Original Article

Network pharmacology analysis of the mechanism of Huisheng oral liquid in the treatment of lung cancer

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Background: To study the active ingredient and possible mechanism of Huisheng oral liquid in the treatment of lung cancer by network pharmacology.

Methods: The active ingredient and drug targets of Huisheng oral liquid were screened using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and Traditional Chinese Medicine Integrated Database (TCMID), and lung cancer targets were screened using the Gene Expression Omnibus (GEO) database. The drug targets of the effective components of Huisheng oral liquid were matched with disease targets and the obtained intersecting targets were imported into the Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) database to construct a protein-protein interaction (PPI) network. R software and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used for Gene Ontology (GO) and KEGG enrichment analyses, and Cytoscape software was used to construct a Huisheng oral liquid component target-lung cancer target network. The function and pathway of the therapeutic target of Huisheng oral liquid for lung cancer were analyzed.

Results: A total of 1,376 differentially expressed genes (DEGs) of lung cancer were obtained, and 185 potential effective components of Huisheng oral liquid in the treatment of lung cancer were obtained, including quercetin, luteolin, kaempferol, and baicalein. There were 36 intersecting targets between Huisheng oral liquid and lung cancer, and the key targets for lung cancer treatment were CDKN1A, CCNB1, MDM2, CDK1, ErbB2, E2F1, EGFR, etc. Huisheng oral liquid mainly regulates the p53 signaling pathway.

Conclusions: The mechanism of Huisheng oral liquid in the treatment of lung cancer is mainly reflected in regulating tumor cell apoptosis, inhibiting angiogenesis, and improving immunity.

Keywords: Huisheng oral liquid; lung cancer; network; pharmacology; key targets

Submitted Aug 02, 2022. Accepted for publication Sep 08, 2022.
doi: 10.21037/tcr-22-2077
View this article at: https://dx.doi.org/10.21037/tcr-22-2077

Introduction

According to the latest data from the National Cancer Center in 2021, the death rate from lung cancer has almost halved, mainly due to a decrease in smoking and improvements in early diagnosis and treatment, especially new drugs for non-small cell lung cancer. Drug therapies such as traditional Chinese medicine (TCM), chemotherapy drugs, small molecule targeting drugs, and large molecule

monoclonal antibody drugs all play an important role.

Clinically, TCM is primarily used in adjuvant radiotherapy and chemotherapy, targeted therapy, palliative treatment, and other first-line treatment programs. Huisheng oral liquid is modified from Hua zheng Hui sheng dan, which is a classical Chinese medicine prescription published in "*Wen bing Tiao Bian*" that is widely used in tumor treatment. In recent years, many clinical trials have studied the effect of Huisheng oral liquid on improving immune function and inhibiting tumor angiogenesis in patients with lung cancer (1,2). It was found that Huisheng oral liquid could enhance immune function, inhibit tumor angiogenesis and tumor cell proliferation, improve the quality of life of patients, and enhance the efficacy and reduce the toxicity of combined chemotherapy. But the mechanism of Huisheng oral liquid on lung cancer is not clear.

The present study analyzed the differentially expressed genes (DEGs) in lung cancer by using the gene expression omnibus (GEO) database and the network pharmacology method to predict the pharmacological mechanism of Huisheng oral liquid in the treatment of lung cancer. We identified the drug target of Huisheng oral liquid, thus providing a scientific basis for its clinical application and further research.

Methods

Database and software

The following databases and software were used in this study: Traditional Chinese Medicine Integrated Database (TCMID) (http://www.megabionet.org-/tcmid/), GEO (https://www.ncbi.nlm.nih.gov/geo/), Traditional Chinese Medicine Systems Pharmacology Data base and Analysis Platform (TCMSP) (http://lsp.nwu.edu.cn/tcmsp.php), Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp), Cytoscape (3.6.0) software (USA) (https://cytoscape.org), Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) (https://string-db.org), UniProt (https://www.uniprot.org), and GEO2R (https://www.ncbi.nlm.nih.gov/geo/-geo2r/). This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Screening lung cancer DEGs

We used the GEO2R platform to screen the lung cancer mechanism dataset and employed the ggplot2 R language package (USA) to construct a volcano map. The statistical analysis threshold was set to an adjusted P<0.05 and |log2 Fold Change| >1; an adjusted P<0.05 and log2 Fold Change >1 indicated that the DEG was significantly up-regulated. Following adjustment, P<0.05 and Log2 Fold Change <–1 indicated that the DEG was significantly down-regulated. FunRich software was used to obtain the common DEGs between two datasets, and a Wayne diagram was constructed using FunRich to display the intersecting DEGs.

