Network pharmacological systems study of Huang-Lian-Tang in the treatment of glioblastoma multiforme

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Abstract. Glioblastoma multiforme (GBM) has a poor prognosis and its recurrence and mortality rates are high. At present, there is no effective clinical method to control its progression and recurrence. Traditional Chinese Medicine has a high status not only in China, but also in the world. Certain drugs are also used in the clinical treatment of tumor diseases. In clinical practice, Huang-Lian-Tang (HLT) has proven efficacy in treating brain diseases and preventing tumor recurrence. However, the mechanisms of action have remained elusive. The present study explored the potential mechanisms of HLT in the treatment of gliomas based on network pharmacology. First, information on the composition of HLT was obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, and the composition and targets of the chemical substances contained in the herbs were analyzed. Subsequently, a pharmacological interaction network for HLT was built. Furthermore, the expressed genes of patients with GBM were obtained from Gene Expression Omnibus database and screened. A protein-protein interaction network was then constructed for both sets of data and they were combined with a topology method for analysis. Finally, the screened genes were subjected to enrichment analysis and pathway analysis. A total of 386 candidate targets and 7 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were screened, which were mainly associated with amino acid metabolism. Gene Ontology enrichment analysis and KEGG signal pathway analysis indicated that these targets are involved in anti-apoptosis, anti-oxidative stress, multicellular biological processes and other physiological and pathological processes related to the occurrence and development of GBM. In conclusion, the present results indicated that the mechanisms of action of HLT against GBM involve multiple targets and signaling pathways that are related to tumorigenesis and progression. The present study not only provided a novel theoretical basis for Traditional Chinese Medicine to treat tumors but also novel ideas for the treatment of GBM.

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Introduction

Gliomas are the most common central nervous system tumors in adults, accounting for 44.69% of intracranial tumors and 70% of them are malignant gliomas (1). The incidence of glioma is ~6.04 cases per 100,000 individuals in 2013, and although this incidence is not high, the mortality rate is very high (2), and therefore, glioma may be considered dangerous. Due to the particularity of the intracranial structure, gliomas exhibit high invasiveness, a high recurrence rate and high resistance to traditional radiochemotherapy, and under the most ideal treatment conditions, the prognosis of patients with glioma is still not ideal (3). The survival time of patients with GBM is only 12-15 months, the vast majority of patients die within 2 years and the 5-year survival rate is <5% (2,4). Due to the characteristics of invasive growth and progressive malignancy of gliomas, it is difficult to completely remove

the tumor with surgical treatment. Adjuvant therapies, such as radiation therapy and chemotherapy can only eliminate tumor cells or delay recurrence to a certain extent and can also cause certain damage to the patient's body (5,6). Although numerous advances have been made in the standardized as well as individualized comprehensive treatment of malignant gliomas, the clinical efficacy remains limited (7).

In the clinic, certain Traditional Chinese Medicine prescriptions have been used to treat serious diseases such as tumors (8). Huang-Lian-Tang (HLT) is a classic Chinese herbal formula. The major ingredients are *Coptis chinensis*, *Hebeninos commutaverunt* and *Gingiberi exaruit*. In China and other Asian countries, HLT has a certain or a subtle effect in numerous diseases (such as tumor, diabetes, arthritis, ischemic stroke and liver disease) (9). Previous studies have indicated that activation of immune cells and metabolic reprogramming the inflammatory response are involved in the mechanism of action (9-11). Studies have also suggested that one of the major components of HLT, *Huanglian*, has a valuable effect in the treatment of Alzheimer's disease and brain injury (12,13). Furthermore, *Huanglian* has also been effective in treating certain types of cancer (14-17).

To improve the treatment of gliomas, the present study turned to Chinese medicine to identify whether it is able to inhibit key molecular targets and signaling pathways associated with the occurrence aggressive growth behavior of gliomas to provide novel approaches for their clinical treatment. The anti-proliferative effect of HLT on cancer cell growth has been demonstrated in human myeloma cells (8-10). However, it has remained elusive whether HLT has any effect on gliomas and their therapeutic targets. In the present study, network pharmacological systems analysis technology was used to explore and analyze the multi-component, multi-target and multi-pathway interaction laws and regulatory networks involved in the inhibition of glioma cell growth, cell-cycle arrest and apoptosis induced by HLT. The present study lays a foundation for future in-depth study of the mechanism of action of HLT in the treatment of gliomas and the development of novel valuable drugs and may provide novel approaches for the clinical treatment of GBM.

Materials and methods

Data preparation. Drug data were obtained from the Traditional Chinese Medicines Systems Pharmacology database (TCMSP; http://tcmspw.com/tcmsp.php) (18). TCMSP is a unique systems pharmacology platform of Chinese herbal medicines that captures the relationships between drugs, targets and diseases. The database includes chemicals, targets and drug-target networks, and associated drug-target-disease networks, as well as pharmacokinetic properties of natural compounds, including oral bioavailability, drug-likeness, intestinal epithelial and blood-brain-barrier permeability, and aqueous solubility.

Enrichment analysis was performed using the Database for the Annotation, Visualization and Integrated Discovery database (DAVID; https://david.ncifcrf.gov/) using tumor gene data from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo).

Traditional Chinese Medicine ingredient screening and target detection. In order to obtain the molecular drug composition of HLT, the TCMIP database was searched to retrieve the name of each active component included in HLT and their chemical structural similarity to commercially available drugs was compared using the US Food and Drug Administration (https://www.fda.gov/). The standard of oral bioavailability ≥30% and drug-likeness ≥0.18 was used to screen Chinese medicine ingredients and target analysis was performed. Using the aforementioned criteria, pharmaceutical ingredients that serve a therapeutic role were screened.

