Observational Study

Medicine

OPEN

Molecular mechanisms underlying the therapeutic effects of Linggui Zhugan decoction in stroke

Insights from network pharmacology and single-cell transcriptomics analysis

Di Sun, MD[a], Fang Luo, MD[a], Chengbing Fang, MD[a], Qingyan Zhu, MD[b], Chong Li, MD[a,*]

Abstract

Linggui Zhugan decoction (LZD), a traditional Chinese medicine formula, has demonstrated significant therapeutic effects in managing poststroke cognitive impairment and hemiplegia. However, the precise molecular mechanisms underlying its efficacy remain incompletely elucidated. The active ingredients and target proteins of LZD were retrieved from the traditional Chinese medicine systems pharmacology database and analysis platform database, which is specifically designed for traditional Chinese medicine research. The stroke-related genes were obtained from publicly available databases. Protein–protein interaction, enrichment analysis, and single-cell data analysis were conducted to identify key cells, targets, and pathways. Molecular docking was employed to assess the binding affinity between key components and targets. Network pharmacology analysis identified 190 active ingredients and 248 targets in LZD. These targets were significantly enriched in processes and pathways such as cellular response to lipid, orexin receptor pathway, and were significantly associated with Cerebral infarction and Middle Cerebral Artery Occlusion. Intersection analysis with 2035 stroke-related genes revealed 144 potential targets, which exhibited 2870 interactions and were significantly enriched in signaling pathways such as PI3K-AKT single pathway, MAPK single pathway, and tumor necrosis factor single pathway. Gene set variation analysis showed that the targets of LZD exhibited higher enrichment scores in microglia, M2 macrophages, endothelial cells, and neutrophils, while lower enrichment scores were observed in oligodendrocytes. Furthermore, molecular docking demonstrated a strong binding affinity between key active ingredients and targets. Network pharmacology and single-cell sequencing analysis elucidated the key cells, pathways, targets, and components involved in the therapeutic mechanism of LZD for the treatment of stroke.

Abbreviations: BBB = blood–brain barrier, LDH = lactate dehydrogenase, LZD = Linggui Zhugan decoction, TCM = traditional Chinese medicine, TNF = tumor necrosis factor, UMAP = uniform manifold approximation and projection.

Keywords: gene set variation analysis, Linggui Zhugan decoction, molecular docking, network pharmacology, stroke

1. Introduction

Cerebrovascular accident, commonly known as stroke, ranks as the second leading cause of mortality and the third leading cause of morbidity among the global adult population.[1] Furthermore, the post-stroke healthcare resource expenditures are considerable.[2] Clinically, stroke is classified into hemorrhagic and ischemic subtypes, with ischemic stroke constituting 70% to 80% of all stroke incidents. This condition involves ischemic necrosis of cerebral tissue due to arterial occlusion, which may be complicated by postinfarction hemorrhage.[3,4] The pathophysiological mechanisms of ischemic stroke encompass disruptions in energy metabolism, oxidative stress, inflammation, neuronal damage, and currently lack definitive therapeutic modalities.[5,6] Presently, clinical emphasis on the management of ischemic stroke primarily centers on ultra-early thrombolysis, acute neuroprotection, and restoration of neurovascular architecture and function during the rehabilitation phase.[7] Recombinant tissue plasminogen activator stands as the sole FDA-approved medication for the

Institutional review board approval and informed consent were not required in the current study because research data are publicly available and all patient data are de-identified.

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

There is no need for informed consent in our study since the unidentified data were free from medical ethics review.

Supplemental Digital Content is available for this article.

[a] *Department of Rehabilitation, Tongde Hospital of Zhejiang Province, Hangzhou, Zhejiang, China,* [b] *Geriatric Medicine Department, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China.*

* *Correspondence: Chong Li, Department of Rehabilitation, Tongde Hospital of Zhejiang Province, Hangzhou, Zhejiang 314408, China (e-mail: lichong20080823@163.com).*

How to cite this article: Sun D, Luo F, Fang C, Zhu Q, Li C. Molecular mechanisms underlying the therapeutic effects of Linggui Zhugan decoction in stroke: Insights from network pharmacology and single-cell transcriptomics analysis. Medicine 2024;103:13(e37482).

Received: 7 November 2023 / Received in final form: 9 February 2024 / Accepted: 13 February 2024

http://dx.doi.org/10.1097/MD.0000000000037482

treatment of acute ischemic stroke.[8] However, its narrow therapeutic window and significant adverse effects substantially constrain its clinical utility. Hence, the exploration of adjunctive and alternative therapeutic strategies assumes critical significance.

Numerous Chinese herbal interventions have demonstrated notable therapeutic efficacy in the management of ischemic stroke, as substantiated by clinical research.[9,10] Among these interventions, Linggui Zhugan Decoction (LZD), a well-established Chinese herbal formula, is widely employed in clinical practice for the treatment of ischemic stroke. Comprising 4 principal herbs—Wolfiporia cocos (Fuling in Chinese), Cinnamomum cassia (Guizhi in Chinese), Atractylodes macrocephala (Baizhu in Chinese), and Glycyrrhiza uralensis (Gancao in Chinese)—LZD is conventionally utilized to tonify yang, resolve fluid retention, strengthen the spleen, and dispel dampness. Notably, a relevant clinical observation has demonstrated that LZD can ameliorate cognitive dysfunction in patients with ischemic stroke-related phlegm turbidity obstructing the orifices, non-dementia type, and enhance their activities of daily living. Additionally, it facilitates the recovery of neurological deficits and enhances complex instrumental abilities in these patients.[11] In the rehabilitation of post-stroke hemiplegic patients, the modified LZD combined with the awakening the mind and opening the orifices acupuncture technique can improve patients' neurological function, hemorheological parameters, muscle tone, and clinical outcomes.[12] Furthermore, the modified LZD combined with acupuncture demonstrates favorable therapeutic effects in the treatment of stroke-associated pneumonia, effectively alleviating systemic inflammatory responses, improving neurological deficits, and exhibiting good safety profiles.[13] Nevertheless, the pharmacological effects of LZD on ischemic stroke have yet to be fully elucidated.