In the UniProt database, the species was set as human and the standard name corresponding to the target gene was screened.

Screening the active ingredient and targets of Huisheng oral liquid

Huisheng oral liquid contains the following 34 kinds of TCM: leonur iherba, zanthoxyli pericarpium, carthami flos, hirudo, sappan lignum, angelicae sinensis radix, sparganii rhizoma, sichuan lovage rhizome, anemone raddeanae, dalbergia odorifera lignum, cyperus rotundus, alpinia officinarum hance, ginseng radix et rhizoma, myrrha, curcumae longae rhizoma, rhei radix et rhizoma, armeniacae semen amarum, artemisiae argyi folium, rehmanniae radix praeparata, ferulae resina, cinnamomi cortex, toxicodendri resina, euodiae fructus, typhae pollen, olibanum, corydalis rhizoma, paeoniae radix alba, trionycis carapax, caryophylli flos, Faeces Trogopterori, tabanus, foeniculif fructus, persicae semen, and perillae fructus.

The above TCMs were searched in the TCMSP and TCMID databases, and toxic metabolokinetics (ADME) screening was performed to identify the chemical components contained in these TCMs. Based on the predicted oral bioavailability (OB) and drug likeness (DL) values of each ingredient, OB ≥30% and DL ≥0.18 were taken as the screening thresholds, and text mining was performed to characterize the screened chemical ingredients as potential effective components of Huisheng oral liquid. The corresponding targets of the effective components were downloaded, and the English names of the effective components were obtained via the PubChem database and a literature search.

Construction of drug-disease target protein-protein interaction (PPI) networks

The target sets of effective components of Huisheng oral

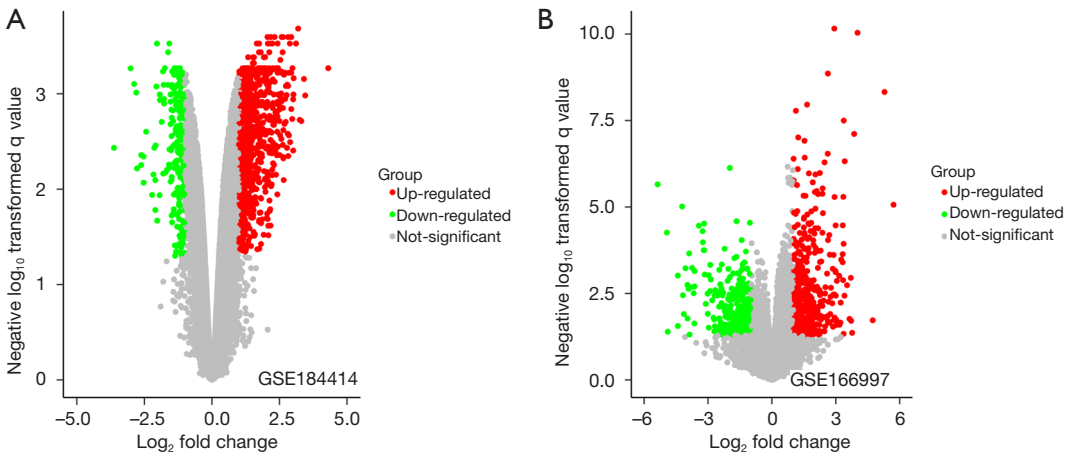

Figure 1 Volcanic maps of lung cancer gene expression.

liquid and lung cancer were cross-examined to obtain the effective target set of Huisheng oral liquid in the treatment of lung cancer. The name of the effective target was entered into the STRING database and the human species was selected, the isolated node was ignored, and relationships with a combined score >0.4 were finally selected. The PPI network was subsequently obtained.

DEGs were analyzed for GO and KEGG enrichment

Normalized drug-disease target gene names were transformed into UniProt ID. R software and the Gene Ontology (GO) and KEGG databases were used for enrichment analysis of the target functions and pathways.

Construction of drug-regulated disease interaction network

Cytoscape (3.6.1) software was used to construct the drug-regulated disease interaction network, and edge links were used to represent the interactions between the TCMs, effective components, targets, diseases, signaling pathways, and biological processes (BPs). The Network Analysis Cytoscape plug-in was used to analyze the Network topology.

Results

DEGs in lung cancer

Two lung cancer mechanism datasets, GSE184414 and GSE166997, were screened on the GEO2R platform, and a total of 1,376 DEGs were identified in these two datasets. GSE184414 contained 2,790 up-regulated genes and 342 down-regulated genes, while GSE166997 contained 713 up-regulated genes and 613 down-regulated genes. The ggplot2 software package of R language was used to draw the respective volcano maps; the red dots represented significantly up-regulated genes and the green dots represented down-regulated genes (*Figure 1*).