Gene screening. The GEO datasets (GSE108474, GSE109857, GSE143263, GSE145940 and GSE150956) included for glioblastoma cells or tissues were compared with those for normal brain tissue in order to identify differentially expressed genes in glioma. In addition, only datasets containing more than 20 samples were selected. The titles and summaries of 350 potentially relevant datasets were screened to identify eligible datasets for further evaluation. Finally, only GSE108474 (72 samples) were selected for further analysis. The GSE108474 dataset based on the GPL570 platform was downloaded from the NCBI GEO database, containing the data of 20 normal brain tissues and 52 tumor samples. The Limma version 3.11 software package (https://bioconductor.org/packages/limma/) was used to screen genes that were differentially expressed between the high-risk group and the low-risk group (adjusted P-value <0.01, fold change at least x2).

Drug-associated active ingredient inspection and target screenings. The names of the major ingredients of HLT, namely *Coptis chinensis*, ume and *dried ginger*, were inputted in the TCMSP database and the chemical composition of each drug was retrieved. The screening conditions were based on oral bioavailability (OB; ≥30%) and drug-likeness (DL ≥0.18), so as to obtain the drug composition and targets. Numerous recent studies have shown that the screening criteria can screen out effective pharmaceutical ingredients (19-22).

Drug target-GBM network construction. The drug-disease network was constructed using the Bisogenet (23) plug-in of Cytoscape software (version 3.7.2) for drug targets that were active through TCMSP and the differentially expressed genes in glioma identified from the GEO dataset. It contains six PPI databases, including their Interaction Protein Database, the Biological Universal Repository of Interaction Data Sets, Human Protein Reference Database, the IntAct Molecular Interaction Database, the Biomolecular Interaction Network Database and the Molecular Interaction Action database. Two PPI interactive networks were built and illustrated using Cytoscape software, including the screened HLT target and the known GBM target. After combining these two networks into candidate networks, topology analysis was used to gradually screen out the central network.

Network topology analysis. The analysis was performed by using the CytoNCA (24) plug-in in Cytoscape software by determining these 6 metrics: Degree centrality (DC), topology intermediateness (BC), closeness centrality (CC), feature vector centrality (EC), local average connectivity (LAC), and network
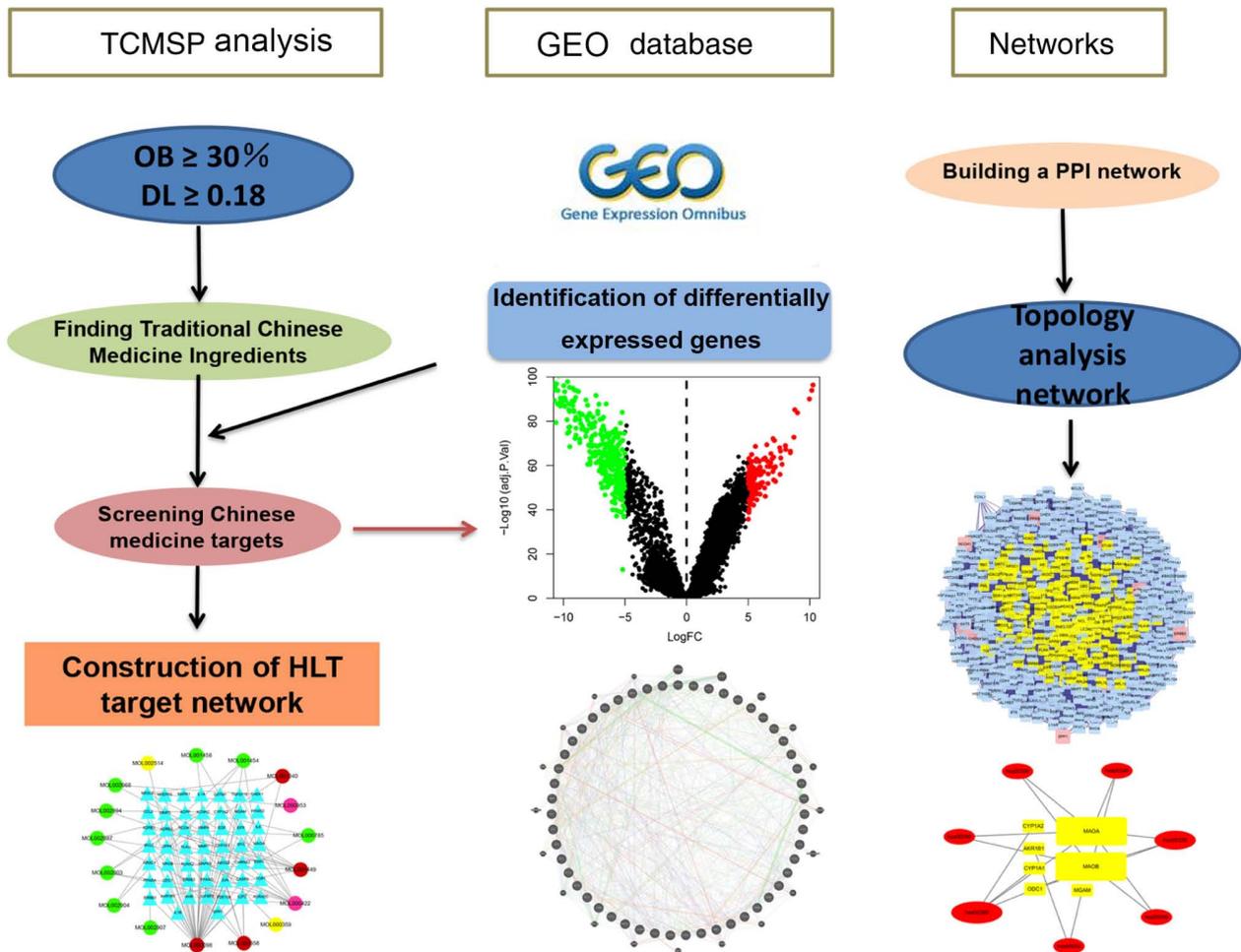
Figure 1. Flowchart of the systematic profiling of the alternative splicing in glioblastoma multiforme in the present study. TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB, oral bioavailability; DL, drug-likeness; GEO, Gene Expression Omnibus database; PPI, protein-protein interaction; HLT, Huang-Lian-Tang.

centrality (NC). The six metrics provide an in-depth analysis of the attributes of all nodes in the interactive network. The definition and calculation equations of all parameters reflect the significance of the nodes. A higher quantitative value indicates a greater significance in the network. Through the above methods, the desired central network was gradually selected.

