Research Article

Network Pharmacology-Based Strategy for Predicting Therapy Targets of Traditional Chinese Medicine Xihuang Pill on Liver Cancer

Xu Zhao,1 Jian Hao,2 and Sinuan Chen1

1Department of Traditional Chinese Medicine, Shenzhen Longgang Central Hospital, Shenzhen, Guangdong Province 518116, China
2Clinical Cancer Therapy Center, The Fourth Central Hospital of NanKai University, Hebei District, 300140 Tianjin, China

Correspondence should be addressed to Xu Zhao; zhaoxutougao@163.com

Received 14 June 2019; Revised 16 January 2020; Accepted 22 January 2020; Published 16 March 2020

Academic Editor: George B. Lenon

Copyright © 2020 Xu Zhao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. To investigate the potential therapy targets and pharmacological mechanism of traditional Chinese medicine (TCM) Xihuang pill in liver cancer based on network pharmacology. Methods. Drug ingredients-target network was constructed based on the target sets of Xihuang pill and liver cancer. The overlapping genes between Xihuang pill targets and liver cancer-related molecular targets were investigated using comparative analysis. Moreover, the PPI network and module was constructed based on overlapping genes and hub nodes, respectively, followed by the pathway enrichment analysis. Results. A drug ingredients-target network was established with 1184 nodes and 11035 interactions. Moreover, a total of 106 overlapping genes were revealed between drug targets and liver cancer molecular targets. Furthermore, a PPI network and 4 modules were further investigated based on overlapping genes, respectively. These hub nodes such as VEGFA and EGFR were mainly enriched in GO functions including positive regulation of MAP kinase activity, activation of protein kinase activity, regulation of MAP kinase activity, and pathways like proteoglycans in cancer, bladder cancer, and estrogen signaling. Conclusion. VEGFA and EGFR might be potential therapy targets of Xihuang pill in liver cancer. Furthermore, the effect of Xihuang pill on liver cancer might be realized by targeting VEGFA and EGFR in pathways like proteoglycans in cancer and estrogen signaling.

1. Introduction

As the second leading cause of death, liver cancer has caused a wide social burden for a long period of time [1]. Although partial surgical resection is the optimal therapy strategy for patients with liver cancer, the recurrence rates after surgery are still very high [2, 3]. Thus, exploring the effective clinical treatment for liver cancer is necessary.

Traditional Chinese medicine (TCM) has been widely used for clinical treatment of various tumors such as liver cancer [4, 5]. Xihuang pill is a complementary and alternative medicine that has been used in TCM due to the inhibition for tumor cell proliferation [6]. Xihuang pill is composed of Ru Xiang (olibanum), Mo Yao (Commiphora myrrha), She Xiang (Moschus), and Niu Huang (calculus bovis), which exert multiple antitumor effects [7]. A previous study shows that Xihuang pill has an anticancer effect on breast tumor [8], gastric cancer, and primary liver cancer [7]. Actually, Xihuang pill could improve quality of life and clinical manifestations of advanced primary liver cancer patients [9]. A previous study indicates that there is a reversal effect of serum containing Xihuang pill on the multidrug resistance of liver cancer cells via P-glycoprotein pathway [10]. Although Xihuang pill is effective in treatment of liver cancer, the drug targets and pharmacological mechanisms of Xihuang pill on liver cancer are still unclear.

The network pharmacology analysis is a useful tool for further understanding of the drug action [11], which may
provide insights into how we can improve drug discovery for complex diseases [12]. In the current study, the potential mechanism of Xihuang pill on the treatment of liver cancer was analyzed by a network-based systematic study (as shown in Supplementary Figure 1). Briefly, the drug ingredients-target network was constructed based on the target sets of Xihuang pill and liver cancer. Then, the overlapping genes were investigated using comparative analysis. Moreover, the networks of protein-protein interaction (PPI) and modules were constructed based on overlapping genes and hub nodes, followed by the GO and KEGG pathway enrichment analysis. We hoped to explore the potential therapy targets of Xihuang pill in the treatment of liver cancer, which can provide the basis for the pharmacological mechanism study of Xihuang pill.

2. Materials and Methods

2.1. Identification of Ingredients of Drug. The chemical ingredients of Xihuang pill were collected from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database (http://lsp.nwsuaf.edu.cn/tcmsp.php) [13] and the Traditional Chinese Medicine Integrated Database (TCMID, http://www.megabionet.org/tcmid/) [14]. According to the relevant parameters of the pharmacokinetic properties, the ingredients were screened according to oral bioavailability (OB) and drug-likeness (DL) values and those ingredients with DL ≥ 0.18 and OB ≥ 30% were selected as the active ingredients [15, 16].