Single-cell RNA sequencing represents an innovative bioinformatics analysis approach that has demonstrated exceptional efficacy in elucidating the etiology and progression of diseases, thereby yielding novel insights into the therapeutic landscape for various medical conditions.[14] The comprehension of cell type-specific alterations and regulatory processes at the individual cellular level holds the potential to unveil the molecular underpinnings governing the pathophysiological cascades associated with stroke.[15] The application of network pharmacology has gained increasing traction in the investigation of the mechanistic principles underlying traditional Chinese medicine, offering elucidation of the intricate multi-component, multi-target modalities of Chinese herbal compounds. Nevertheless, the body of knowledge pertaining to single-cell level network pharmacology research remains limited.[16] Consequently, the primary objective of this investigation is to integrate network pharmacology and bioinformatics analysis to delineate the mechanistic actions of LZD in the management of stroke at the single-cell resolution.

2. Materials and methods

2.1. Identification of active ingredients and targets of LZD

The active constituents and targets of LZD were identified through individual queries of its 4 medicinal components in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (https://old.tcmsp-e.com/tcmsp.php). Active ingredients were selected based on thresholds of oral bioavailability ≥ 20% and drug-likeness ≥ 0.1, and their respective target information was retrieved. Subsequently, the symbol of target genes were standardized using the Uniprot database (https://www.uniprot.org/), with nonhuman genes being excluded from the analysis.

2.2. Identification of stroke-related genes

Genes associated with stroke were sourced from 5 public databases, namely GeneCards (https://www.genecards.org/), Online Mendelian Inheritance in Man (https://www.omim.org/), PharmGKB (https://www.pharmgkb.org/), Therapeutic Target Database (https://db.idrblab.net/ttd/), and DisGeNET (https://www.disgenet.org/). The amalgamation of these 5 datasets constituted the pool of stroke-related genes utilized for subsequent analysis.

2.3. Protein–protein interaction and enrichment analysis

The overlapping target proteins of LZD and stroke-related genes were identified as potential targets, and protein-protein interaction data was retrieved from the STRING database (https://string-db.org/cgi/input.pl). Subsequently, enrichment analysis of the potential targets was performed using either the ClusterProfiler package or the Metascape online tool, incorporating the Benjamini-Hochberg correction method. Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes pathways with adjusted P-values < .05 were chosen, and a subset of the results was visualized using barplots and bubble plots.

2.4. Single-cell sequencing analysis

The single-cell sequencing dataset GSE234052 pertaining to stroke was retrieved from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/gds/) and subjected to analysis using the Seurat package, in accordance with established methodologies. Specifically, cells featuring fewer than 200 attributes and exhibiting a mitochondrial content exceeding 10% were excluded. Subsequent to log-normalization, the optimal number of clusters was determined through the utilization of the findneighbors and findclusters functions. Uniform Manifold Approximation and Projection was executed with consistent dimensions to mitigate the risk of over-clustering or under-clustering. Cell annotation was conducted by referencing cell type-specific genes documented in the literature.[17–25] The GSVA package was employed to assess the enrichment levels of the target proteins of LZD across various cell subtypes.

2.5. Molecular docking analysis

The molecular docking analysis was conducted employing the AutoDock Vina software. Initially, the 3D structural information of the active compounds was sourced from the PubChem database, and subsequently transformed from the.sdf format to the. pdbqt format using the Open Babel software. The 3D structure data of the target protein was obtained from the PDB database, and the removal of solvent molecules and other peptide segments from the protein structure was executed using the PyMOL software. Following this, the getBox plugin was employed to generate the molecular docking grid. Preceding the molecular docking process, the protein molecules underwent hydrogenation and charge calculation procedures. Subsequently, a genetic algorithm was applied to conduct conformational searches for molecular docking, thereby yielding optimal configurations for the compound-target complex.

2.6. Network analysis

The network construction and analysis were conducted using Cytoscape software, with a primary emphasis on establishing the herbal medicine-component-target network, the protein–protein interaction network of potential targets, and the pathway-target-component-herbal medicine network. Network topology analysis was performed using the Analyze Network tool, while core network analysis was carried out using the

Cytohubba plugin. Furthermore, network integration was achieved through the utilization of the Merge tool.

3. Results

3.1. The regulatory network of active ingredients and targets of LZD

A total of 190 active ingredients from LZD were obtained from the traditional Chinese medicine systems pharmacology database and analysis platform database, comprising 21 from Fuling, 14 from Baizhu, 34 from Guizhi, and 125 from Gancao (Table S1, Supplemental Digital Content, http://links.lww.com/MD/L922). Subsequent target collection yielded 283 target genes associated with 143 active ingredients. The specific ingredient-target network is depicted in Figure 1A, featuring 248 targets for Gancao, 116 for Guizhi, 31 for Fuling, and 41 for Baizhu. Enrichment analysis unveiled the enrichment of these genes in biological processes or pathways pertinent to stroke, including cellular response to lipid, the orexin receptor pathway, and nuclear receptors meta-pathways (Fig. 1B). Furthermore, disease analysis based on the DisGeNET dataset revealed significant enrichment of these genes in cerebral infarction and middle cerebral artery occlusion (Fig. 1C). These findings underscore the potential of LZD in stroke treatment.

3.2. Potential targets of LZD in the treatment of ischemic stroke

In our quest to identify genes associated with ischemic stroke, we conducted comprehensive searches across 5 prominent public databases, namely GeneCards, OMIM, TTD, PharmGKB, and DisGeNET. The collective results from these databases yielded 1029, 84, 27, 27, and 1159 genes, respectively. Upon performing an intersection analysis, we successfully identified a total of 2035 genes linked to ischemic stroke (Fig. 2A and Table S2, Supplemental Digital Content, http://links.lww.com/MD/L923). Subsequently, through the intersection of these ischemic stroke-related genes with those targeted by LZD, we pinpointed 144 potential targets for the treatment of ischemic stroke (Fig. 2B). Furthermore, our analysis of the protein-protein interaction network unveiled a total of 2870 interactions among the 143 target proteins, averaging 20 interactions per target. Notably, insulin and tumor necrosis factor (TNF) emerged as the most central hub proteins, each engaging in 108 interactions (Fig. 2C).