Screening the collection of core Protein-Protein Interaction (PPI) networks. The tightly connected area of molecular complexes in the huge PPI network obtained was defined as topological modules with pure network characteristics (25,26). The aggregation of similar nodes and related nodes of drugs in the same group are defined as the pharmacology modules. Networks that destroy cell functions or lead to the GBM phenotype are defined as pathogenic modules. In the topology analysis, pharmacology modules and pathogenic modules are defined in the same network. Thus, the pathogenic module is considered to play a role in interference and destruction. The final core PPI network cluster was obtained by analyzing the corresponding network using the MCODE plug-in in Cytoscape software (26).

Gene ontology (GO) and pathway enrichment analysis. Functional enrichment analyses of the screened genes were performed through GO analysis (27) in three categories, namely biological processes (BP), cellular component (CC) and molecular function (MF), and DAVID was used to perform gene and genomic encyclopedia of the Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis. Functional terms and pathways with $P \leq 0.05$ were considered important. The ggplot2 plugin package for R software (vision 3.6.2) was used to visualize the results.

Results

Research process. The present study was divided into three phases in sequence, and Fig. 1 presents all of the processes in the systematic analysis. First, the drug components of HLT were downloaded from TCMIP and the components were analyzed to build a drug network. Furthermore, the data of patients with GBM were downloaded from GEO and the differentially expressed genes were screened to construct a network. Next, the two networks were merged together and a topology analysis was applied to filter out the desired core network. The network contained a total of 171 nodes and 6,309 edges. Finally, GO and KEGG analyses were performed on these targets to determine the mechanism of action of HLT against GBM.

Table I. Top 10 most upregulated genes.

Gene name	logFC	Average expression	t	P-value	Adjusted P-value	B
RPS25 (Ribosomal Protein S25)	10.26617	10.03903	186.0101	5.71×10^{-100}	3.86×10^{-97}	200.7867
STARD7 (StAR Related Lipid Transfer Domain Containing 7)	10.16257	9.964207	171.7686	1.96×10^{-97}	9.94×10^{-95}	197.2763
YTHDF1 (YTH N6-Methyladenosine RNA Binding Protein 1)	9.965356	9.821776	151.0904	2.36×10^{-93}	8.60×10^{-91}	191.1616
QRICH1 (Glutamine Rich 1)	8.783873	8.968483	128.8207	2.76×10^{-88}	6.65×10^{-86}	182.8256
CRKL (CRK Like Proto-Oncogene, Adaptor Protein)	9.000407	9.124869	123.2493	7.00×10^{-87}	1.51×10^{-84}	180.383
KHDC4 (KH Domain Containing 4, Pre-MRNA Splicing Facto)	8.712287	8.916781	86.2513	1.42×10^{-75}	1.58×10^{-73}	158.9653
MRPL33 (Mitochondrial Ribosomal Protein L33)	7.022553	7.696419	84.3207	7.39×10^{-75}	7.83×10^{-73}	157.5233
SYF2 (SYF2 Pre-MRNA Splicing Factor)	7.127974	7.772555	82.1461	4.94×10^{-74}	5.05×10^{-72}	155.8492
PYGO2 (Pygopus Family PHD Finger 2)	6.51068	7.326732	77.2753	4.18×10^{-72}	3.91×10^{-70}	151.8939
PHF2 (PHD Finger Protein 2)	8.01312	8.411828	76.1175	1.25×10^{-71}	1.12×10^{-69}	150.9093

There were a total of 2,751 upregulated genes. FC, fold change.

Identification of differentially expressed genes in GBM. The dataset GSE108474 was selected and downloaded from the GEO database as a sample, which contained the data of 20 normal tissues and 52 tumor tissues. In order to screen for differentially expressed genes, a differential expression analysis on the data was performed obtained using the limma software package. By performing this analysis, it was determined that compared with normal brain tissue samples, a total of 5,491 genes were significantly differentially expressed in GBM, 2,751 of which were upregulated genes and 2,740 were downregulated genes. Tables I and II list the top 10 most upregulated and downregulated genes, respectively. According to the fold change, Ribosomal Protein S25 (RPS25), StAR Related Lipid Transfer Domain Containing 7 (STARD7) and YTH N6-Methyladenosine RNA Binding Protein 1 (YTHDF1) are the three genes with the highest degree of upregulation, while High Mobility Group Nucleosomal Binding Domain 2 (HMGN2), Staufen Double-Stranded RNA Binding Protein 1 (STAU1) and Ferritin Heavy Chain 1 Pseudogene 5 (FTH1P5) were the three genes with the highest degree of downregulation. A heat map (Fig. 2A) and a volcano plot (Fig. 2B) were drawn to visualize the differentially expressed genes.