2.2. Drug Target. Usually, the ingredients of drugs play a role in related biological functions via targets. To predict the targets of ingredients of Xihuang pill, the small molecular structure information of active ingredients in Xihuang pill was retrieved on the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [17]. Subsequently, the targets were screened with a screening online tool called the Swiss Target Prediction (http://www.swisstargetprediction.ch/) [18]. Finally, all the target information was standardized using UniProt (http://www.UniProt.org/).

2.3. Drug Ingredients-Target Network Construction. The drug ingredients-target network was constructed by Cytoscape software (version: 3.0.0, http://chianti.ucsd.edu/cytoscape-3.0.0/) [11]. The topological parameters of the network, including the degree were analyzed using Degree Centrality (DC) based on CytoNCA software [19].

2.4. Liver Cancer Targets. The main source of disease targets for liver cancer was obtained from IPA (http://www.ingenuity.com). The targets of liver cancer were retrieved after deleting duplicate data. Then, the target datasets were compared by Geneweaver (http://www.geneweaver.org) [20], and the overlapping targets of both liver cancer and the drug ingredients were considered potential targets of Xihuang pill for the treatment of liver cancer.

2.5. PPI Network Construction and Module Analysis. According to STING database (http://www.string-db.org/) [21] with the species limited to “Homo sapiens” and the confidence score > 0.9, the protein interaction pairs associated with overlapping target genes between Xihuang pill and liver cancer were screened. And the PPI network was constructed via utilizing the network visualization software Cytoscape (version: 3.0.0, http://chianti.ucsd.edu/cytoscape-3.0.0/) [11], and the topological properties of the PPI network were analyzed through NetworkAnalyzer (default settings). Furthermore, MCODE (version 1.5.1) [22] was used to screen the significant enriched modules from the PPI network.

2.6. Hub Gene Investigation in PPI Network. The hub genes in PPI network were further investigated by a Cytoscape plugin cytoHubba [23], and 11 network topology parameters, including Degree, Edge Percolated Ingredients, Maximum Neighborhood Ingredients, Maximal Clique Centrality Bottleneck, Eccentricity, Closeness, Radiality, Betweenness, and Stress were selected for importance calculation. After screening the core nodes in PPI network, the rank sum ratio (RSR) [24] was used to rank the nodes synthetically to get the core nodes of the network. The more important the output of a node, the higher the value of this node in the network.

2.7. Enrichment Analysis for the Hub Genes. In order to reveal the potential biological function of Xihuang pill in the treatment of liver cancer, GO (Gene Ontology) functions [25] and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway [26] enrichment analysis were performed. Specially, GO terms were grouped into the following three categories: biological process (BP), molecular function (MF), and cellular component (CC) [25]. And the KEGG analysis was conducted according to the enrichment analysis tool of the Database for Annotation, Visualization and Intergrated Discovery (DAVID, version: 6.8) software [27]. A p value < 0.05 was considered as the cutoff criterion.

3. Results

3.1. Drug Ingredients-Target Network Analysis. A total of 53 ingredients and 1131 targets of Xihuang pill, as well as 566 molecular targets of liver cancer, were obtained in the current study. Based on these data, the drug ingredients-target network was constructed. As shown in Figure 1, the network consists of 1184 nodes and 11035 interactions (see details in Supplementary File 1). Moreover, the top 50 nodes including 3 targets were further selected to construct the module of the drug ingredients-target network (Figure 2; Supplementary File 2).

3.2. Overlapping Genes between Drug Targets and Liver Cancer Molecular Targets. After the comparative analysis, the overlapping genes, which might be potential drug therapy target for Xihuang pill in liver cancer, between drug targets and liver cancer therapy targets were obtained. The result
showed that a total of 106 overlapping genes (Attachment 1), including VEGFA, EGFR, ESR1, PLG, and MAPK3, were revealed in the current study.

Additionally, the KEGG pathway enrichment analysis showed that these overlapping genes were mainly enriched in pathways, such as metabolic pathways, pathways in cancer, proteoglycans in cancer, estrogen-signaling pathway, and HIF-1-signaling pathway. The top 20 pathways enriched by overlapping genes were listed in Table 1.

**3.3. PPI Network Analysis.** Based on the potential pharmacodynamic target of Xihuang pill for liver cancer, a PPI network was constructed by using the STRING. The result showed that there were 102 nodes and 766 interactions in the network (Figure 3). Moreover, a total of 4 modules including module 1 (score = 9.913), module 2 (score = 2.143), module 3 (score = 1.5), and module 4 (score = 1.333) were further investigated from PPI network using MOCDE (Figures 4(a)–4(d)).

