in achieving optimal prediction accuracy using bioinformatics models alone, therefore, additional approaches must be employed to comprehensively investigate the mechanisms underlying traditional Chinese medicine's efficacy in disease treatment.

In summary, this study used bioinformatics methods to analyze and predict the key targets of Shaoyao Gancao Decoction in the treatment of depression, and conducted animal experiments to verify that Shaoyao Gancao Decoction can regulate inflammatory factors—IL-10 and TNF-α, The regulation of BDNF and its effect on key proteins FGFR2, SMAD3, and FGFR1 provide a mechanistic explanation for the treatment of depression with Shaoyao Gancao Decoction which a traditional Chinese medicine. However, the validation results of animal experiments are not entirely consistent with the predicted results of bioinformatics, which may be a

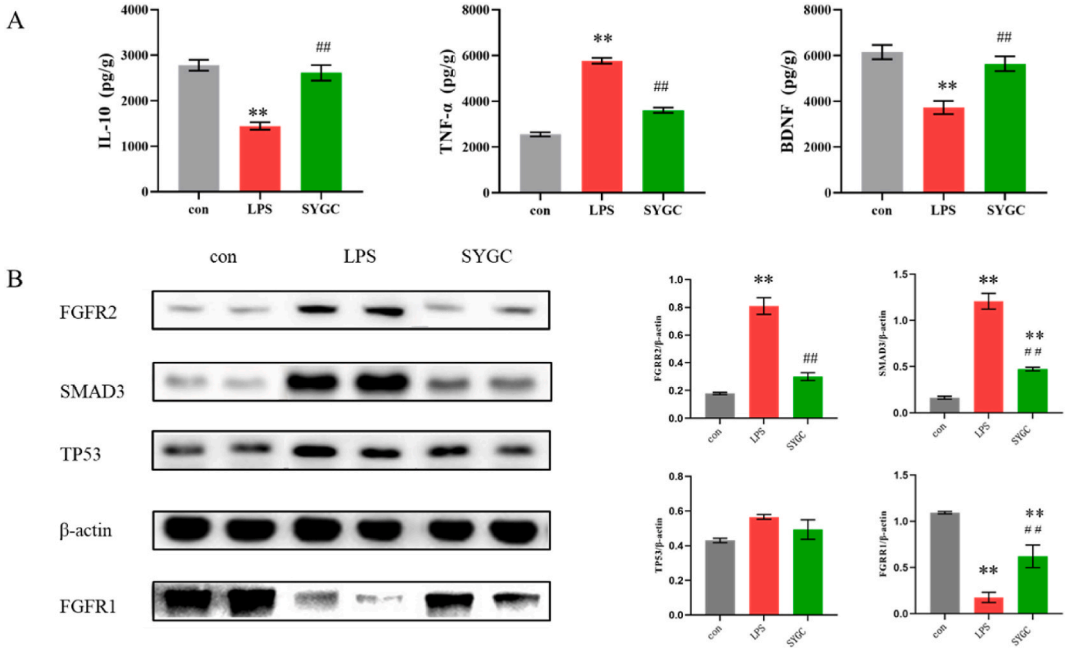

Fig. 8. ELISA and Western blot result of hippocampal sample. (A)The expression quantity of IL-10,TNF-α and BDNF with ELISA. (B) The expression quantity of FGFR2, SMAD3, TP53 and FGFR1 with Western blot. The data are presented as mean ± SEM. n = 8 mice per group. One-way ANOVA; **P < 0.01 vs Con. ##P < 0.01 vs LPS. Experimenters were blinded to the treatment.

limitation of this work.

Data availability statement

All data generated or analyzed during this study are included in this article.

CRediT authorship contribution statement

Long Li: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Jin Wang:** Data curation. **Shanbo Ma:** Data curation. **Meiling Zheng:** Formal analysis, Data curation. **Feiyan Wang:** Data curation. **Xiaodi Guo:** Data curation. **Shan Miao:** Writing – review & editing, Formal analysis. **Xiaopeng Shi:** Writing – review & editing, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34865.