Chemical composition of HLT and traditional Chinese medicine target prediction. A total of 468 chemical ingredients of 7 Chinese herbs in HLT were collected through the TCMIP database of Chinese medicinal ingredients, and similarity scores with drugs in the DrugBank database were obtained. Similar drugs with a score ≥0.8 were considered to be highly similar to the chemical ingredients contained in HLT. A total of 3,939 'component-target' pairs of HLT were retrieved. Among them, there were 14 components of *Coptis chinensis* with 87 predicted targets, 38 components of *dried ginger* with 26 predicted targets, 12 components of cinnamon sticks with 57 predicted targets, 41 components of *Pinellia ternata* with 237 predicted targets, 72 components of *jujube* with 1,172 predicted targets, 158 components of ginseng with 2,000 predicted targets and 133 components of licorice with 360 predicted targets. The components and targets in the obtained associations were assigned to the single drugs contained in HLT to obtain the target information corresponding to each drug. Among them, *Coptis chinensis* had 67 component targets and *Guizhi* had 46 component targets. *Dried ginger*, *Pinellia ternata*, *ginseng*, *jujube* and *licorice* each contained 7, 196, 876, 429, 115 component targets. The common targets between the two constituent drugs were analyzed (Table III). The results suggested that there were different numbers of common targets among the 7 components, of which 57 were common targets of *Coptis chinensis* and Ginseng, accounting for 85.1% of the total number of targets of *Coptis chinensis*. There were 152 targets for *Pinellia ternata* and ginseng.

Table II. Top 10 most downregulated genes.

Id	logFC	Average expression	t	P-value	Adjusted P-value	B
HMGN2 (High Mobility Group Nucleosomal Binding Domain 2)	-12.813	3.559164	-296.316	8.37×10^{-115}	1.19×10^{-110}	216.6827
STAU1 (Staufen Double-Stranded RNA Binding Protein 1)	-11.6436	3.234342	-290.265	3.80×10^{-114}	2.70×10^{-110}	216.1427
FTH1P5 (Ferritin Heavy Chain 1 Pseudogene 5)	-12.9861	3.607250	-272.269	4.17×10^{-112}	1.98×10^{-108}	214.3746
CHERP (Calcium Homeostasis Endoplasmic Reticulum Protein)	-11.5667	3.212962	-260.047	1.21×10^{-110}	3.86×10^{-107}	213.0173
RP5-930J4.4 (RP5 Pre-MRNA Splicing Factor)	-12.3119	3.419980	-259.647	1.36×10^{-110}	3.86×10^{-107}	212.9705
RPL34 (Ribosomal Protein L34)	-12.4815	3.467075	-251.274	1.51×10^{-109}	3.56×10^{-106}	211.9538
UBA3 (Ubiquitin Like Modifier Activating Enzyme 3)	-11.0044	3.056778	-243.291	1.61×10^{-108}	2.96×10^{-105}	210.9144
RPL27A (Ribosomal Protein L27a)	-12.5865	3.496262	-243.174	1.67×10^{-108}	2.96×10^{-105}	210.8986
BTBD1 (BTB Domain Containing 1)	-11.792	3.275543	-241.309	2.93×10^{-108}	4.63×10^{-105}	210.645
NSUN2 (NOP2/Sun RNA Methyltransferase 2)	-10.8641	3.017812	-240.118	4.21×10^{-108}	5.99×10^{-105}	210.481

There were a total of 2,740 downregulated genes. FC, fold change.

Compound regulation network of HLT. A compound regulation network of the active components and molecular relationships of HLT was built and 766 related events and 68 nodes were obtained. There are 17 drug nodes (round nodes; *dried ginger* is yellow, *Coptis* is green, *ume* is pink, and various drug combinations are red) and 51 molecular nodes (triangular nodes). The core target network was mapped through Cytoscape (Fig. 3).

Construction and analysis of PPI network. With the rise of network pharmacology, its role in systems biology research is increasing. In the present study, the HLT drug target network and the PPI network for GBM were fused (Fig. 4A and B). In order to reveal the pharmacological mechanism of HLT against GBM, Cytoscape software was used to combine two huge networks to build a new and complex network (2,954 nodes and 72,642 edges), which resembled a merge of the HLT and GBM networks (Fig. 4B). Subsequently, the CytoNCA plugin in Cytoscape was used to confirm the core goals. The selection criteria were set as follows: BC value >450, CC >0.6, DC >70, EC >0.02, NC >20 and LAC >18 (Fig. 4C and D). Finally, a core network comprising 171 nodes and 6,309 edges was screened out.

Enrichment analysis and KEGG signaling pathways of candidate targets for HLT against glioma. The 386 candidate targets for HLT in treating GBM were subjected to a GO analysis in the DAVID database to explore the links between the functional units, their potential significance in the biological systems network. The mechanism of action is clarified through this functional enrichment analysis The GO terms were determined in the following categories: Biological processes (Fig. 5A), cellular component (Fig. 5B) and molecular function (Fig. 5C). In the category biological process, the candidate genes were associated with response to nutrient levels, response to steroid hormone, response to lipopolysaccharide and response to molecule of organism process. In the category cellular component, enriched terms were transcription factor complex, RNA polymerase II transcription factor complex, nuclear transcription factor complex, myelin sheath, nuclear chromatin and apical plasma membrane. Finally, in the category molecular function, genes were associated with repression of transcription factor binding and RNA polymerase II transcription factor binding. A KEGG enrichment analysis was also performed on these genes, revealing that they are mainly related to amino acid metabolism (Fig. 5D).