Effective components and targets of Huisheng oral liquid

Using the TCMSP and TCMID databases, the compounds and corresponding targets of each TCM component in Huisheng oral liquid were obtained. A total of 431 effective components met the screening conditions. Leonur Iherba contains eight effective components; Carthami Flos contains 22 effective components; Zanthoxyli Pericarpium contains five effective components; Angelicae Sinensis Radix contains two effective components; SparganIIRhizoma contains five effective components; Anemone Raddeanae contains five effective components; Sichuan Lovage Rhizome contains seven effective components; Dalbergia odorifera lignum contains 37 effective components; CyperusRotundus contains 18 effective components; Ginseng Radix et Rhizoma contains 22 effective components; Alpinia Officinarum Hance contains 13 effective components; curcumae LongaeRhizoma contained three effective components; Myrrha contains 45 effective components; Armeniacae semen amarum contains 19 effective components; Rhei Radix et rhizoma contains 16 effective components; Perillae

fructus contains 16 effective components; foeniculifFructus contains three effective components; Persicae semen contains 23 effective components; Caryophylli Flos contains six effective components; Corydalis rhizoma contains 49 effective components; Paeoniae Radix Alba contains 13 effective components; typhaePollen contains eight effective components; Olibanum contains eight effective components; Euodiae fructus contains 30 effective components; Artemisiae Argyi Folium contains nine effective components; and Rehmanniae Radix Praeparata contains two effective components. The above active components corresponded to 3,137 targets.

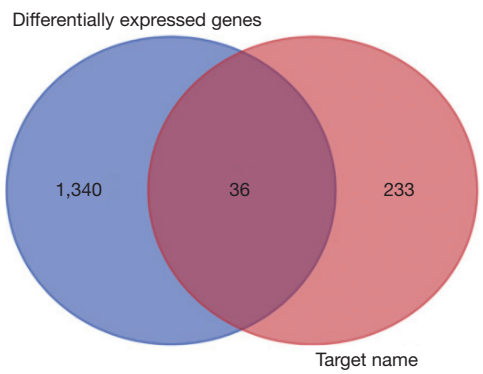

Figure 2 Venn diagram.

Screening of therapeutic targets of Huisheng oral liquid for lung cancer and construction of the PPI network

The intersecting corresponding targets of the effective components of Huisheng oral liquid and the DEGs of lung cancer were obtained (*Figure 2*). Thirty-six targets of Huisheng oral liquid for lung cancer were obtained, and these targets corresponded to 185 effective components (*Table 1*). These 36 target genes were imported into the STRING database to establish the PPI network (*Figure 3*).

GO analysis and KEGG signaling pathway analysis of the target genes

The cytological component (CC) analysis showed that the DEGs were mainly enriched in the extracellular space, cytoplasm, endoplasmic reticulum, nucleus, and spindle microtubules (*Figure 4*). The molecular function (MF) analysis results showed that the DEGs were mainly enriched in enzyme binding, protein binding, protein kinase activity, protein kinase binding, ubiquitin protein ligase binding, etc. (*Figure 5*). Also, the BP analysis results showed that the DEGs were mainly enriched in cell hypoxia reaction, drug reaction, negative regulation of apoptosis, positive regulation of fibroblast proliferation, DNA damage reaction, and cell cycle arrest caused by the signal

Table 1 The top 10 effective components of Huisheng oral liquid

Molecule ID	Effective components	OB%	DL	Number of targets
MOL000098	Quercetin	46.43	0.28	21
MOL000006	Luteolin	36.16	0.25	14
MOL000422	Kaempferol	41.88	0.24	8
MOL002714	Baicalein	33.52	0.21	7
MOL000787	Fumarine	59.26	0.83	5
MOL002565	Medicarpin	49.22	0.34	5
MOL002961	(3S)-7-hydroxy-3-(2,3,4-trimethoxyphenyl)chroman-4-one	48.23	0.32	5
MOL002981	Duartin	70.63	0.34	5
MOL002985	Isoduartin	74.11	0.34	5
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39	5

OB, oral bioavailability; DL, drug-likeness.