**3.4. Hub Nodes Investigation.** According to the PPI network, the core nodes of the network were screened based on the characteristics of the network’s topological structure (Figures 5 and 6). The top 20 important nodes including VEGFA (RSR = 0.1218), EGFR (RSR = 0.1382), ESR1 (RSR = 0.1427), PLG (RSR = 0.1436), and MAPK3 (RSR = 0.1464) were summarized in Table 2. Moreover, the association among top 5 hub nodes, drug ingredients, and CTM was listed in Table 3.
3.5. Investigation of Pathways Enriched by Hub Nodes.

The top 5 hub nodes including VEGFA, EGFR, ESR1, PLG, and MAKP3 were used for further GO function and pathway enrichment analyses. The result showed that the hub genes could be assigned to different GO terms for BP, CC, and MF categories. The prominent functions enriched by hub genes were positive regulation of MAP kinase activity, activation of protein kinase activity, regulation of MAP kinase activity and phosphatidylinositol bisphosphate kinase activity, of which the top 10 functions for GO terms are presented in Figure 7. Moreover, these hub nodes were mainly enriched in pathways like proteoglycans in cancer (ID: 05205; count = 4; FDR = 8.93E-06), bladder cancer (ID: 05219; count = 3; FDR = 8.93E-06), and estrogen-signaling pathway (ID: 04915; count = 3; FDR = 6.48E-05). The top 10 pathways are listed in Table 4.

4. Discussion

Liver cancer is one of the most lethal cancers having worldwide prevalence, and the effect of clinical treatment for this disease is not satisfactory [28]. The current study, for the first time, explored the potential therapy targets of Xihuang pill on liver cancer based on network pharmacology analysis. The results showed that a drug ingredients-target network was established with 1184 nodes and 11035 interactions.

Figure 2: The module constructed by the TOP 50 nodes from the drug ingredients-target network. The triangle represented the ingredients of drug, and the square represented the targets of disease. The darker the color, the more significant it is.

Table 1: The top 20 pathways enriched by the overlapping genes between Xihuang pill targets and liver cancer molecular targets.

| ID   | Pathway description                  | Count | FDR       |
|------|-------------------------------------|-------|-----------|
| 1100 | Metabolic pathways                  | 35    | 2.81E-16  |
| 5200 | Pathways in cancer                  | 24    | 4.18E-19  |
| 5205 | Proteoglycans in cancer             | 18    | 4.38E-15  |
| 4151 | PI3K-Akt-signaling pathway           | 15    | 4.16E-09  |
| 4915 | Estrogen-signaling pathway           | 13    | 9.30E-14  |
| 4015 | Rap1-signaling pathway               | 13    | 1.04E-09  |
| 4066 | HIF-1-signaling pathway              | 12    | 9.29E-12  |
| 4510 | Focal adhesion                       | 11    | 1.12E-07  |
| 4014 | Ras-signaling pathway                | 11    | 2.20E-07  |
| 1230 | Biosynthesis of amino acids          | 10    | 9.36E-11  |
| 5218 | Melanoma                            | 10    | 9.36E-11  |
| 5215 | Prostate cancer                     | 10    | 5.39E-10  |
| 4068 | FoxO-signaling pathway               | 10    | 1.29E-08  |
| 5206 | MicroRNAs in cancer                 | 10    | 5.79E-08  |
| 5219 | Bladder cancer                      | 8     | 4.16E-10  |
| 4370 | VEGF-signaling pathway               | 8     | 1.34E-08  |
| 5212 | Pancreatic cancer                    | 8     | 1.64E-08  |
| 4914 | Progesterone-mediated oocyte maturation | 8 | 1.12E-07 |
| 4912 | GnRH-signaling pathway               | 8     | 2.20E-07  |
| 5210 | Colorectal cancer                    | 7     | 2.20E-07  |

Count, the number of genes enriched in certain pathways; FDR, false discovery rate.
Moreover, a total of 106 overlapping genes were revealed between drug targets and liver cancer molecular targets. Furthermore, a PPI network and 4 modules networks were further investigated based on overlapping genes and hub nodes, respectively. These hub nodes such as VEGFA, EGFR, ESR1, PLG, and MAPK3 were mainly enriched in pathways like proteoglycans in cancer, bladder cancer, and estrogen-signaling pathway.