KEGG relationship network construction. To clarify the overall mechanisms of HLT in the treatment of gliomas, the above-mentioned steps provided a complete approach based on the current knowledge of the pathogenesis of gliomas (Fig. 6). Through layer-by-layer screening, 7 genes (oval) and 7 KEGG
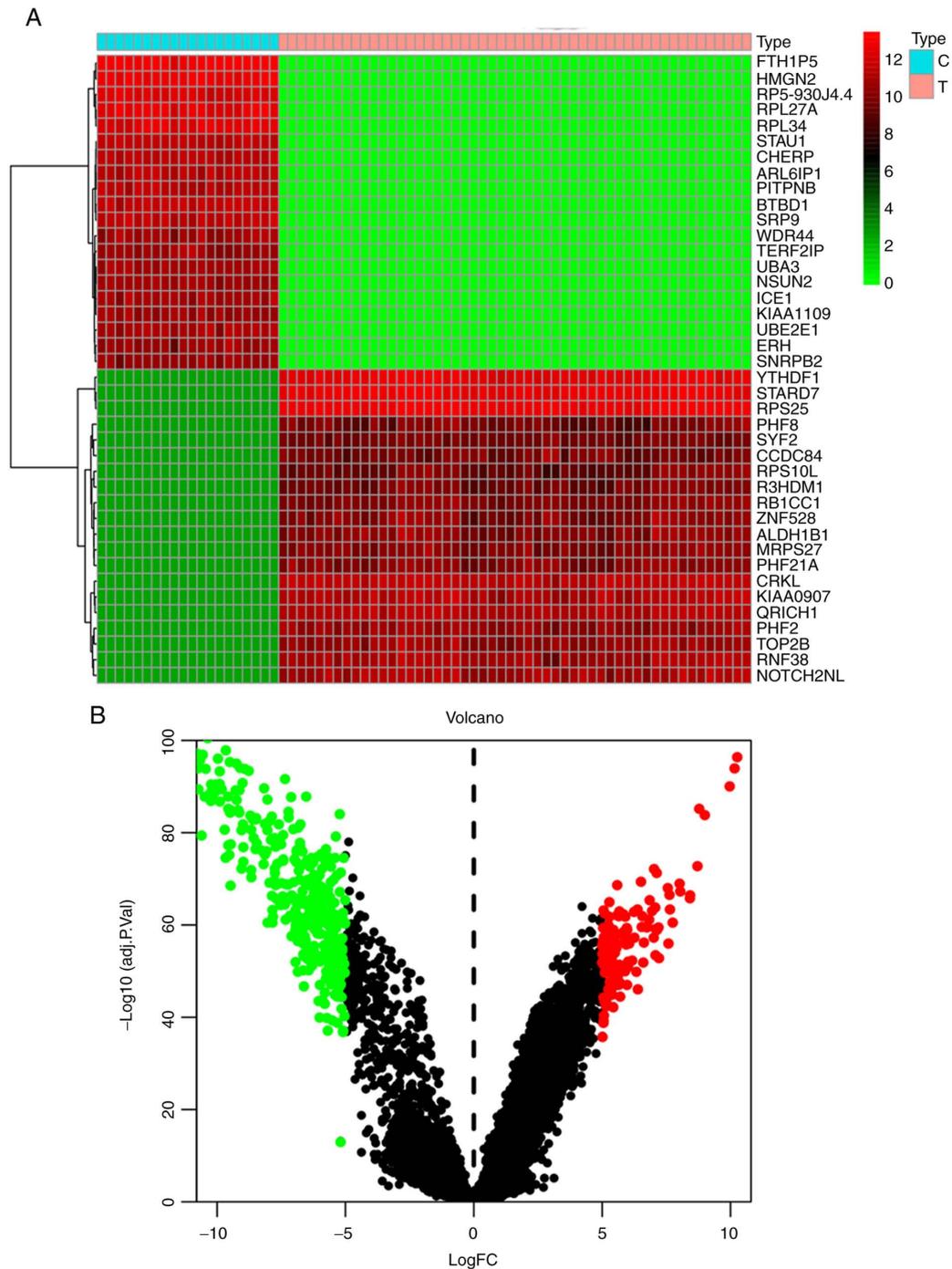
Figure 2. Heatmap and volcano plot of the differentially expressed genes. (A) Heatmap of the genes with differential expression between tumor and control samples. (B) Volcano plot. Black dots indicate genes that are not differentially expressed between 20 normal brain tissue samples and 52 tumor samples, and red and green dots indicate genes that are up- and down-regulated in tumor samples, respectively. FC, fold change; C, control; T, tumor; P.val, P-value.

signaling pathways (rectangular) associated with them were obtained; the size of the symbols is associated with the number of adjacent nodes. The 7 genes were MAOA (Monoamine Oxidase A), MAOB (Monoamine Oxidase B), MGAM (Maltase-Glucoamylase),CYP1A2 (Cytochrome P450 Family 1 Subfamily A Member 2), AKR1B1(Aldo-Keto Reductase Family 1 Member B), CYP1A1(Cytochrome P450 Family 1 Subfamily A Member 1) and ODC1(Ornithine Decarboxylase 1). The 7 KEGG signaling pathways were hsa00380, hsa0052, hsa00260, hsa00330, hsa00340, hsa00350 and hsa00360.

Discussion

In the present study, target genes associated with GBM were first screened. It was analyzed which genes influence the occurrence, development and prognosis of GBM. The active components of HLT were also identified through an online pharmacology database. Combining the interaction networks of the two provided the potential mechanisms of action of HLT in the treatment of GBM. The present study provided a foundation for further research on Traditional Chinese Medicine treatments for GBM to improve the prognosis of affected

Table III. Common pharmacology-targets between any two components of Huang-Lian-Tang.