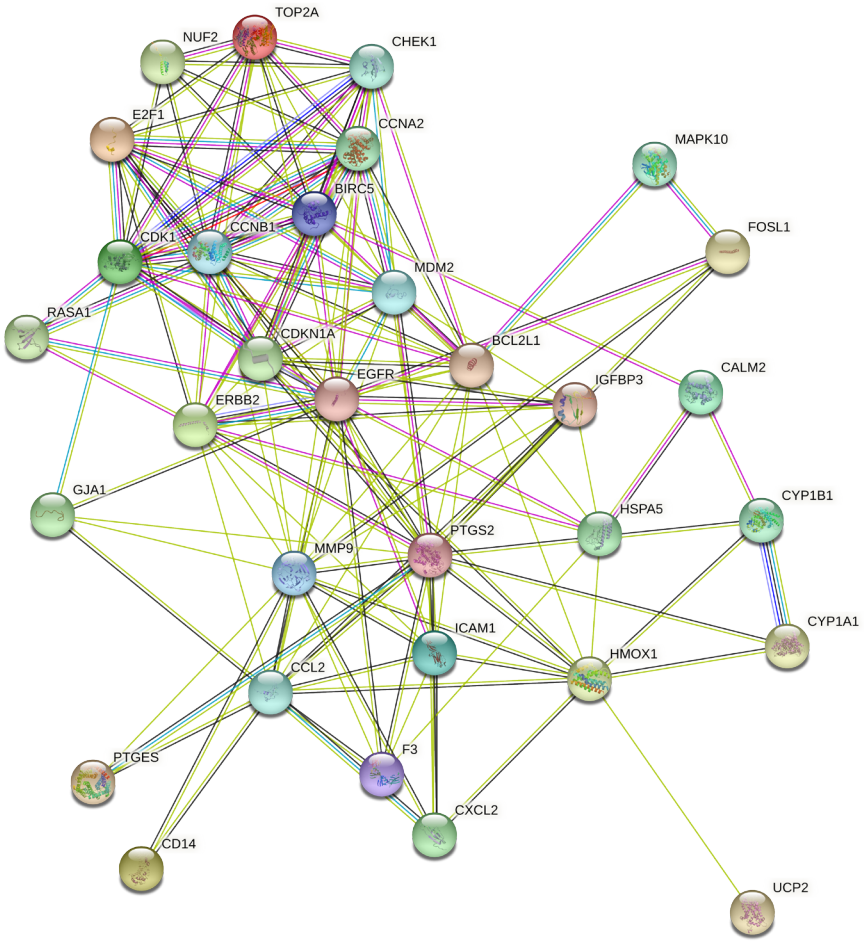

Figure 3 PPI network. PPI, protein-protein interaction.

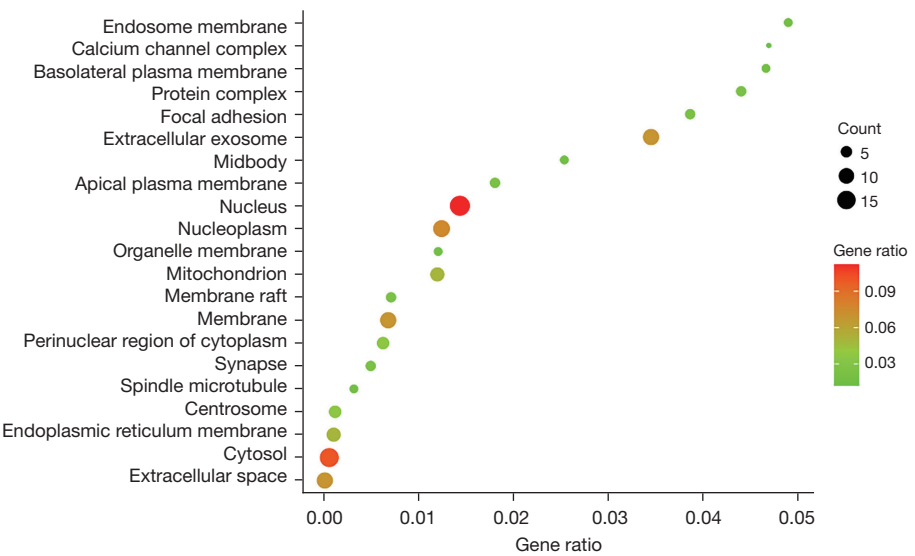

Figure 4 Enrichment analysis of CC. CC, cytological components.