Vascular endothelial growth factor (VEGF) family plays a major role in angiogenesis, which are essential for both healing of injured tissue and proliferation of carcinoma cells [29]. The previous meta-analysis indicates that there is a prognostic role for VEGFA in various cancers including cervical cancer, gastric cancer, epithelial ovarian cancer, and liver cancer [30–33]. Taniguchi et al. indicates that VEGF promotes proliferation of hepatocytes through reconstruction of liver sinusoids by proliferation of sinusoidal endothelial cells [33]. Importantly, VEGF is an important drug target of various TCM [34, 35]. A previous study shows that the TCM Fuzhenghuayu has a decoction effect on VEGF secretion in hepatic stellate cells [36]. However, the association between VEGF and Xihuang pill in liver cancer is unknown. Moreover, epidermal growth factor receptor (EGFR) is a transmembrane protein associated with the epidermal growth factor family [37]. The compartmentalization and biological function of EGFR in liver plasma membrane have been focused [38]. A previous study indicates that there is an increment of serum EGFR level in liver cancer patients [39]. Actually, there is a dual role of EGFR in liver injury and regeneration after acetaminophen overdose.
in mice [40]. Zhu et al. indicate that the EGFR status is independently correlated with TCM treatment in non-small-cell lung cancer patients [41]. Although previous study has confirmed the relation between EGFR expression and TCM active compounds using cell membrane chromatography [42], the effect of Xihuang pill on EGFR expression in liver cancer is still unclear. In the current study, VEGFA and EGFR were not only overlapping genes revealed between Xihuang pill target sets and liver cancer target sets but also hub nodes in PPI modules. Thus, we speculated that genes such as VEGFA and EGFR might be potential therapy targets of Xihuang pill in liver cancer.

Proteoglycans perform multiple functions in cancer and angiogenesis due to their polyhedral nature [43]. The deterioration of liver function is accompanied by an increase in the amount of chondroitin sulfate proteoglycans [44].

Figure 4: The modules networks of PPI. The square represented the targets; the line between two nodes represented the interaction. (a) Module 1. (b) Module 2. (c) Module 3. (d) Module 4.
Table 2: The top 20 core targets genes in the drug-target gene network.

| Name   | RSR     |
|--------|---------|
| VEGFA  | 1.22E-01|
| EGFR   | 1.38E-01|
| ESR1   | 1.43E-01|
| PLG    | 1.44E-01|
| MAPK3  | 1.46E-01|
| IGF1   | 1.49E-01|
| GAPDH  | 1.54E-01|
| SRC    | 1.55E-01|
| MAPK1  | 1.84E-01|

Table 2: Continued.

| Name   | RSR     |
|--------|---------|
| HGF    | 1.88E-01|
| PTGS2  | 1.90E-01|
| MMP9   | 2.08E-01|
| FGF2   | 2.17E-01|
| TIMP1  | 2.25E-01|
| MMP2   | 2.25E-01|
| SERPINE1 | 2.33E-01|
| HSP90AA1 | 2.35E-01|
| TOP2A  | 2.44E-01|
| RHOA   | 2.47E-01|

RSR, the rank sum ratio.
Table 3: The drug ingredients corresponding to hub nodes.

| Hub nodes | Code          | CTM      | Ingredients |
|-----------|---------------|----------|-------------|
| VEGFA     | Swiss-Prot:P15692 | Niuhuang | MOL008847   |
| VEGFA     | Swiss-Prot:P15692 | Ruxiang | MOL001280   |
| VEGFA     | Swiss-Prot:P15692 | Ruxiang | MOL001264   |
| VEGFA     | Swiss-Prot:P15692 | Ruxiang | MOL001266   |
| VEGFA     | Swiss-Prot:P15692 | Ruxiang | MOL001265   |
| VEGFA     | Swiss-Prot:P15692 | Ruxiang | MOL001268   |
| VEGFA     | Swiss-Prot:P15692 | Moyao   | MOL001003   |
| VEGFA     | Swiss-Prot:P15692 | Moyao   | MOL000997   |
| VEGFA     | Swiss-Prot:P15692 | Moyao   | MOL001023   |
| VEGFA     | Swiss-Prot:P15692 | Moyao   | MOL001005   |
| VEGFA     | Swiss-Prot:P15692 | Moyao   | MOL000989   |
| VEGFA     | Swiss-Prot:P15692 | Moyao   | MOL001124   |
| EGFR      | Swiss-Prot:P00533 | Niuhuang | MOL008847   |
| EGFR      | Swiss-Prot:P00533 | Ruxiang | MOL000858   |
| EGFR      | Swiss-Prot:P00533 | Ruxiang | MOL001281   |
| EGFR      | Swiss-Prot:P00533 | Moyao   | MOL001003   |
| EGFR      | Swiss-Prot:P00533 | Moyao   | MOL000989   |
| EGFR      | Swiss-Prot:P00533 | Moyao   | MOL001039   |
| ESR1      | Swiss-Prot:P03372 | Shexiang | tcm03_006384|
| ESR1      | Swiss-Prot:P03372 | Shexiang | tcm03_006339|
| ESR1      | Swiss-Prot:P03372 | Shexiang | tcm05_001854|
| ESR1      | Swiss-Prot:P03372 | Shexiang | tcm03_003968|
| ESR1      | Swiss-Prot:P03372 | Niuhuang | MOL008836   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001276   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001272   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001275   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001264   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001266   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001265   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001268   |
| ESR1      | Swiss-Prot:P03372 | Ruxiang | MOL001277   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001023   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001038   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001079   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001057   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001003   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL000997   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001032   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001036   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001023   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001037   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001019   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001005   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001008   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001041   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001196   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL000989   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001079   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001124   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001134   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001072   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL001080   |
| ESR1      | Swiss-Prot:P03372 | Moyao   | MOL000993   |
| PLG       | Swiss-Prot:P00747 | Shexiang | tcm03_002894|
| PLG       | Swiss-Prot:P00747 | Shexiang | tcm03_002835|
| MAPK3     | Swiss-Prot:P27361 | Ruxiang | MOL001266   |
| MAPK3     | Swiss-Prot:P27361 | Ruxiang | MOL001265   |
| MAPK3     | Swiss-Prot:P27361 | Ruxiang | MOL001268   |
| MAPK3     | Swiss-Prot:P27361 | Moyao   | MOL000997   |
| MAPK3     | Swiss-Prot:P27361 | Moyao   | MOL001036   |
Table 3: Continued.