Component (target number)	Dried ginger (7)	Ginseng (876)	Guizhi (46)	Jujube (429)	Coptis chinensis (67)	Pinellia (196)	Licorice (115)
Dried ginger (7)	/	7	0	1	0	1	0
Ginseng (876)	7	/	22	265	57	152	110
Guizhi (46)	0	22	/	20	7	26	24
Jujube (429)	1	265	20	/	57	28	105
Coptis chinensis (67)	0	57	7	57	/	7	54
Pinellia (196)	1	152	26	28	7	/	24
Licorice (115)	0	110	24	105	54	24	/
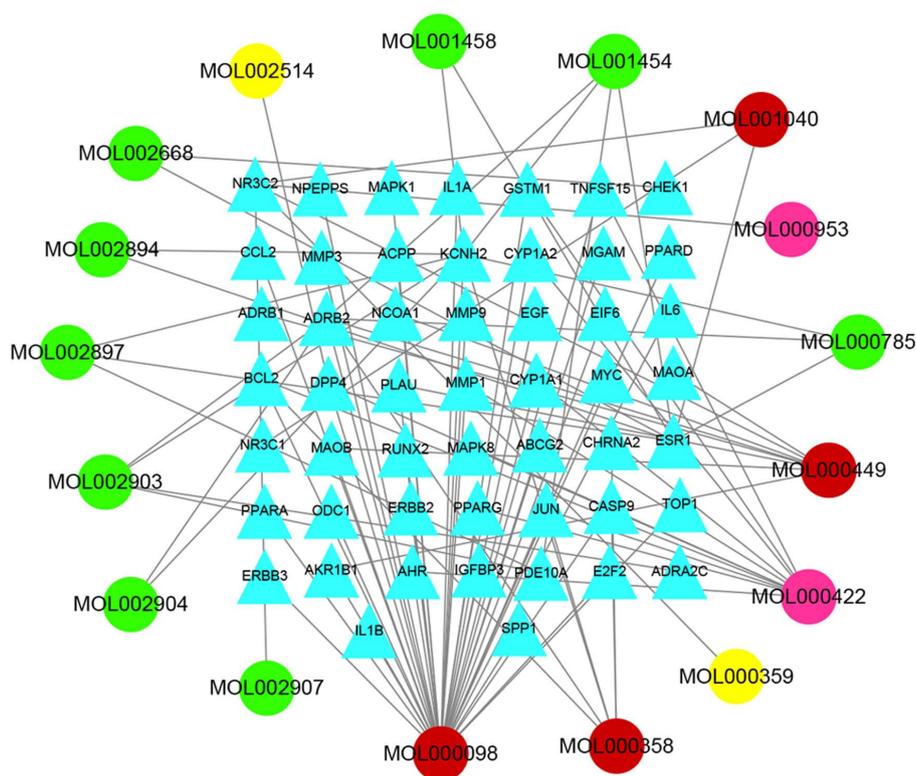
Figure 3. Network of active components of Huang-Lian-Tang and molecular targeting relationships. There are 17 drug nodes (round nodes, *dried ginger* is yellow, Coptis is green, *ume* is pink, and various drug collections are red) and 51 molecular nodes (triangular nodes).

patients. The target genes of HLT identified in the present study hold promise for the development of novel targeted therapies for GBM.

Glioma is characterized by rapid growth and resistance to treatment, and patients usually have a poor prognosis (28). Diffuse glioma is the most common intracranial malignancy, accounting for >60% of cases (29). GBM is the most common and malignant type of primary brain tumor, accounting for ~80% of malignant astrocytomas (30). GBM cells vary in shape and size, which is known as glioblastoma polymorphisms (30). GBM may develop rapidly from undiagnosed, less malignant precursor lesions or may progress slowly from preexisting low-grade glioma (31). In spite of the progress in the treatment of GBM, including chemotherapy, the prognosis remains poor (32,33). Therefore, it is necessary to identify

potential pathways for the development of this cancer type and to prevent the occurrence of GBM.

In the previous decades, scientists have studied the potential activity of drugs and conducted basic research or clinical trials for tumor treatment (34). The introduction of the concept of big data and the continuous development of pharmacology has provided the opportunity to analyze the relationship between drugs and molecular targets of diseases *in silico* (35). Network pharmacology is helpful for exploring multi-channel signaling pathway regulation, improve drug efficacy and the success rate of clinical trials and reduce drug development costs. Chinese herbal medicines and plant ingredients have positive prospects in the treatment of a variety of complex diseases (36,37). Network pharmacology has been extensively applied to study the
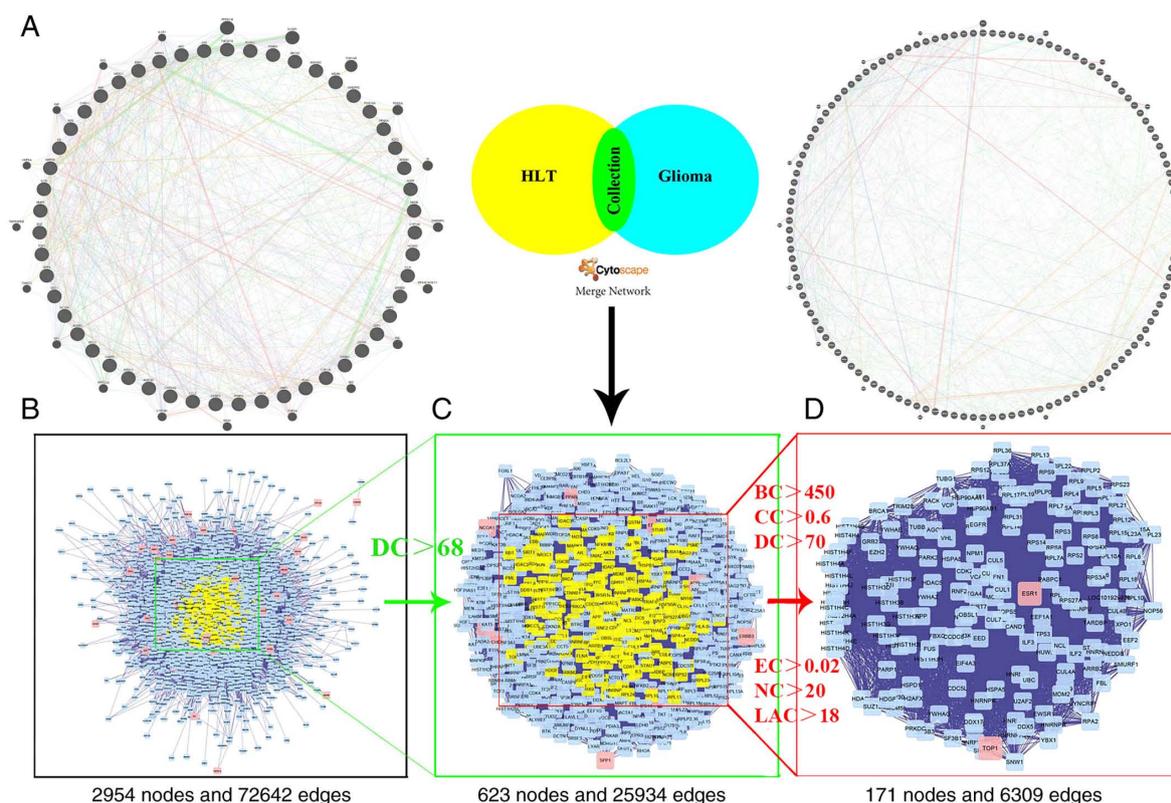
Figure 4. Identification of a core PPI network for the mechanism/drug-target interactions. (A) Construction of two PPI networks, one for HLT targets and the other for glioblastoma multiforme. (B) The interactive PPI network of HLT and glioblastoma multiforme targets comprising 2,954 nodes and 72,642 edges. (C) PPI network of significant proteins extracted from B; this network comprises 623 nodes and 25,934 edges. (D) PPI network of significant proteins extracted from C; this network is made up of 171 nodes and 6,309 edges. BC, betweenness centrality; CC, closeness centrality; DC, degree centrality; EC, eigenvector centrality; NC, network centrality; LAC, local average connectivity; PPI, protein-protein interaction; HLT, Huang-Lian-Tang.