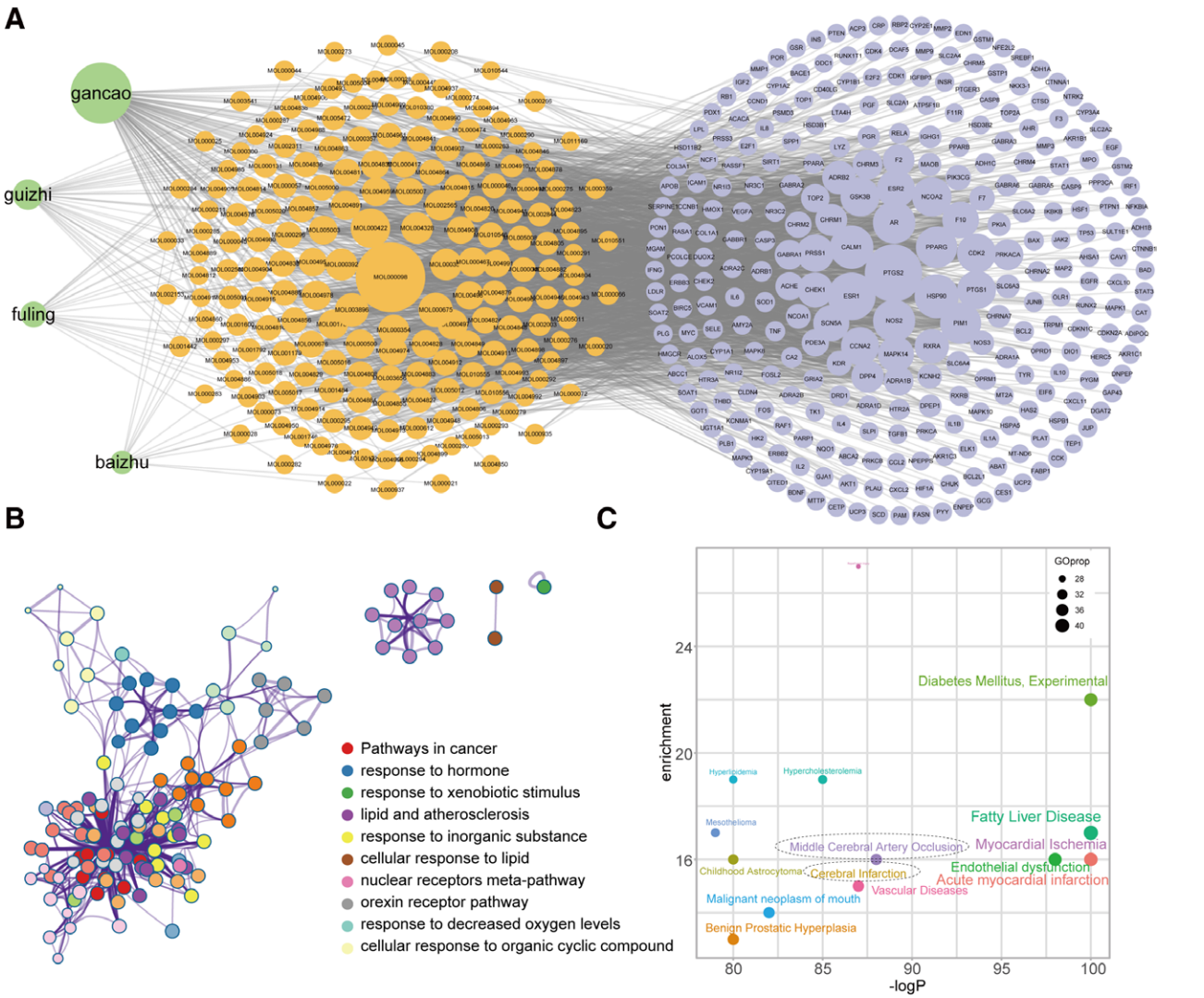

Figure 1. Active ingredients and targets of LZD. (A) Medicinal herbs-components-targets network of LZD. (B) Gene Ontology (GO) enrichment results of targets of LZD. (C) Enrichment results of biological processes and pathways of targets of LZD. (D) Disease enrichment results of components and targets of LZD. LZD = Linggui Zhugan decoction.

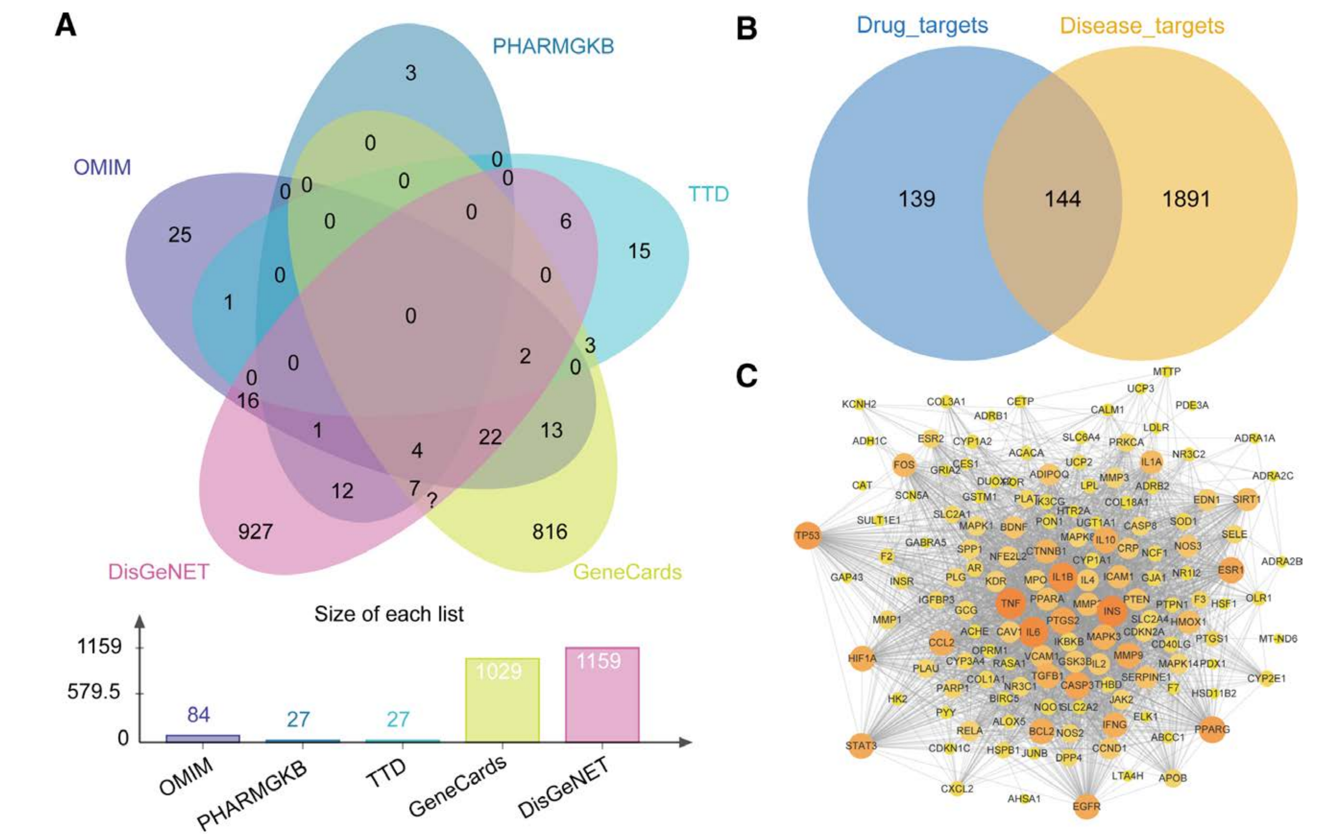

Figure 2. Identification of potential targets of LZD for the treatment of stroke. (A) Venn diagram showing stroke-related genes obtained from GeneCards, TTD, PharmGKB, OMIM, and DisGeNET databases. (B) Venn diagram depicting the overlap between stroke-related genes and targets of LZD. (C) Protein-protein interaction network of the 144 potential targets of LZD for the treatment of stroke. LZD = Linggui Zhugan decoction.