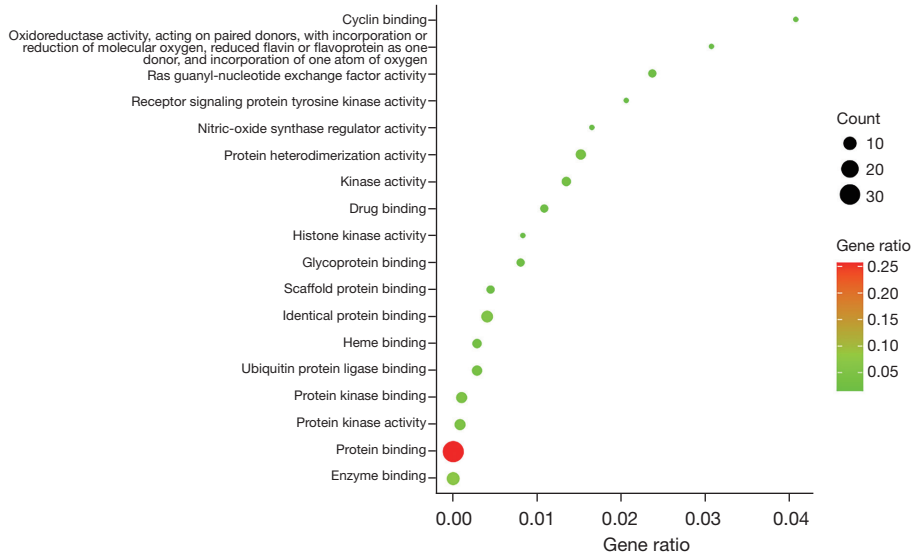

Figure 5 Enrichment analysis of MF. MF, molecular function.

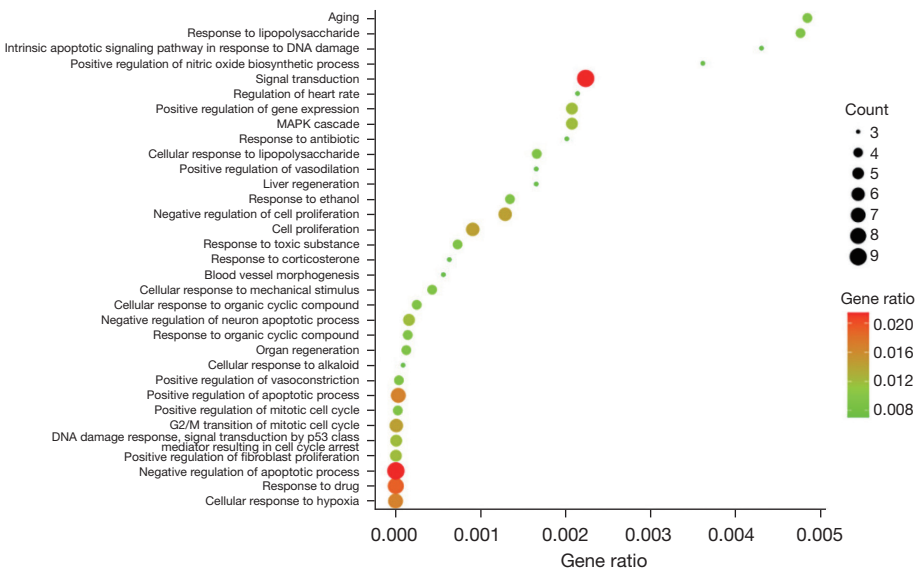

Figure 6 Enrichment analysis of BP. BP, biological processes.

transduction of p53 mediators (*Figure 6*).

Twenty-nine related signaling pathways were obtained by KEGG enrichment analysis (*Figure* 7). Among them, the enrichment degree of the p53 signaling pathway and pathways in the cancer was the highest. The p53 signaling pathway targets were as follows CDKN1A, CCNB1, IGFBP3, CHEK1, MDM2, and CDK1. The cancer target pathways included MAPK10, CDKN1A, ERBB2, MDM2, E2F1, BIRC5, PTGS2, MMP9, EGFR, and BCL2L1. The key targets of Huisheng oral liquid in the treatment of lung cancer included CDKN1A, CCNB1, MDM2, CDK1, ERBB2, E2F1, and EGFR.

Network diagram of lung cancer regulation by Huisheng oral liquid

The data of drug-disease target, effective components molecule ID, drug, target-signal pathway, and target-

