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Discussion on Molecular Mechanism of Siwei Xiaoliuyin in Treating Glioma Based on Network Pharmacology and Molecular Docking

Biaogang Han[1, 2], Xiaohong Wu[1, 2], Xiaopei Zhang[1, 2], Shihua Liu[1, 2], Yongqing Shen[3], Aixia Sui[2, *]

[1]College of Postgraduate, Hebei North University, Zhangjiakou, China

[2]Department of Oncology, Hebei General Hospital, Shijiazhuang, China

[3]School of Nursing, Hebei University of Chinese Medicine, Shijiazhuang, China

Email address:

BiaogangHan@163.com (Biaogang Han), xiaohongtougao@163.com (XiaohongWu), 18832078432@163.com (Xiaopei Zhang), lsh18732548669@163.com (Shihua Liu), shyq2005@sina.com (Yongqing Shen), suiaxhebei@126.com (Aixia Sui)

[*]Corresponding author

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Abstract: *Background*. Siwei Xiaoliuyin, a traditional Chinese medicine, is effective in treating glioma, but its molecular mechanism of action is still unclear. In this paper, we will explore the molecular mechanism of Siwei Xiaoliuyin in the treatment of glioma through network pharmacology. *Methods*. The potential active components and molecular targets of Siwei Xiaoliuyin were collected through the pharmacological database and analysis platform of traditional Chinese medicine system and TCMID database; glioma-related target genes were obtained through the GenCards database, OMIM database and Disgenet database; the intersection of drug action targets and disease genes was extracted using R software, and Venn diagram was drawn; the key targets were imported into the String database to construct a protein interaction network; the key targets were imported into R software using clusterProfiler for GO and KEGG enrichment analysis; the main components of Siwei Xiaoliuyin were molecularly docked with the Hub gene by AutoDock Vina technology. *Results*. Siwei Xiaoliuyin consists of four components, which are Curcuma zedoaria, Tianlong, Solanum nigrum and Smilax glabra and a total of 26 potential active components and 56 targets were identified from it, 5750 glioma-related genes and 47 key target genes crossed between Siwei Xiaoliuyin and glioma. The results of enrichment analysis showed that GO entries involved fatty acid metabolic processes, response to steroid hormones and other processes. KEGG analysis identified key genes mainly enriched in PI3K-Akt signaling pathway, estrogen signaling pathway and HIF-1 signaling pathway, etc. The results of molecular docking showed that Diosgenin, the main component of Siwei Xiaoliuyin, docked well with the AHR gene. *Conclusions*. Through network pharmacology prediction, Siwei Xiaoliuyin may regulate multiple signaling pathways such as PI3K-Akt, estrogen and HIF-1 through multiple targets EGFR, ESR1, VEGFA, AHR and AR, thus affecting the function of multiple cells and playing an important role in the treatment of glioma.

Keywords: Siwei Xiaoliuyin, Glioma, Network Pharmacology, Molecular Docking

1. Introduction

Glioma is the most common primary intracranial tumor, accounting for 28% of all brain tumors and 80% of malignant brain tumors [1] and surgical resection and adjuvant chemotherapy with temozolomide combined with radiotherapy are the standard treatment strategies for the disease. However, these resistance to chemotherapy and radiotherapy lead to high recurrence rates and Milena Sant et al. found no improvement in the survival rate of malignant central nervous system tumors during the 14-year study period [2] and therefore, patients with malignant gliomas benefit little from standard treatment [3]. Aiming at the bottleneck stage of glioma treatment, conventional treatment combined with traditional Chinese medicine (TCM) can effectively

reduce the side effects of surgery, chemotherapy and radiotherapy, improve the body's defense ability, ensure the quality of life of patients and consolidate and strengthen the therapeutic effect of tumors. Studies have demonstrated that adjuvant therapy with traditional Chinese medicine has its unique advantages in its prevention and treatment [4], but the molecular mechanism of its treatment is not clear, which may be due to the characteristics of multiple components and multiple targets of traditional Chinese medicine.