3.3. Biological processes and pathways related to the treatment of stroke by LZD

To gain insights into the underlying biological processes and pathways associated with these potential targets, we conducted Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses. The findings revealed a significant enrichment of these potential targets in key biological processes such as oxidative stress response, response to nutrient levels, and cellular response to chemical stress. Moreover, these targets exhibited enrichment in various molecular functions, including signaling receptor activator activity, receptor ligand activity, and DNA-binding transcription factor binding activity (Fig. 3A, Table S3, Supplemental Digital Content, http://links.lww.com/MD/L924). Furthermore, we identified the top 30 significantly enriched signaling pathways, with notable representation of genes in pathways such as the PI3K-AKT single pathway, MAPK single pathway, and TNF single pathway (Fig. 3B, Table S4, Supplemental Digital Content, http://links.lww.com/MD/L925).

3.4. Single-cell level analysis of the therapeutic effect of LZD on stroke

To gain insight into the underlying mechanism of action, we employed GSVA analysis on the stroke single-cell sequencing dataset GSE234052, focusing on the target genes of LZD. Through clustering analysis and the utilization of known cell type markers or specific gene combinations, we successfully identified 13 distinct cell subtypes, including microglia (*Itgam*⁺, *Aif1*⁺, *Ptprc*⁺), endothelial cells (*Pecam1*⁺), oligodendrocytes (*Mog*⁺, *Olig2*⁺), pericytes (*Pdgfrβ*⁺, *Rgs5*⁺, *Acta2*⁻), astrocytes (*Gfap*⁺, *Slc1a2*⁺), M2 macrophages (*Itgam*⁺, Aif1⁺, *Ptprc*⁺, *Arg1*⁺), neuroblasts (*Dcx*⁺), smooth muscle cells (*Pdgfrβ*⁺, *Acta2*⁺), neutrophils (*Fpr1*⁺,*Ly6g*⁺), perivascular macrophages (*Mrc1*⁺), T-cells (*Cd3e*⁺), B-cells (*Citta*⁺, *Cd79a*⁺) and fibroblasts (*Col1a1*⁺,*Pdgfr*⁺, *Pdgfr*⁺) (Fig. 4A). The expression levels of these marker genes across different subtypes are illustrated in Figure 4B. Remarkably, the GSVA analysis unveiled higher enrichment scores of LZD target genes in microglia, M2 macrophages, endothelial cells, and neutrophils, while lower enrichment scores were observed in oligodendrocytes and other cell types (Figs. 4C and 5D).

3.5. Affinity assessment of critical active constituents and targets for LZD

In order to elucidate the crucial mechanisms underlying the therapeutic effects of LZD on stroke, we established a comprehensive network encompassing medicinal herbs, constituents, genes, and pathways using significantly enriched signaling cascades (Fig. 5A). This network comprises 4 medicinal herb nodes, 127 constituent nodes, 47 pathway nodes, 95 gene nodes, as well as 47 non-constituent target protein nodes. By applying Cytohubba analysis, we identified the top 10 gene nodes (PTGS2, MAPK14, INS, GSK3B, ESR1, IL6, PPARG, TNF, MAPK3, NOS2) critical in this network (Fig. 5B). Consequently, we proceeded to evaluate the binding affinity between these 10 targets and the 10 constituents exhibiting the highest degree values. As depicted in Figure 5C, with the exception of PTGS2 and formonenetin, the binding energies of the remaining constituent-target complex conformations were all below -5kcal/mL, indicative of their pronounced affinity. Notably, certain active constituents including licochalcone A, kaempferol, and oleic acid displayed enhanced binding affinity towards specific key targets.