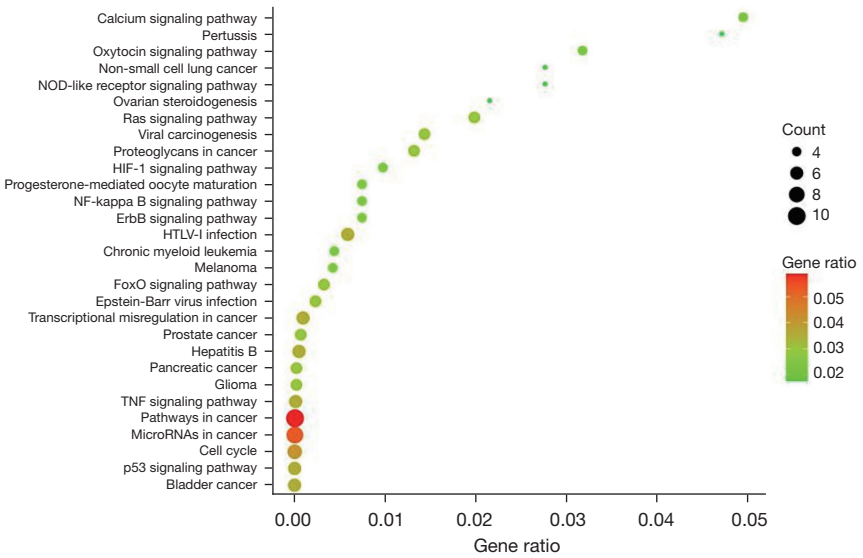

Figure 7 KEGG signaling pathway analysis. KEGG, Kyoto Encyclopedia of Genes and Genomes.

biological process were imported into Cytoscape (3.6.0), and the association function (Merge) in this software was used to obtain the network diagram of Huisheng oral liquid's regulation of lung cancer (*Figure 8*).

Discussion

Network pharmacology is a new subject that reveals the effect of TCM on the regulatory network of the body at the systemic level, and it provides a bridge for the study of the relationship between TCM and modern pharmacology. In the field of TCM, network pharmacology is used to screen the active components of TCM and compound prescription, to predict the target of utility, to analyze the drug target and pathway of compound prescription in treating diseases, and to analyze the potential mechanism of compound prescription in treating diseases. Network pharmacology has promoted the development of drug reorientation, natural small molecule compounds with good drug properties, new compound and secondary development of Chinese patent medicines. It provides the possibility for the new use of old drugs and the design of multi-target drugs, which is one of the strategic contents of the modernization development of TCM.

Based on the topological analysis of the "component-target-disease" network of the 185 effective components and 36 intersecting targets, we found that quercetin, luteolin, kaempferol, and baicalein may be the effective components in Huisheng oral liquid for the treatment of lung cancer. These components are flavonoids. Quercetin inhibits lung cancer A549 cell proliferation by up-regulating microRNA-16 and down-regulating claudin-2 protein and mRNA expression (3). It also inhibits lung cancer A549 cell growth by regulating Aurora B (4), and promotes apoptosis by up-regulating Trailr, Fas, and the tumor necrosis factor receptor and down-regulating cell the survival-related genes Ikk and Akt (5). Furthermore, quercetin inhibits lung cancer invasion and metastasis via the Src/fibroblast growth factor-inducible molecule 14/nuclear factor-kappa B (Src/Fn14/NF-κB) pathway by down-regulating Src, Fn14, P-IkBa kinase B (P-IKKB), and NF-κB expression and up-regulating IkBa expression (6).

Luteolin can inhibit the growth of A549 human lung cancer cells, induce cell arrest in the G1 phase (7), and reduce the activities of protein kinase, PLK1, cyclin B1, CCNA2, PCNA, CDKN1A, CCND1, CDC2, and CDK2, and decrease the expression of anti-apoptotic genes (8,9). It is also involved in tumor cell repair and regulates cell signal cascade reactions (10).

kaempferol regulates the expression of the MicroRNA-21/phosphatase and tensin homolog (miR21/PTEN) signaling pathway-related genes, including miR21, PTEN, P-AKT, C-myC, and Cyclin D1, and inhibits the proliferation of lung cancer A549 cells (11). It also provides targeted inhibition of (su(var)-3-9,enhancer-of-zeste,trithorax) domain-containing protein 7/9 (SET7/9), activation of the protein phosphatase 2A/protein kinase B/glycogen synthase kinase 3 (PP2AC/AKT/GSK-3β)