| Hub nodes | Code               | CTM     | Ingredients     |
|-----------|--------------------|---------|-----------------|
| MAPK3     | Swiss-Prot:P27361  | Moyao   | MOL001023       |
| MAPK3     | Swiss-Prot:P27361  | Moyao   | MOL001037       |
| MAPK3     | Swiss-Prot:P27361  | Moyao   | MOL001019       |
| MAPK3     | Swiss-Prot:P27361  | Moyao   | MOL001124       |

CTM, Chinese traditional medicine; VEGFA, vascular endothelial growth factor A; EGFR, epidermal growth factor receptor; ESR1, estrogen receptor 1; PLG, plasminogen; MAPK3, mitogen-activated protein kinase 3.

Figure 7: Gene ontology (GO) term enrichment for hub genes. GO terms including biological process (BP), cellular component (CC), and molecular function (MF) enriched by hub genes, respectively.

Table 4: The top 10 pathways enriched by the top 5 hub nodes in PPI network.

| ID   | Description                          | Count | FDR             |
|------|--------------------------------------|-------|-----------------|
| 05205| Proteoglycans in cancer              | 4     | 8.93E – 06      |
| 05219| Bladder cancer                       | 3     | 8.93E – 06      |
| 05212| Pancreatic cancer                    | 3     | 2.40E – 05      |
| 04915| Estrogen-signaling pathway           | 3     | 6.48E – 05      |
| 04066| HIF-1-signaling pathway              | 3     | 7.06E – 05      |
| 04014| Ras-signaling pathway                | 3     | 3.80E – 04      |
| 04015| Rap 1-signaling pathway              | 3     | 3.80E – 04      |
| 04320| Dorso-ventral axis formation         | 2     | 3.80E – 04      |
| 04510| Focal adhesion                       | 3     | 3.80E – 04      |

FDR, false discovery rate; Count, the number of nodes enriched in certain pathway.
altered proteoglycan composition interferes with the physiologic function of the liver on several levels [45]. Actually, the biological function of proteoglycans has been widely investigated in VEGF-induced diseases [46, 47]. A previous study shows that the activation of EGFR contributes to the inhibition of axon regeneration [48]. Furthermore, estrogen plays a vital role in the progression of liver cancer [49]. Kim et al. indicate that estrogen-related receptor $\gamma$ is upregulated in liver cancer [50]. Previous studies show that VEGF and EGFR expression are all associated with estrogen receptor status in patients with cancer [51, 52]. Meanwhile, cytoplasmic expression of estrogen receptor can predict poor outcome of EGFR-TKI therapy in metastatic lung adenocarcinoma [53]. In this study, the pathway analysis showed that the potential targets such as VEGFA and EGFR were mainly enriched in pathways including proteoglycans in cancer and estrogen-signaling pathway. Thus, we speculated that the effect of Xihuang pill on liver cancer might be realized by targeting VEGFA and EGFR in pathways like proteoglycans in cancer and estrogen signaling. However, there were some limitations in this study such as small sample size and lack of verification analysis. Thus, a further verification study based on a large sample size is needed.

In conclusion, VEGFA and EGFR might be potential therapy targets of Xihuang pill in liver cancer. Furthermore, the effect of Xihuang pill on liver cancer might be realized by targeting VEGFA and EGFR in pathways like proteoglycans in cancer and estrogen signaling.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. (1) VEGFA and EGFR might be potential therapy targets of Xihuang pill in liver cancer. (2) Proteoglycans in cancer and estrogen signaling might be associated with liver cancer. (3) Totally, 106 genes were explored between drug targets and liver cancer targets.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by Longgang District Special Funds for Economic and Technological Development of Shenzhen City (LGKCYLWS2019000876).