biological mechanisms of certain Traditional Chinese Medicine prescriptions and ingredients (38,39). Therefore, the network pharmacology method was used to understand the biological mechanisms of action HLT against GBM at the molecular level.

In southeast Asia, HLT has a significant role in the treatment of brain diseases. Studies have indicated that HLT is able to nourish the nerve cell, restore brain function and improve blood circulation in the brain (40). Studies have also suggested that HLT is an anti-apoptotic, anti-oxidant and anti-inflammatory agent (41-43). HLT has also been indicated to promote neurotrophic factor expression (44) and regulate neurogenesis of neural precursor cells (45), which is conducive to the recovery of neural function. In the present study, not only network pharmacology, but also bioinformatics were used, and a topology analysis was performed. The central network with 171 nodes and 6,309 edges was screened out. GO analysis was performed and HLT was indicated to be involved in numerous biological processes associated with tumorigenesis and development, including RNA polymerase transcription factor complex and response to nutrient levels. The KEGG pathway analysis of the core PPI network was used to identify pathways by which HLT exerts its effects in treating GBM by regulating tumorigenesis and progression. According to the P-value of each enriched pathway and its role in GBM, there were seven related signaling pathways that were significant, and these pathways were all closely associated with the amino acid metabolism of tumorigenesis

and progression. It is well known that cancer cells promote the 'Warburg effect' (46), which is enhanced glycolysis or aerobic glycolysis, even when the surrounding oxygen supply is sufficient. In addition, it has been determined that dysfunctional anabolism/catabolism of fatty acids and amino acids, particularly glutamine, serine and glycine, have a role as metabolic regulators in supporting cancer cell growth (47,48). Furthermore, extensive crosstalk was reported between dysfunctional metabolic networks and cancer cell signaling (49,50).

The occurrence and development of tumors are related to a large number of cellular processes, which ultimately lead to the formation or recurrence of tumors, including the induction of cell cycle processes, evasion of apoptotic programs and activation of cell survival pathways (51). In the present study, it was observed that HLT disrupts the process by which these cancer cells are being established, thereby limiting their growth *in vitro* and *in vivo*. It remains elusive how HLT specifically reduces tumor growth under normal cellular conditions, so that it does not cause any systemic imbalances or serious side effects for patients. However, it may be speculated that individual active ingredients in the prescription have a mutual synergy, and that the tumor cells are affected by drugs at a concentration below the lethal dose for normal cells.