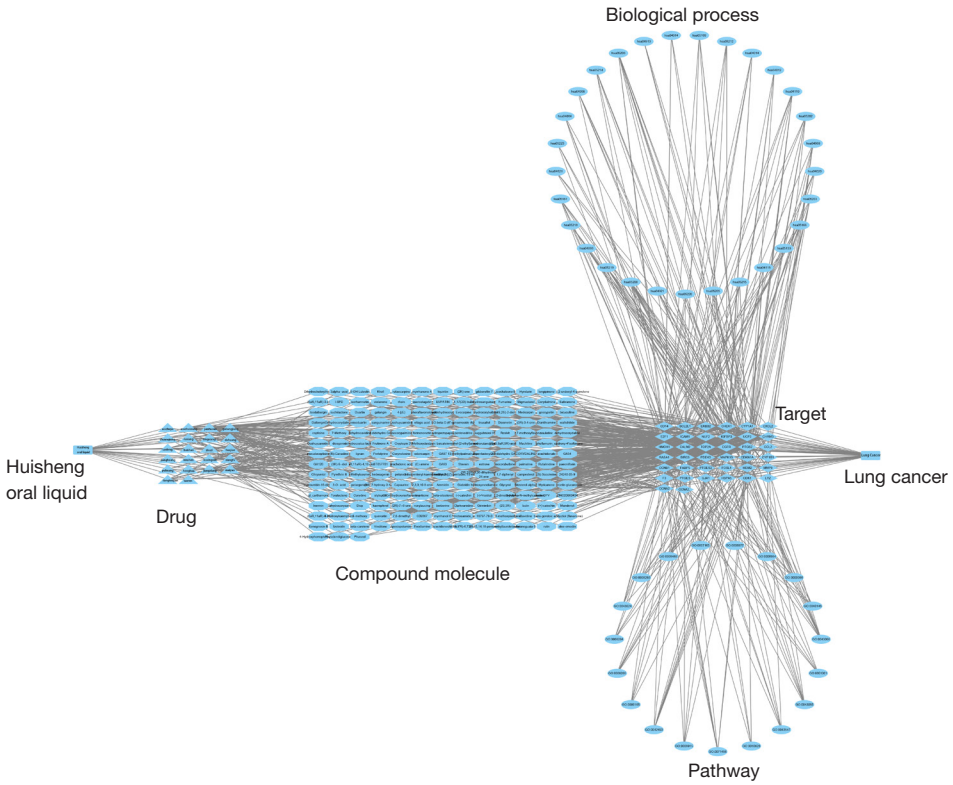

Figure 8 Huisheng oral liquid therapy for lung cancer compound target pathway- disease network.

signaling pathway, promotes the release of Cytochrome C from mitochondria, and accelerates tumor cell apoptosis. The expression of vascular endothelial growth factor (VEGF) and endothelial growth factor can be inhibited via the extra cellular signal-regulated kinase/nuclear factor-kappa B/c-MYC/P21(ERK/NF-κB/C-MYC/P21) pathway, and the expression of VEGF and angiogenesis can be inhibited (12), thereby exerting an anti-tumor effect.

Baicalein down-regulates the expression of CyclinD1 and CDK1, up-regulates the expression of cadherin mRNA and protein in epithelial cells, hinders the invasion of A549 and H1299 cells and epithelial-mesenchymal transformation, and inhibits the Notch signaling pathway (13). It also down-regulates miR-424-3p, targets the PTEN/PI3K/Akt pathway, and inhibits tumor cell growth (14). Moreover, baicalein reduces the protein expression of 12-lipoxygenase and VEGF and inhibits lung cancer cells by regulating multiple pathways (15). Most of these key ingredients belong to flavonoids, and thus, flavonoids may become key drugs for lung cancer treatment.

The PPI network results showed that the core targets of Huisheng oral liquid in the treatment of lung cancer included CDKN1A, CCNB1, MDM2, CDK1, ErbB2, E2F1, EGFR, etc. CDKN1A, a cyclin-dependent kinase inhibitor 1A, inhibits CDK4/6 complex and CDK2, mediates p53-dependent G1/S growth stagnation, and induces apoptosis (16). CDK1 is cyclin-dependent kinase 1, CCNB1 is cyclin-b1, and CCNB1 is an important cell cycle regulator that is related to the G2/M detection point. CCNB1 regulates CDK1, initiates cells from the G1/S phase to the G2/M phase, and promotes mitosis. MDM2 is a major endogenous negative regulator of p53, which affects the degradation, nucleation, and mitochondrial transport of p53, and MDM2 deletion promotes the activation of p53-dependent apoptosis-related genes (17). The kinase catalytic regions of ErbB2 and EGFR exhibit high homology and similar biochemical dynamics, and these two signaling pathways play a synergistic role in tumorigenesis. ErbB2 gene mutations form a heterodimer with EGFR and promote cell proliferation (18). Also, E2F1 plays the role of an oncogenic gene and exerts a key function in regulating cell cycle progression and apoptosis. After activation of E2F1, cells enter the S phase from the G1 phase, and after entering the S phase, E2F1 is inactivated and cells then

enter the G2 phase (19). In terms of cell apoptosis, E2F1 activates P14/P19, prevents p53 degradation, inhibits downstream CDK activity, interrupts the cell proliferation cycle, and induces cell apoptosis. In addition, E2F1 regulates the expression of VEGF and participates in and plays an inhibitory role in angiogenesis (20). The above targets are mainly related to cell cycle and angiogenesis, indicating that Huisheng oral liquid can regulate tumor cell apoptosis and inhibit angiogenesis in the treatment of lung cancer.