Supplementary Materials

Supplementary Figure 1: workflow of network pharmacology analysis. Supplementary File 1: all nodes and interactions in drug ingredients-target network. Supplementary File 2: the top 50 nodes in drug ingredients-target network. (Supplementary Materials)

References

[1] K. M. Iburg, “Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013,” The Lancet, vol. 385, no. 9963, pp. 117–171, 2015.

[2] M. Omata, A. L. Cheng, N. Kokudo et al., "Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update," Hepatology International, vol. 11, no. 4, pp. 317–370, 2017.

[3] J. Tejeda-Maldonado, I. García-Juárez, J. Aguirre-Valadez et al., “Diagnosis and treatment of hepatocellular carcinoma: an update,” World Journal of Hepatology, vol. 7, no. 3, pp. 362–376, 2015.

[4] W. F. Lin, J. Y. Lu, B. B. Cheng, and C. Q. Ling, "Progress in research on the effects of traditional Chinese medicine on the tumor microenvironment," Journal of Integrative Medicine, vol. 15, no. 4, pp. 282–287, 2017.

[5] Y. H. Liao, C. C. Lin, T. C. Li, and J. G. Lin, "Utilization pattern of traditional Chinese medicine for liver cancer patients in Taiwan," BMC Complementary & Alternative Medicine, vol. 12, no. 1, p. 146, 2012.

[6] X. Zheng, M. Zhang, J. Wang, and S. Lv, "Study of qualitative and quantitative methods for Xihuang pills," Chinese Journal of Pharmaceutical Analysis, vol. 31, no. 7, pp. 1410–1413, 2011.

[7] Q. Guo, J. Lin, R. Liu et al., “Review on the applications and molecular mechanisms of Xihuang pill in tumor treatment,” Evidence-Based Complementary and Alternative Medicine, vol. 2015, Article ID 854307, 10 pages, 2015.

[8] G. Pan, W. Wang, L. Wang et al., “Anti-breast cancer effects and mechanisms of Xihuang pill on human breast cancer cell lines,” Journal of Traditional Chinese Medicine, vol. 33, no. 6, pp. 770–778, 2013.

[9] Z. Q. Cheng, “Clinical observation on Xihuang pill in treating 23 cases of advanced primary hepatic cancer,” China Journal of Traditional Chinese Medicine & Pharmacy, vol. 1, 2010, in Chinese.

[10] L. Gui-zhi, D. Yi, H. Ren-bao, T. Zhu-ting, Y. Meng-qun, and Z. Fei, “In vitro study on the reversal effect of serum containing Xihuang pill on the multidrug-resistance of hepatic cancer cells via P-glytoprotein pathway,” Chinese Journal of Integrated Traditional and Western Medicine on Digestion, vol. 2, 2016, in Chinese.

[11] R. Z. Zhang, S. J. Yu, H. Bai, and K. Ning, "TCM-Mesh: the database and analytical system for network pharmacology analysis for TCM preparations,” Scientific Reports, vol. 7, no. 1, p. 2821, 2017.

[12] L. Chen, Y. Cao, H. Zhang et al., “Network pharmacology-based strategy for predicting active ingredients and potential targets of Yangxinshi tablet for treating heart failure,” Journal of Ethnopharmacology, vol. 219, pp. 359–368, 2018.

[13] J. Ru, P. Li, J. Wang et al., “TCMSP: a database of systems pharmacology for drug discovery from herbal medicines,” Journal of Cheminformatics, vol. 6, no. 1, p. 13, 2014.

[14] R. Xue, Z. Fang, M. Zhang, Z. Yi, C. Wen, and T. Shi, “TCMID: traditional Chinese medicine integrative database for herb molecular mechanism analysis,” Nucleic Acids Research, vol. 41, no. D1, pp. D1089–D1095, 2012.

[15] A. Y. Lee, W. Park, T.-W. Kang, M. H. Cha, and J. M. Chun, “Network pharmacology-based prediction of active compounds and molecular targets in Yijin-Tang acting on hyperlipidaemia and atherosclerosis,” Journal of Ethnopharmacology, vol. 221, pp. 151–159, 2018.
Evidence-Based Complementary and Alternative Medicine

[16] X. Xu, W. Zhang, C. Huang et al., “A novel chemometric method for the prediction of human oral bioavailability,” *International Journal of Molecular Sciences*, vol. 13, no. 6, pp. 6964–6982, 2012.