The search for the main components for the treatment of glioma from natural medicines is also a current research hotspot [5]. Adjuvant treatment of glioma with traditional Chinese medicine is more widely used, for example, the pharmacological mechanism of cinobufotalin mainly treats glioma by inhibiting cell cycle, promoting apoptosis and regulating immunity through multiple targets such as RAC1, FOS and NOS3 [6]. Matrine can induce apoptosis and autophagy by inhibiting the PI3K/AKT and Wnt-β-catenin pathways and down-regulating the expression of circ-104075 and Bcl-9 in glioma cells [7]. While Siwei Xiaoliuyin consists of Curcuma zedoaria, Tianlong, Solanum nigrum and Smilax glabra. Curcuma zedoaria has the effects of inhibiting tumor cell proliferation, growth, metastasis, invasion and angiogenesis, of which the extract has the effect of multi-target regulation of abnormal proteins in esophageal cancer cells [8]. Tianlong, also known as gecko, has been demonstrated to exert various functions such as inhibiting tumor proliferation and invasion and metastasis, promoting apoptosis, anti-angiogenesis and immune regulation, inducing tumor cell apoptosis and down-regulating VEGF and bFGF protein expression to play an anti-tumor role [9-11]. The extract of Solanum nigrum has the properties of inducing apoptosis, anti-proliferation, invasion and metastasis and has anticancer properties in osteosarcoma, glioma, liver cancer and cervical cancer, which may induce apoptosis through the mitochondrial pathway and alter the levels of apoptosis-related proteins in human cholangiocarcinoma cells [12-15]. Smilax glabra can effectively inhibit the phosphorylation of Akt (Thr308) through Akt-mediated signaling pathway and then inhibit the proliferation and invasion of gastric cancer cells [16] and it can be seen that each component of Siwei Xiaoliuyin inhibits the proliferation and invasion of tumor cells through a variety of signaling pathways. Relevant studies have confirmed that the mechanism by which Siwei Xiaoliuyin inhibits glioma angiogenesis may be achieved by regulating VEGF and down-regulating the expression of vascular endothelial growth factor [17]. Under the intervention of Siwei Xiaoliuyin combined with temozolomide, the expression of miRNA-21 and miRNA-221 in the tumor tissues of tumor-bearing mice is inhibited, so that the transplanted tumors are significantly reduced [18] and this drug can inhibit glioma cell proliferation, invasion and angiogenesis through a variety of signaling pathways.

Network pharmacology can explore the systematic role of TCM by combining biological, pharmacological and bioinformatics methods and these analyses provide potential biological processes and pathways by which TCM may act [19]. Network pharmacology approaches have been used to study "compound-protein/gene-disease" pathways, enabling the description of the complexity between biological systems, drugs and diseases from a network perspective. The application of systems biology methods to determine the pharmacological effects, mechanism of action and safety of TCM is of incalculable value for the research and development of modern TCM [20]. The aim of this study was to explore the efficacy of Siwei Xiaoliuyin and systematically evaluate the therapeutic target and mechanism of Siwei Xiaoliuyin in glioma through network pharmacology and molecular docking techniques.

2. Materials and Methods

2.1. Identification and Target Prediction of Active Ingredients of Siwei Xiaoliuyin

With the help of Traditional Chinese Medicine Systems Pharmacology Database and Analysis Plat (TCMSP database) (https://old.tcmsp-e.com/tcmsp.php) and Traditional Chinese Medicines Integrated Database (TCMID database) (http://www.megabionet.org/tcmid/), the active ingredients of Siwei Xiaoliuyin were searched, oral bioavailability (OB ≥ 30%) and drug-like properties (DL ≥ 0.18) were used as screening conditions for active compounds and potential targets were obtained from the Universal Protein Resource (Uniprot database).

2.2. "Ingredient-Target"Network Construction Analysis

Active ingredients and targets were imported into Cytoscape 3.6.0 software (https://cytoscape.org/) to establish a visual association between active ingredients and targets.

2.3. Analysis of Glioma-Related targets and Venn Map

Glioma-related genes were searched in GenCards database (https://www.genecards.org/), OMIM database (https://www.omim.org/) and Disgenet database (https://www.disgenet.org/) using "glioma" as the keyword. R software (version 3.5.3) (htps:/www.r-project.org) was used to extract the target and disease genes, obtain cross genes (key target genes) and draw Venn diagram.

2.4. Construction of PPI Network and Network Topology Analysis

The key targets were imported into String database (https://cn.string-db.org/) to construct protein interaction network (PPI) and the PPI picture was saved to draw the topological attribute map of Degree's top target network in network topology analysis.

2.5. GO and KEGG Analysis

The key target genes were imported into R software for Gene ontology (GO, http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG,

https://www.kegg.jp/) pathway enrichment analyses [21-22] to study the main metabolic pathways and biological processes of Siwei Xiaoliuyin components in the treatment of glioma targets and to analyze the biological significance of cross-genes.

2.6. Molecular Docking

In this study, Cytoscape software was used to construct a "component-target" network. To further identify the central genes among potential targets, we identified the top four Hub genes using cytoHubba software. The components with the largest degree value of the "component-target" network of the first four Hub genes were subjected to molecular docking using AutoDock Vina (https://vina.scripps.edu/) and the binding energy score of docking was calculated and visualized using DS and PyMol (https://pymol.org/2/).