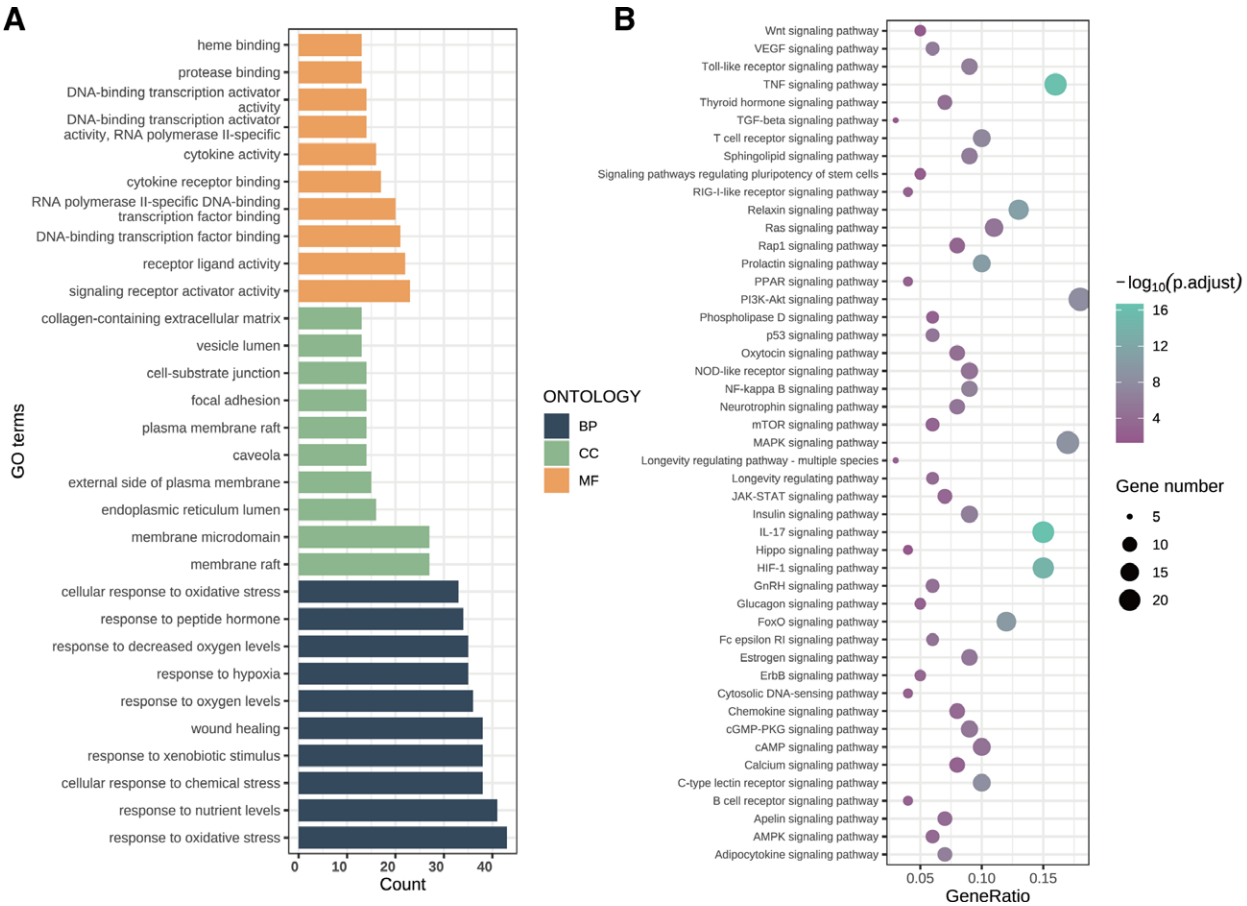

Figure 3. Enrichment analysis results of potential targets of LZD for the treatment of stroke. (A) Top 10 significantly enriched biological process (BP), cellular component (CC), and molecular function (MF) terms among the 144 targets. (B) Top 30 significantly enriched signaling pathways among the 144 potential targets. LZD = Linggui Zhugan decoction.

4. Discussion

Stroke is a critical cerebrovascular ailment, necessitating both acute and recovery phase interventions. While pharmacological approaches are pivotal in stroke management, the utilization of traditional Chinese medicine (TCM) has garnered significant attention. Numerous TCM formulations, such as Sanhuang Xiexin decoction,[26] Xinglou Chengqi decoction,[27] and Buyang Huanwu Decoction,[28] have displayed clinical efficacy in stroke treatment. Specifically, LZD has demonstrated favorable outcomes in ameliorating post-stroke cognitive impairment and hemiplegia. Therefore, this investigation employs network pharmacology and bioinformatics methodologies to elucidate the underlying mechanisms responsible for its therapeutic effects. Through meticulous analysis, we have identified 143 bioactive constituents and their corresponding 283 target genes from 4 herbs within LZD. Additionally, we have inferred 144 potential target genes associated with biological processes and pathways relevant to stroke treatment. Subsequent biological analysis has shed light on the significantly influenced cell subgroups affected by LZD. Furthermore, network analysis has unveiled key mechanisms through which LZD exerts its therapeutic effects in stroke treatment. Finally, molecular docking analysis has been employed to assess the binding affinity between pivotal bioactive constituents and their respective targets.

LZD is a herbal formula consisting of 4 botanical ingredients, namely *Wolfiporia cocos*, *Cinnamomum cassia*, *Atractylodes macrocephala*, and *Glycyrrhiza uralensis*. In the context of ischemic stroke treatment, lactate dehydrogenase inhibitors have gained significant attention, with natural products being recognized as potential sources for such inhibitors. Notably, Wolfiporia cocos has been identified to contain 5 compounds, namely dehydrotrametenolic acid, trametenolic acid, dehydroeburicoic acid, eburicoic acid, and ergosterol peroxide, which exhibit lactate dehydrogenase inhibitory activity.[29] Studies have demonstrated that extracts derived from Cinnamomum cassia possess the ability to modulate inflammatory and autophagic pathways subsequent to ischemic stroke.[30] Additionally, Glycyrrhiza uralensis, commonly referred to as Licorice, exhibits diverse pharmacological activities including neuroprotective, antifungal, and anticaries effects. Pretreatment with Licorice has been found to regulate apoptosis-related proteins, thereby conferring protection against cerebral ischemic injury in mice.[31] The blood-brain barrier (BBB) functions as a crucial physical barrier that maintains homeostasis in the central nervous system by separating it from the blood. Preservation of BBB integrity represents a novel therapeutic approach. Liquiritin, a flavonoid compound isolated from Licorice, has been observed to stimulate cell proliferation, migration, and angiogenesis while mitigating apoptosis. These effects may be attributed to its ability to inhibit oxidative stress and endoplasmic reticulum stress, consequently playing a role in maintaining BBB integrity.[32]

The efficacy of TCM in stroke treatment has been demonstrated through the identification of active ingredients such as Panax notoginseng saponins[33]and salvianolic acid IIA.[34] Network pharmacology analysis has further revealed 144 potential active components in LZD, among which quercetin, kaempferol, and oleic acid, exhibited the most targeted effects. Intravenous thrombolysis has emerged as an effective therapeutic strategy for acute ischemic stroke within a