[17] S. Kim, P. A. Thiessen, E. E. Bolton et al., “PubMed substance and compound databases,” *Nucleic Acids Research*, vol. 44, no. D1, pp. D1202–D1213, 2016.

[18] D. Geller, O. Michielin, and V. Zoete, “Shaping the interaction landscape of bioactive molecules,” *Bioinformatics*, vol. 29, no. 23, pp. 3073–3079, 2013.

[19] M. L. Yu Tang, J. Wang, Yi Pan, and F.-X. Wu, “CytoNCA: a cytoscape plug-in for centrality analysis and evaluation of biological networks,” *BioSystems*, vol. 11, p. 5, 2014.

[20] E. J. Baker, J. J. Jay, J. A. Buibier, M. A. Langston, and E. J. Chesler, “GeneWeaver: a web-based system for integrative functional genomics,” *Nucleic Acids Research*, vol. 40, no. D1, pp. D1067–D1076, 2012.

[21] D. Szklarczyk, J. H. Morris, H. Cook et al., “The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible,” *Nucleic Acids Research*, vol. 45, no. D1, pp. D362–D368, 2017.

[22] G. D. Bader and C. Hogue, “An automated method for finding molecular complexes in large protein interaction networks,” *BMC Bioinformatics*, vol. 4, no. 1, p. 2, 2003.

[23] C. H. Chin, S. H. Chen, H. H. Wu, C. W. Ho, M. T. Ko, and C. Y. Lin, “cytoHubba: identifying hub objects and sub-networks from complex interactome,” *BMC Systems Biology*, vol. 8, no. S4, p. S11, 2014.

[24] H. Wang and W. Q. Jiang, “Research on two-stage approach based on rank-sum ratio in multi-attribute decision condition,” *Mathematics in Practice & Theory*, vol. 5, no. 10, pp. 199–203, 2015.

[25] M. Ashburner, C. A. Ball, J. A. Blake et al., “Gene ontology: tool for the unification of biology. The Gene Ontology Consortium,” *Nature Genetics*, vol. 25, no. 1, pp. 25–29, 2000.

[26] M. Kanehisa and S. Goto, “KEGG: kyoto encyclopedia of genes and genomes,” *Nucleic Acids Research*, vol. 28, no. 1, pp. 27–30, 2000.

[27] D. W. Huang, B. T. Sherman, and R. A. Lempicki, “Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources,” *Nature Protocols*, vol. 4, no. 1, pp. 44–57, 2009.

[28] L. Boix, Z. Maríño, F. Torres, X. Forns, J. Bruix, and M. Reig, “Liver cancer emergency associated with antiviral treatment: an immune surveillance failure?,” *Seminars in Liver Disease*, vol. 32, no. 5, pp. 1013–1031, 2012.

[29] J. Zhang, J. Liu, C. Zhu et al., “Prognostic role of vascular endothelial growth factor in cervical cancer: a meta-analysis,” *Oncotarget*, vol. 8, no. 15, pp. 24797–24803, 2017.

[30] M. Zhuang, Z. Peng, J. Wang, and X. Su, “Vascular endothelial growth factor gene polymorphisms and gastric cancer risk: a meta-analysis,” *Journal of BUON: Official Journal of the Balkan Union of Oncology*, vol. 22, no. 3, p. 714, 2017.

[31] H. Komatsu, T. Oishi, H. Itamochi et al., “Serum vascular endothelial growth factor-A as a prognostic biomarker for epithelial ovarian cancer,” *International Journal of Gynecological Cancer*, vol. 27, no. 7, pp. 1325–1332, 2017.

[32] E. Taniguchi, S. Sakisaka, K. Matsuo, K. Tanikawa, and M. Sata, “Expression and role of vascular endothelial growth factor in liver regeneration after partial hepatectomy in rats,” *Journal of Histochemistry & Cytochemistry*, vol. 49, no. 1, pp. 121–129, 2001.

[33] J. W. Guo, C. Chen, Y. Huang, and B. Li, “Combinatorial effects of Naomai Yihao capsules and vascular endothelial growth factor gene-transfected bone marrow mesenchymal stem cells on angiogenesis in cerebral ischemic tissues in rats,” *Journal of Traditional Chinese Medicine*, vol. 32, no. 1, pp. 87–92, 2012.

[34] Z.-X. Qi and L. Chen, “Effect of Chinese drugs for promoting blood circulation and eliminating blood stasis on vascular endothelial growth factor expression in rabbits with glucocorticoid-induced ischemic necrosis of femoral head,” *Journal of Traditional Chinese Medicine*, vol. 29, no. 2, pp. 137–140, 2009.