There are still certain deficiencies in the present study that require to be addressed. First, the present study was only performed *in silico*. In the next step, a pharmacology model
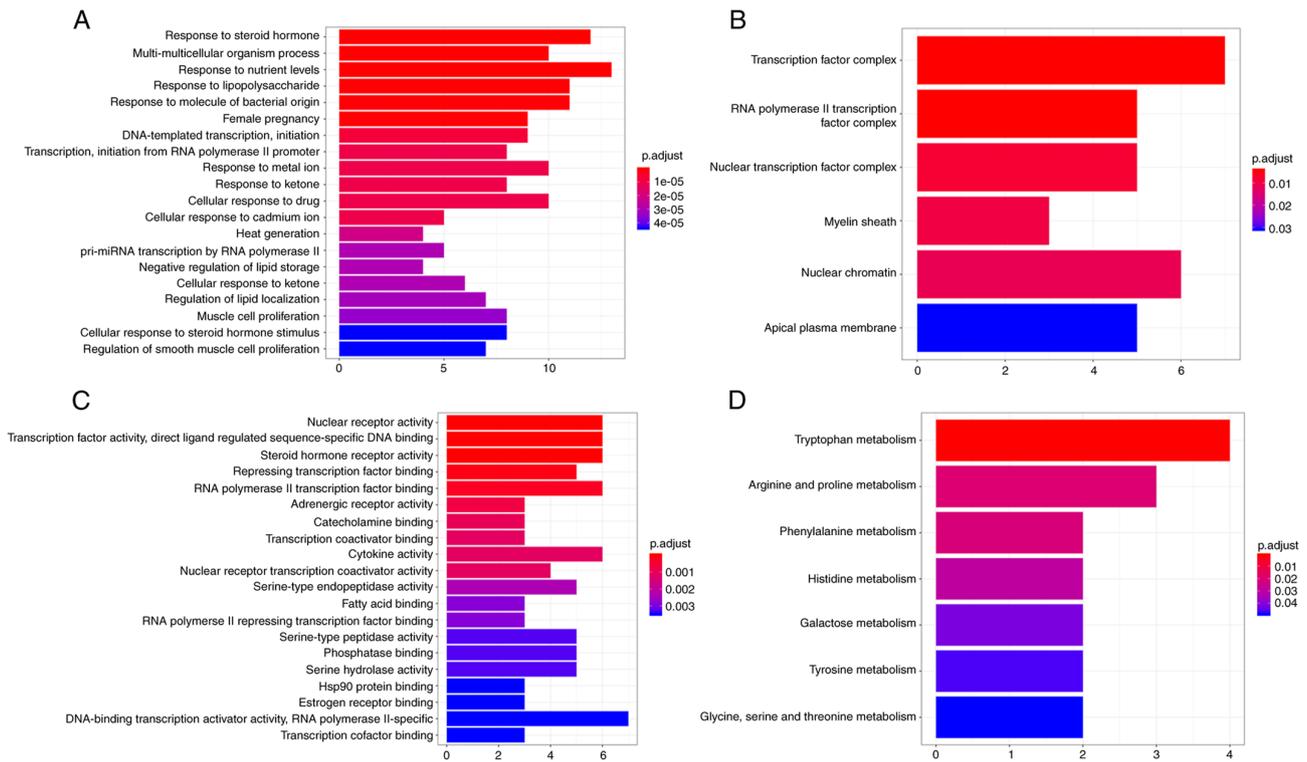
Figure 5. GO analysis and KEGG pathway analyses were performed on screened genes. The top 10 terms in (A-C) the GO categories (A) biological process, (B) cellular component and (C) molecular function, and (D) KEGG pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes. P. adjust, adjusted P-value; HSP, heat shock protein.
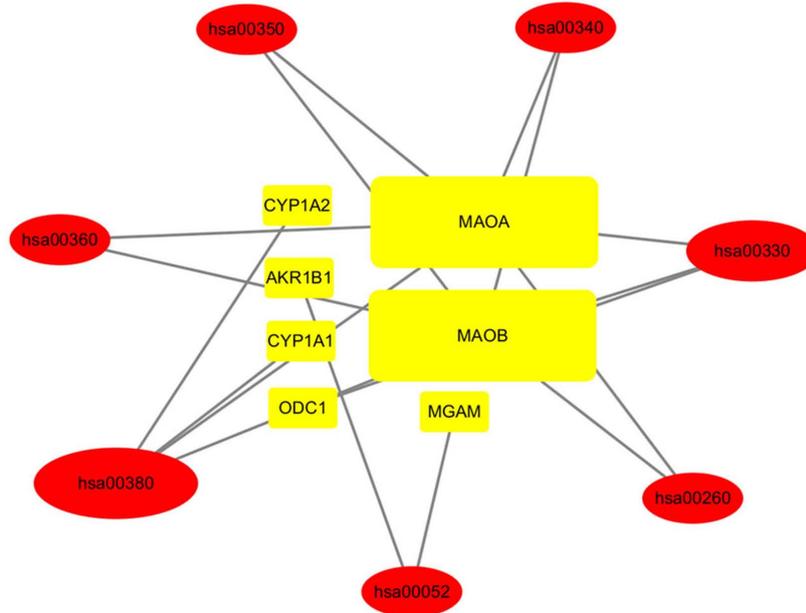
Figure 6. Network of compounds of Huang-Lian-Tang-drug targets-atherosclerosis targets-signaling pathways. A total of 7 genes (oval nodes) and 7 Kyoto Encyclopedia of Genes and Genomes signaling pathways (rectangular nodes) associated with them were obtained. The size of the nodes is proportional to the number of adjacent nodes. Hsa, *Homo sapiens*. MAOA, Monoamine Oxidase A; MAOB, Monoamine Oxidase B; MGAM, Maltase-Glucoamylase; CYP1A2, Cytochrome P450 Family 1 Subfamily A Member 2; CYP1A1, Cytochrome P450 Family 1 Subfamily A Member 1; AKR1B1, Aldo-Keto Reductase Family 1 Member B; ODC1, Ornithine Decarboxylase 1.

will be built to further confirm the therapeutic effect of HLT on GBM through basic experiments. Furthermore, the optimal dosage of HLT for treating GBM patients and contraindications require to be established. In the future, in-depth research

on the most important targets or pathways of the final drug candidates will be performed.

In conclusion, the present network pharmacology analysis on the effect of HLT in GBM indicated that it may be a promising

chemotherapeutic drug whether it is able to slow/inhibit tumor growth and recurrence and whether it is used as an adjuvant treatment for patients subjected to other standard treatments remains to be elucidated. HLT have few side effects (52), so they may be used in GBM-associated clinical trials.

Availability of data and materials

The datasets generated and analyzed during the current study are available in the [GEO and TCM] repositories, [https://www.ncbi.nlm.nih.gov/geo/;https://tcmspw.com/tcmsp.php].

Authors' contributions

Conceptualization: WRL, WHX and CYL. Data collection and interpretation: WRL, WHX, JFX and RYH. Formal analysis: HNH, XJL, WRL, WHX, JFX and KH. Funding acquisition: HNH, XJL, WRL and WHX. Methodology: WRL, WHX, CYL, JFX and RYH. Project administration: HNH, XJL, KH, HNH, XJL, JFX and RYH. Resources: HNH, XJL, JFX, RYH and KH. Supervision: JFX, KH and RYH. Writing: WRL, WHX, CYL, JFX, RYH and KH. Review and editing: HNH and XJL. Responsible for all aspects of the work to ensure that issues related to the accuracy or completeness of any part of the work are properly investigated and resolved: HNH and XJL. All authors were involved in writing the manuscript and read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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