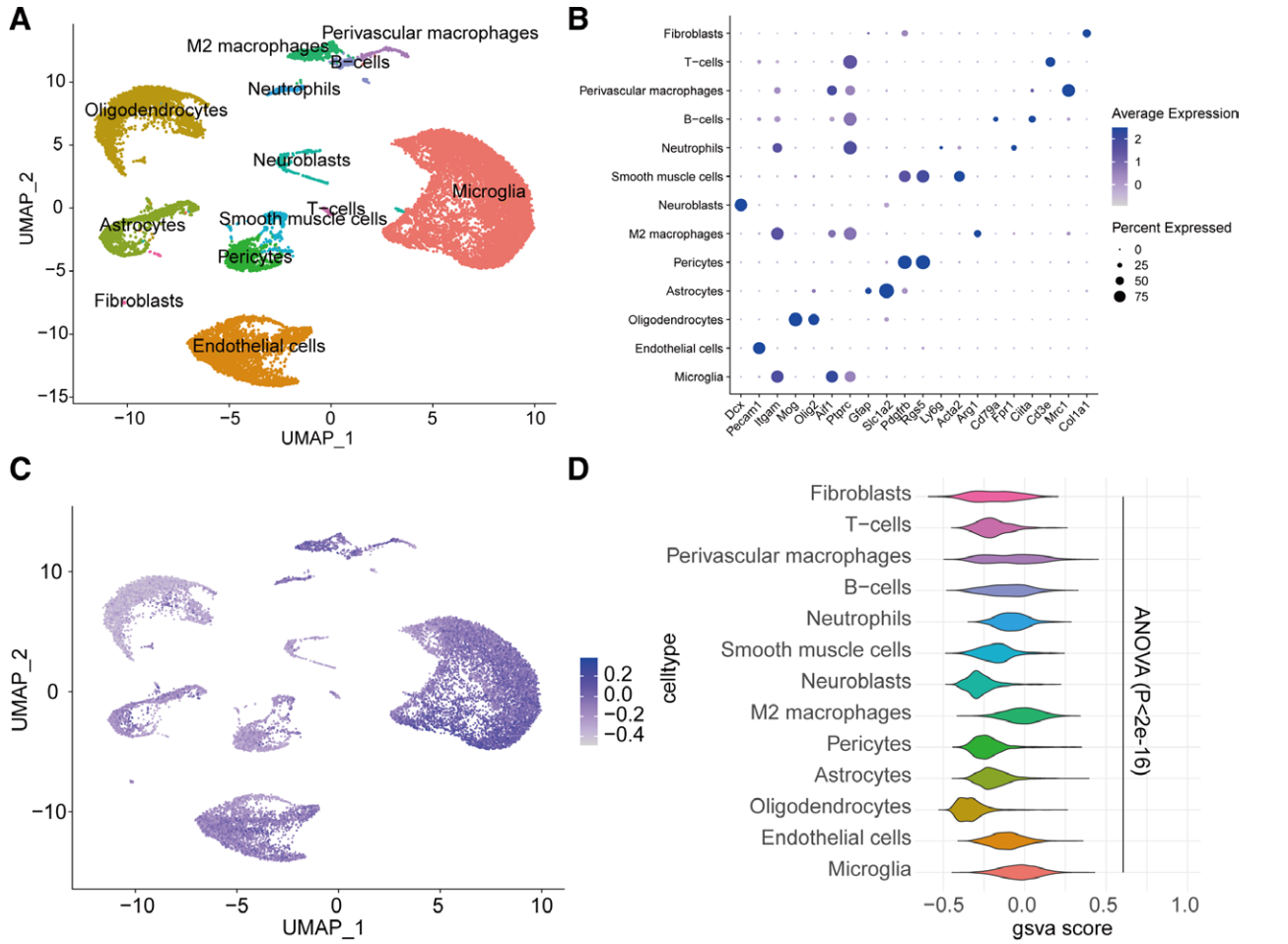

Figure 4. Single-cell level analysis of the therapeutic effect of LZD on stroke. (A) UMAP clustering visualization of the GSE234052 dataset after cell annotation. (B) Gene expression profiles of identified cell types and genes determining cell types through clustering analysis. (C) UMAP visualization of GSVA scores of candidate genes in the GSE234052 dataset. (D) Violin plot comparing GSVA scores of candidate genes across different cell types. UMAP = uniform manifold approximation and projection.

well-defined time window. Quercetin, a widely distributed flavonoid found in vegetables and fruits, has been found to inhibit the secretion of inflammatory cytokines by immune cells, consequently reducing platelet aggregation and limiting the formation of inflammatory thrombi. Preclinical studies on patients with ischemic brain injury have provided evidence of the neuroprotective effects of quercetin[35] in stroke management. Quercetin, being a naturally occurring flavonoid compound, has demonstrated notable neuroprotective effects in ischemic stroke. Its impact on stroke pathology has been attributed to the downregulation of the JAK1/STAT3 pathway, leading to the inhibition of peripheral blood and brain neutrophil activation and accumulation in rat models.[36] Although specific investigations on the therapeutic effects of oleic acid in stroke treatment are currently lacking, clinical analyses have shown an inverse correlation between higher plasma levels of oleic acid and lower incidence rates of stroke. This intriguing finding suggests the potential value of oleic acid supplementation in improving stroke outcomes.[37]

The single-cell data analysis conducted in this study has revealed a distinct enrichment pattern of LZD targets, particularly in microglia, M2 macrophages, endothelial cells, and neutrophils. Conversely, a lower enrichment score was observed in oligodendrocytes, implying potential effects of LZD on these specific cell types. Extensive research has elucidated the significance of these cells in the pathogenesis, progression, and treatment of stroke. For instance, as the primary immune cells within the brain, resident microglia promptly respond to ischemic stroke-induced pathological and physiological changes. Activation of microglia plays a pivotal role in neurogenesis, angiogenesis, and synaptic remodeling, subsequently facilitating functional recovery post-cerebral ischemia.[38] Microglia/macrophages exhibit a dual role in brain injury and repair subsequent to ischemia/reperfusion. Encouraging a shift from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype in microglia/macrophages is considered a potential therapeutic strategy for ischemic stroke.[39] In a murine stroke model, endothelial cells regulate astrocyte-to-neuronal progenitor cell differentiation, thereby presenting a novel vascular regulatory mechanism for neural plasticity that may offer treatment prospects for improving post-stroke prognosis.[40] Notably, the composition of neutrophils in the peripheral blood of stroke patients significantly increases shortly after stroke onset, and higher neutrophil counts are associated with a poorer prognosis.[41] Oligodendrocytes, which are responsible for myelin formation in the central nervous system, play a crucial role in maintaining axonal integrity and function. They are particularly susceptible to injury in adult stroke, periventricular white matter rarefaction, and post-stroke cognitive impairment, thereby highlighting their significance as critical therapeutic targets.[42]

Ultimately, we have elucidated the putative mechanism and cellular carrier underlying the therapeutic effects of LZD in