According to the GO analysis results, cell hypoxia response, negative regulation of apoptosis, ubiquitin protein ligase binding, protein kinase, positive regulation of fibroblast proliferation, DNA damage response, and p53 signaling leading to cell cycle arrest were confirmed to be closely related to lung cancer. Cell hypoxia activates the hypoxia signaling pathway, and p53 interacts with this pathway to affect the occurrence and development of tumors. Under hypoxic stress, t1/2 of the p53 protein is increased, which accumulates and is activated in cells. P53 selectively inhibits transcription or induces target genes, and regulates cell cycle arrest, apoptosis, autophagy, DNA repair, cell migration/invasion, and other cellular responses. Xiao *et al.* (21) found that Huisheng oral liquid reduces Cyclin D1, the key protein of the Wnt pathway in lung cancer cells, blocks lung cancer cells in the G1 phase, and inhibits the growth of lung cancer cells in lactacystin model mice. Liu *et al.* (22) showed that Huisheng oral liquid can significantly down-regulate the expression of VEGF in the serum of advanced lung cancer and inhibit the angiogenesis of lung cancer. Jia *et al.* (23) showed that Huisheng oral liquid up-regulates the serum levels of Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-18 (IL-18), and transforming growth factor-α (TNF-α) in lung cancer, enhances immune function, and assists chemotherapy in the treatment of advanced non-small cell lung cancer.

The KEGG pathway analysis results showed that Huisheng oral liquid was most highly correlated with the p53 signaling pathway, the cancer signaling pathway, the cell cycle, miRNAs in cancer, the TNF signaling pathway, etc. MiRNAs in exosomes play an important role in the occurrence, development, invasion, and metastasis of lung cancer, as well as the phenotype and function of immune cells. Malregulated expression of miRNA can promote the occurrence, angiogenesis, invasion, and metastasis of lung cancer and immune escape. MiR449a targets E2F3, up-regulates miR449a to inhibit E2F3, and causes cell cycle arrest and senescence (24). Mir-660 and miR641 promote lung cancer cell apoptosis by targeting the MDM2 upstream gene of the p53 pathway (25). Also, MiR494 promotes lung cancer angiogenesis by targeting tensin homologues and phosphatases (26). MiR23a induces hypoxia-inducible factor-1α by targeting prolyl hydroxylase 1 and prolyl hydroxylase 2, activates the VEGF pathway, and induces angiogenesis in lung cancer (27). Moreover, miRNAs regulate the related functions of adaptive and innate immune cells. MiRNAs such as miR243p, miR106a5p, and MIR20a5P inhibit T-helper1 differentiation, down-regulate the mitogen-activated protein kinase (MAPK) pathway, and affect the secretion of cytokines such as IL-6, interferon-γ (IFN-γ), IL-2, and IL-10. MiRNAs reduce the antitumor effect (28). Baicalein exerts anti-lung cancer function by down-regulating the mir424-3p expression level, up-regulating the expression level of the tumor suppressor gene PTEN, and inhibiting the phosphorylation of the PI3K/AKT pathway (29).

In conclusion, Huisheng oral liquid achieves its therapeutic effect on lung cancer via multiple pathways and targets, primarily by regulating tumor cell apoptosis, inhibiting angiogenesis, and improving immunity. Through network pharmacology analysis, the possible mechanism of Huisheng oral liquid in the treatment of lung cancer can be comprehensively expounded, and the theoretical basis for the clinical application of Huisheng oral liquid can be provided. The components of TCM used in experiment or clinic are more complex than those of single TCM, so the accuracy of target obtained by network pharmacology method is lower. Therefore, it is necessary to verify whether the active ingredient of Huisheng oral liquid are consistent with the key targets of treating lung cancer in animal and cell experiments, which is our next work plan.

Acknowledgments

The authors acknowledge AME Editing Service for editing the manuscript.
Funding: This study was supported by the Hebei Province Foundation for "333 Talent Project" (No. A202001019), the Hebei Province Natural Science Foundation project (No. H2021405002), and the Scientific Research Project of the Hebei Administration of Traditional Chinese Medicine (No. 2021184).

Footnote

Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2077/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editor: A. Kassem)

Cite this article as: Yan J, Ma N, Liu KQ, Qiao TT, Wang Y, Xue JX, Hao XQ, Zheng MD. Network pharmacology analysis of the mechanism of Huisheng oral liquid in the treatment of lung cancer. Transl Cancer Res 2022;11(9):3287-3297. doi: 10.21037/tcr-22-2077