References

[1] B. Amal, A. Abdulrahman, Z. Maria, et al., Integrative bioinformatics and artificial intelligence analyses of transcriptomics data identified genes associated with major depressive disorders including NRG1, Neurobiology of stress 26 (2023) 100555, https://doi.org/10.1016/j.ynstr.2023.100555.
[2] G.M. S, J.M. John, Depression, Lancet (London, England) (2018) 2299–2312, https://doi.org/10.1016/S0140-6736(18)31948-2.
[3] C.S. Liang, P. Gałecki, K.P. Su, Unresolved systemic inflammation, long COVID, and the common pathomechanisms of somatic and psychiatric comorbidity, J. Clin. Med. 11 (17) (2022) 5114, https://doi.org/10.3390/jcm11175114.
[4] P. Sara, P. Mariagrazia, M.M. Gennaro, et al., Long-term consequences of COVID-19 on cognitive functioning up to 6 months after discharge: role of depression and impact on quality of life, Eur. Arch. Psychiatr. Clin. Neurosci. 272 (2022) 773–782, https://doi.org/10.1007/S00406-021-01346-9.
[5] T. Takeuchi, Matrix analysis and risk management to avert depression and suicide among workers, Biopsychosoc. Med. 4 (1) (2010) 1–6, https://doi.org/10.1186/1751-0759-4-15.