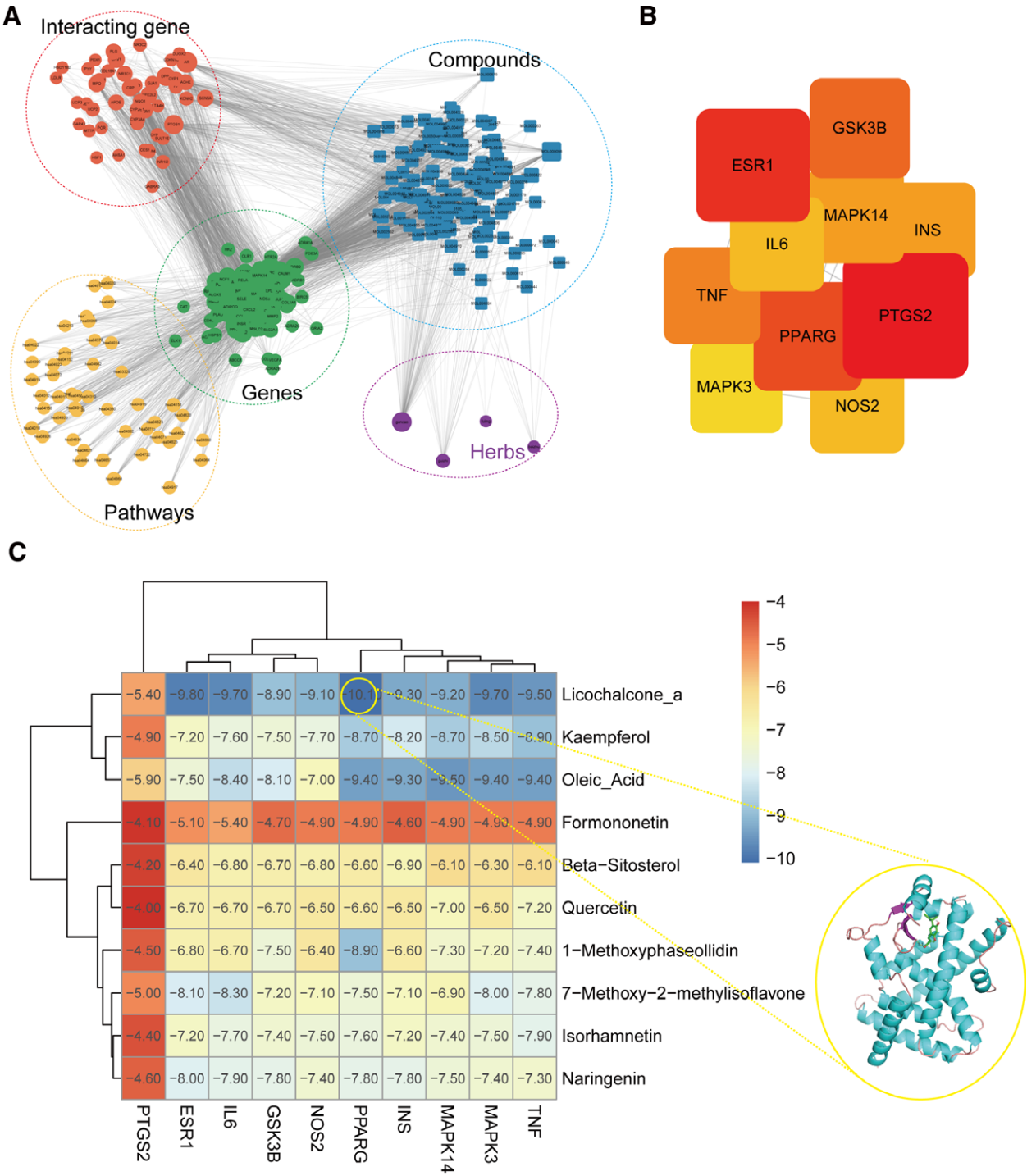

Figure 5. Binding affinity of key active ingredients and targets in Linggui Zhugan Decoction (LZD). (A) Network diagram illustrating the key active ingredients, targets, and pathway of LZD in the treatment of stroke. (B) Binding affinity between key active ingredients and targets. (C) Heatmap of the binding affinity between key active ingredients and targets, along with the 3D representation of the optimal complex configuration.

the management of stroke, employing network pharmacology and bioinformatic analysis. Nonetheless, this investigation is not without its constraints. Primarily, all inferences stem exclusively from computational methodologies, without corroboration through in vitro and in vivo experimentation, specifically pertaining to the modulation of distinct cellular pathways and phenotypic expressions by the therapeutic agent. Subsequently, the deductions derived from computational approaches are insufficient to ascertain the directionality of regulatory effects exerted by LZD on potential cell targets, necessitating further empirical inquiries.

5. Conclusion

This study employed network pharmacology to investigate the molecular basis and target mechanisms underlying the therapeutic effects of LZD in stroke treatment. Through the integration of single-cell transcriptomics analysis, diverse cell types, including microglia, M2 macrophages, and oligodendrocytes, were identified as potential cellular carriers mediating the clinical efficacy of LZD. Moreover, this study proposed key mechanisms of action through which LZD exerts its therapeutic effects in stroke. However, further validation of these findings is required through additional cellular, animal, and clinical experiments in the future.

Author contributions

Conceptualization: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Data curation: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Formal analysis: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Investigation: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Methodology: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Project administration: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu, Chong Li.
Resources: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Software: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Supervision: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Validation: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu.
Visualization: Di Sun, Fang Luo, Chengbing Fang, Qingyan Zhu, Chong Li.
Writing – original draft: Di Sun, Chong Li.
Writing – review & editing: Di Sun, Chong Li.