[35] C. Liu, C. M. Jiang, C. H. Liu, P. Liu, and Y. Y. Hu, “Effect of Fuzhenghuayu decoction on vascular endothelial growth factor secretion in hepatic stellate cells,” *Hepatology & Pancreatic Diseases International*, vol. 1, no. 2, pp. 207–210, 2002.

[36] P. Wee and Z. Wang, “Epidermal growth factor receptor cell proliferation signaling pathways,” *Cancers*, vol. 9, no. 12, p. 52, 2017.

[37] Y. Wang, B. I. Posner, and A. Balbis, “Compartmentalization of epidermal growth factor receptor in liver plasma membrane,” *Journal of Cellular Biochemistry*, vol. 107, no. 1, pp. 96–103, 2009.

[38] T. I. Sung, Y. J. Wang, C. Y. Chen, T. L. Hung, and H. R. Guo, “Increased serum level of epidermal growth factor receptor in liver cancer patients and its association with exposure to arsenic,” *Science of the Total Environment*, vol. 424, pp. 74–78, 2012.

[39] B. Bhushan, H. Chavan, P. Borude et al., “Dual role of epidermal growth factor receptor in liver injury and regeneration after acetaminophen overdose in mice,” *Toxicological Sciences*, vol. 155, no. 2, pp. 363–378, 2017.

[40] Y. J. Zhu, H. B. Zhang, L. R. Liu et al., “Yin-cold or yang-heat syndrome type of traditional Chinese medicine was associated with the epidermal growth factor receptor gene status in non-small cell lung cancer patients: confirmation of a TCM concept,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 7063859, 7 pages, 2017.

[41] H. He, S. Han, T. Zhang, J. Zhang, S. Wang, and J. Hou, “Screening active compounds acting on the epidermal growth factor receptor from Radix scutellariae via cell membrane chromatography online coupled with HPLC/MS,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 62, no. 6, pp. 196–202, 2012.

[42] R. V. Iozzo and R. D. Sanderson, “Proteoglycans in cancer biology, tumour microenvironment and angiogenesis,” *Journal of Cellular & Molecular Medicine*, vol. 15, no. 5, pp. 1013–1031, 2011.

[43] O. H. Weiner, M. Zoremba, and A. M. Gressner, “Gene expression of syndecans and betaglycan in isolated rat liver cells,” *Cell & Tissue Research*, vol. 285, no. 1, pp. 11–16, 1996.

[44] K. Baghy, P. Tátrai, E. Regős, and I. Kovalszky, “Proteoglycans in liver cancer,” *World Journal of Gastroenterology*, vol. 23, no. 1, pp. 379–393, 2016.

[45] S. L. Jan, M. Hayashi, Z. Kasza et al., “Functional overlap between chondroitin and heparan sulfate proteoglycans during VEGF-induced sprouting angiogenesis,” *Arteriosclerosis, Thrombosis & Vascular Biology*, vol. 32, no. 5, pp. 1255–1263, 2012.

[46] N. Beckouche, M. Bignon, V. Lelarge et al., “The interaction of heparan sulfate proteoglycans with endothelial
transglutaminase-2 limits VEGF165-induced angiogenesis,” *Science Signaling*, vol. 8, no. 385, p. ra70, 2015.

[48] V. Koprivica, K. S. Cho, J. B. Park et al., “EGFR activation mediates inhibition of axon regeneration by myelin and chondroitin sulfate proteoglycans,” *Science*, vol. 310, no. 5745, pp. 106–110, 2005.

[49] E. Villa, “Role of estrogen in liver cancer,” *Women’s Health*, vol. 4, no. 1, pp. 41–50, 2008.

[50] J. H. Kim, Y. K. Choi, J. K. Byun et al., “Estrogen-related receptor γ is upregulated in liver cancer and its inhibition suppresses liver cancer cell proliferation via induction of p21 and p27,” *Experimental & Molecular Medicine*, vol. 48, no. 3, p. e213, 2016.

[51] D. Fuckar, A. Dekanic, S. Stifer et al., “VEGF expression is associated with negative estrogen receptor status in patients with breast cancer,” *International Journal of Surgical Pathology*, vol. 14, no. 1, pp. 49–55, 2006.

[52] P. Tanjak, A. Thiantanawat, P. Watcharasit, and J. Satayavivad, “Genistein reduces the activation of AKT and EGFR, and the production of IL6 in cholangiocarcinoma cells involving estrogen and estrogen receptors,” *International Journal of Oncology*, vol. 53, no. 1, pp. 177–188, 2018.

[53] X. Ding, L. Li, C. Tang et al., “Cytoplasmic expression of estrogen receptor β may predict poor outcome of EGFR-TKI therapy in metastatic lung adenocarcinoma,” *Oncology Letters*, vol. 16, no. 2, pp. 2382–2390, 2018.