[6] J.S. Buyukdura, S.M. Mcclintock, P.E. Croarkin, Psychomotor retardation in depression: biological underpinnings, measurement, and treatment, Progress in Neuro-Psychopharmacology & Biological Psychiatry: An International Research, Review and News Journal 35 (2) (2011) 395–409, https://doi.org/10.1016/j.pnpbp.2010.10.019.
[7] M. Maj, Why the clinical utility of diagnostic categories in psychiatry is intrinsically limited and how we can use new approaches to complement them, World Psychiatr. 17 (2018) 121–293, https://doi.org/10.1002/wps.20771.
[8] J. Hsieh, A.J. Eisch, Epigenetics, hippocampal neurogenesis, and neuropsychiatric disorders: unraveling the genome to understand the mind, Neurobiol. Dis. 39 (1) (2010) 73–84, https://doi.org/10.1016/j.nbd.2010.01.008.
[9] E. Holtzheimer Paul, Helen S. Mayberg, Stuck in a rut: rethinking depression and its treatment, Trends in neurosciences 34 (1) (2011) 1–9, https://doi.org/10.1016/j.tins.2010.10.004.
[10] P. Sara, P. Mariagrazia, M.M. Gennaro, et al., Long-term consequences of COVID-19 on cognitive functioning up to 6 months after discharge: role of depression and impact on quality of life, Eur. Arch. Psychiatr. Clin. Neurosci. 272 (5) (2022) 773–782, https://doi.org/10.1007/s00406-021-01346-9.
[11] X. Chi, S. Wang, Z. Baloch, et al., Research progress on classical traditional Chinese medicine formula Lily Bulb and Rehmannia Decoction in the treatment of depression, Biomed. Pharmacother. 112 (2019) 108616, https://doi.org/10.1016/j.biopha.2019.108616.
[12] Y.Q. Zhang, Q.Y. Guo, Q.Y. Li, et al., Main active constituent identification in Guanxinjing capsule, a traditional Chinese medicine, for the treatment of coronary heart disease complicated with depression, Acta Pharmacol. Sin. 39 (2017) 975–987, https://doi.org/10.1038/aps.2017.117.
[13] L. Zhang, Z. Yang, G. Fan, et al., Antidepressant-like effect of citrus sinensis (L.) osbeck essential oil and its main component limonene on mice, J. Agric. Food Chem. 67 (50) (2019) 13817–13818, https://doi.org/10.1021/acs.jafc.9b00650.
[14] Z. Hualin, L. Zhong, Z. Zhongliu, et al., Antidepressant-like effects of ginsenosides: a comparison of ginsenoside Rb3 and its four deglycosylated derivatives, Rg3, Rh2, compound K, and 20(S)-protopanaxadiol in mice models of despair, Pharmacology, biochemistry, and behavior 140 (2016) 17–26, https://doi.org/10.1016/j.pbb.2015.10.018.
[15] K. Azad Rajeev, Shulaev Vladimir, Metabolomics technology and bioinformatics for precision medicine, Briefings Bioinf. 20 (6) (2019) 1957–1961, https://doi.org/10.1093/bib/bbx170.
[16] Z. LiuXia, D. Jie, W. Hui, et al., TCMSID: a simplified integrated database for drug discovery from traditional Chinese medicine, J. Cheminf. 14 (1) (2022) 89, https://doi.org/10.1186/s13321-022-00670-z.
[17] M.E. Ritchie, P. Belinda, W. Di, et al., Limma powers differential expression analyses for RNA-sequencing and microarray studies, Nucleic Acids Res. 43 (7) (2015) e47, https://doi.org/10.1093/nar/gkv007.
[18] P. Langfelder, S. Horvath, P. Langfelder, S. Horvath, WGCNA: an R package for weighted correlation network analysis, BMC Bioinf. 9 (559) (2009) 559, https://doi.org/10.1186/1471-2105-9-559.
[19] Y. Zhou, B. Zhou, L. Pache, et al., Metascape provides a biologist-oriented resource for the analysis of systems-level datasets, Nat. Commun. 10 (1) (2019) 1523, https://doi.org/10.1038/s41467-019-09234-6.
[20] T.G.O. Consortium, Gene ontology: tool for the unification of biology, Nat. Genet. 25 (1) (2000) 25–29, https://doi.org/10.1038/75556.
[21] H. Ogata, S. Goto, K. Sato, et al., KEGG: Kyoto Encyclopedia of genes and genomes, Nucleic Acids Res. 27 (1999) 29–34, https://doi.org/10.1093/nar/27.1.29.
[22] M. Kanehisa, M. Furumichi, Y. Sato, et al., KEGG: integrating viruses and cellular organisms, Nucleic Acids Res. 49 (2021) 545–551, https://doi.org/10.1093/nar/gkaa970.
[23] M. Kanehisa, Toward understanding the origin and evolution of cellular organisms, Protein Sci. 28 (2019) 1947–1951, https://doi.org/10.1002/pro.3715.
[24] B. Chen, M.S. Khodadoust, C.L. Liu, et al., Profiling tumor in filtrating immune cells with CIBERSORT, Methods Mol. Biol. 1711 (2018) 243–259, https://doi.org/10.1007/978-1-4939-7493-1_12.
[25] L. Long, M. Shanbo, W. Jin, et al., Inflammatory factors predicted the resilient phenotype in social defeat-induced depression of male mouse, Neuroscience 519 (2023) 38–46, https://doi.org/10.1016/j.neuroscience.2023.02.022.
[26] B. Ben, S. Selvaraj, F. Yang, et al., Minocycline alleviates depression-like symptoms by rescuing decrease in neurogenesis in dorsal hippocampus via blocking microglia activation/phagocytosis, Brain Behav. Immun. 91 (2021) 519–530, https://doi.org/10.1016/j.bbi.2020.11.009.
[27] X. Hu, H. Pang, J. Liu, et al., A network medicine-based approach to explore the relationship between depression and inflammation, Front Psychiatry 14 (2023) 1184188, https://doi.org/10.3389/fpsyt.2023.1184188.
[28] M. Colasanto, S. Madigan, D.J. Korczak, Depression and inflammation among children and adolescents: a meta-analysis, J Affect Disorders 277 (2020) 940–948, https://doi.org/10.1016/j.jad.2020.09.025.
[29] J. Chen, G. Coppola, Bioinformatics and genomic databases, Handb. Clin. Neurol. 147 (2018), https://doi.org/10.1016/B978-0-444-63233-3.00007-5.