References

[1] Disease G, Incidence I, Monasta L, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392:1789–858.
[2] Sherzai AZ, Elkind MS. Advances in stroke prevention. Ann N Y Acad Sci. 2015;1338:1–15.
[3] Guzik A, Bushnell C. Stroke epidemiology and risk factor management. Continuum (Minneap Minn). 2017;23:15–39.
[4] Purroy F, Montalà N. Epidemiology of stroke in the last decade: a systematic review. Rev Neurol. 2021;73:321–36.
[5] Crilly S, Zille M, Kasher PR, et al. Editorial: innovative models of stroke pathology. Front Neurol. 2023;14:1266075.
[6] Han D, Liu H, Gao Y, et al. Targeting brain-spleen crosstalk after stroke: new insights into stroke pathology and treatment. Curr Neuropharmacol. 2021;19:1590–605.
[7] Kuriakose D, Xiao Z. Pathophysiology and treatment of stroke: present status and future perspectives. Int J Mol Sci . 2020;21:7609.
[8] Fugate JE, Rabinstein AA. Absolute and relative contraindications to IV rt-PA for acute ischemic stroke. Neurohospitalist 2015;5:110–21.
[9] Zhang Q, Wang Y, Chen A, et al. Xiaoxuming decoction: a traditional herbal recipe for stroke with emerging therapeutic mechanisms. Front Pharmacol. 2021;12:802381.
[10] Zheng L, Meng L, Liang H, et al. Sanhua decoction: current understanding of a traditional herbal recipe for stroke. Front Neurosci. 2023;17:1149833.
[11] Ying Z. Study on the curative effect of Linggui Zhugan decoction on non-dementia-type cognitive dysfunction after ischemic stroke. Shanghai Univ Tradit Chin Med 2023.
[12] Chan L, Yongke W. Effect of Linggui Shu Gantang combined with Xingnao Kaiqiao acupuncture on neuromuscular rehabilitation of hemiplegic limbs after stroke. Clinical Research and Practice. 2019;4:130–1.
[13] Lingzhi W, Wendong Z, Fei L, et al. Modified Linggui Zhugan decoction combined with acupuncture and moxibustion to treat 34 cases of Yang deficiency cold drink Fu lung type stroke-related pneumonia. Anhui Med Pharm J. 2021;25:1667–70.
[14] Jovic D, Liang X, Zeng H, et al. Single-cell RNA sequencing technologies and applications: a brief overview. Clin Transl Med. 2022;12:e694.
[15] Qiu M, Zong JB, He QW, et al. Cell heterogeneity uncovered by single-cell RNA sequencing offers potential therapeutic targets for ischemic stroke. Aging Dis 2022;13:1436–54.
[16] Song Z, Gao P, Zhong X, et al. Identification of five hub genes based on single-cell RNA sequencing data and network pharmacology in patients with acute myocardial infarction. Front Public Health. 2022;10:894129.
[17] de Saint BG, Geissmann F, Flori E, et al. Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3. J Clin Invest. 2004;114:1512–7.
[18] Dorrier CE, Aran D, Haenelt EA, et al. CNS fibroblasts form a fibrotic scar in response to immune cell infiltration. Nat Neurosci. 2021;24:234–44.
[19] Jablonski KA, Amici SA, Webb LM, et al. Novel markers to delineate murine M1 and M2 Macrophages. PLoS One. 2015;10:e145342.
[20] Buizza C, Enström A, Carlsson R, et al. The transcriptional landscape of pericytes in acute ischemic stroke. Transl Stroke Res. 2023. doi: 10.1007/s12975-023-01169-x
[21] Leslie J, Millar BJ, Del CPA, et al. FPR-1 is an important regulator of neutrophil recruitment and a tissue-specific driver of pulmonary fibrosis. JCI Insight. 2020;5:e125937.
[22] Liu XS, Chopp M, Zhang XG, et al. Gene profiles and electrophysiology of doublecortin-expressing cells in the subventricular zone after ischemic stroke. J Cereb Blood Flow Metab. 2009;29:297–307.
[23] Sharma A, Kazim SF, Larson CS, et al. Divergent roles of astrocytic versus neuronal EAAT2 deficiency on cognition and overlap with aging and Alzheimer's molecular signatures. Proc Natl Acad Sci U S A. 2019;116:21800–11.
[24] Vanlandewijck M, He L, Mäe MA, et al. A molecular atlas of cell types and zonation in the brain vasculature. Nature. 2018;554:475–80.
[25] Wang K, Wei G, Liu D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. Exp Hematol Oncol. 2012;1:36.
[26] Song J, Chen X, Lyu Y, et al. Sanhuang Xiexin decoction promotes good functional outcome in acute ischemic stroke. Brain Behav. 2019;9:e1185.
[27] Chen P, Jiang L, Geng H, et al. Effectiveness of Xinglou Chengqi decoction on constipation in patients with acute ischemic stroke: a randomized controlled trial. J Tradit Chin Med. 2020;40:112–20.
[28] Li Y, Miao L, Guo R, et al. To explore the regulatory effect of Buyang Huanwu Decoction on cerebral infarction based on quantitative proteomics. J Proteomics. 2023;277:104850.
[29] Li S, Zhang J, Li S, et al. Extraction and separation of lactate dehydrogenase inhibitors from Poria cocos (Schw.) Wolf based on a hyphenated technique and in vitro methods. J Sep Sci. 2017;40:1773–83.
[30] Luo C, Chen Q, Liu B, et al. The extracts of angelica sinensis and cinnamomum cassia from oriental medicinal foods regulate inflammatory and autophagic pathways against neural injury after ischemic stroke. Oxid Med Cell Longev. 2021;2021:9663208.
[31] Lim C, Lim S, Lee B, et al. Licorice pretreatment protects against brain damage induced by middle cerebral artery occlusion in mice. J Med Food. 2018;21:474–80.
[32] Li M, Ke J, Deng Y, et al. The protective effect of liquiritin in hypoxia/reoxygenation-induced disruption on blood brain barrier. Front Pharmacol. 2021;12:671783.
[33] Feng L, Han F, Zhou L, et al. Efficacy and safety of panax notoginseng saponins (xueshuantong) in patients with acute ischemic stroke (EXPECT) Trial: rationale and Design. Front Pharmacol. 2021;12:648921.
[34] Kaiser EE, Waters ES, Yang X, et al. Tanshinone IIA-loaded nanoparticle and neural stem cell therapy enhances recovery in a pig ischemic stroke model. Stem Cells Transl Med 2022;11:1061–71.
[35] Zhang L, Ma J, Yang F, et al. Neuroprotective effects of quercetin on ischemic stroke: a literature review. Front Pharmacol. 2022;13:854249.
[36] Zhang SS, Liu M, Liu DN, et al. Network pharmacology analysis and experimental validation of kaempferol in the treatment of ischemic stroke by inhibiting apoptosis and regulating neuroinflammation involving neutrophils. Int J Mol Sci . 2022;23:12694.
[37] Samieri C, Féart C, Proust-Lima C, et al. Olive oil consumption, plasma oleic acid, and stroke incidence: the Three-City Study. Neurology. 2011;77:418–25.
[38] Qin C, Zhou LQ, Ma XT, et al. Dual functions of microglia in ischemic stroke. Neurosci Bull. 2019;35:921–33.
[39] Li L, Gan H, Jin H, et al. Astragaloside IV promotes microglia/macrophages M2 polarization and enhances neurogenesis and angiogenesis through PPARγ pathway after cerebral ischemia/reperfusion injury in rats. Int Immunopharmacol. 2021;92:107335.
[40] Li W, Mandeville ET, Durán-Laforet V, et al. Endothelial cells regulate astrocyte to neural progenitor cell trans-differentiation in a mouse model of stroke. Nat Commun. 2022;13:7812.
[41] Cai W, Liu S, Hu M, et al. Functional dynamics of neutrophils after ischemic stroke. Transl Stroke Res. 2020;11:108–21.
[42] Huang S, Ren C, Luo Y, et al. New insights into the roles of oligodendrocytes regulation in ischemic stroke recovery. Neurobiol Dis. 2023;184:106200.