3. Results

3.1. Target Prediction of Chemical Constituents of Siwei Xiaoliuyin

With the help of TCMSP database and TCMID database, 26 potential active components of Curcuma zedoaria, Tianlong, Solanum nigrum and Smilax glabra were selected, integrated and de-duplication to obtain 56 potential targets.

3.2. "Ingredient-Target" Network Construction Analysis

Twenty-six components as well as the corresponding target genes were imported into Cytoscape software for network construction and visualization and images were saved (Figure 1). The top six by composition were diosgenin, solanocapsine, stigmasterol, enhydrin, neoastilbin and beta-carotene.

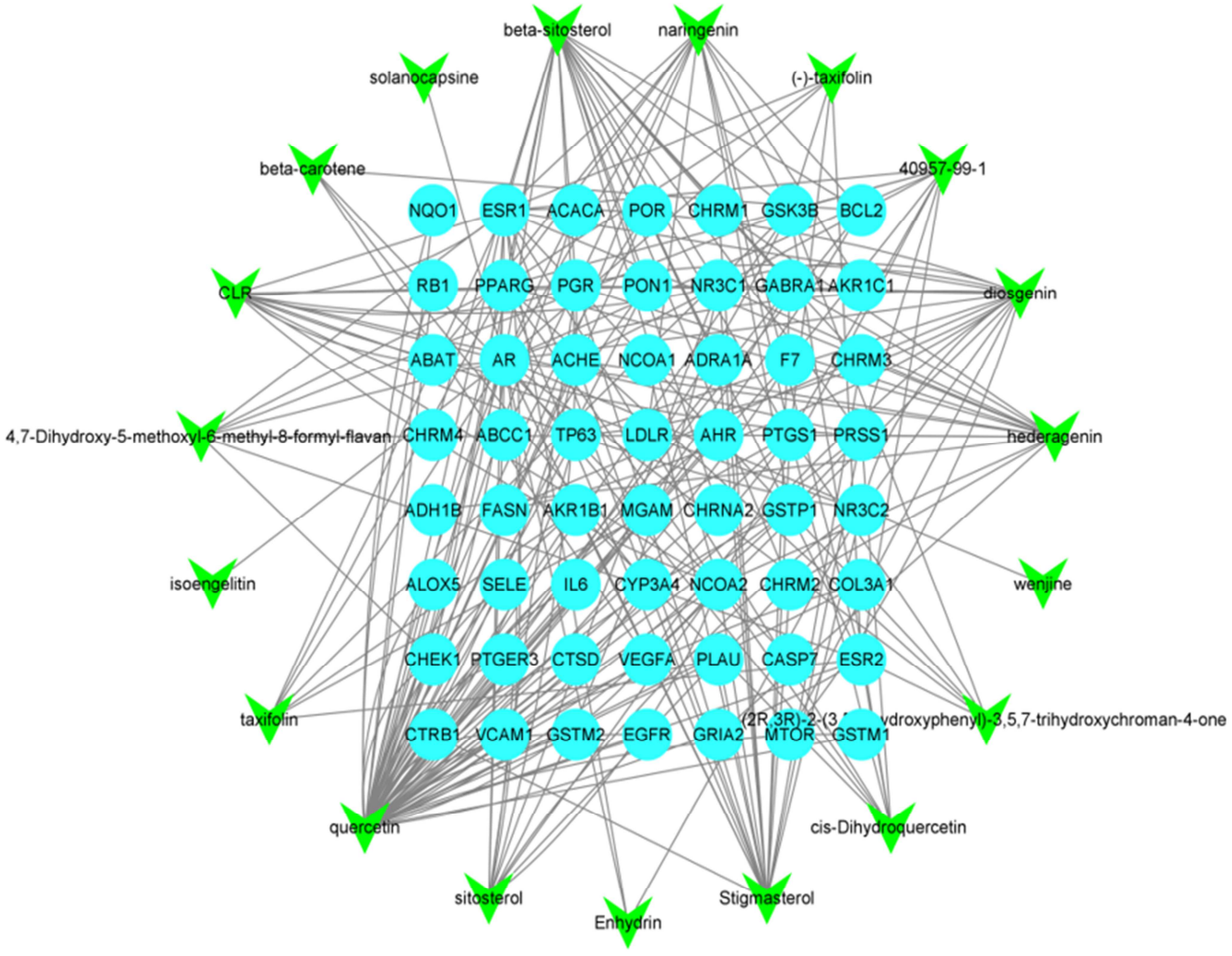

Figure 1. *Network diagram of "Ingredient-Target". (The green arrow is the active ingredient of Siwei Xiaoliuyin and the blue circle is the target of Siwei Xiaoliuyin.).*

3.3. Key Targets of Glioma and Venn Diagram Drawing

A total of 5750 glioma-related target genes were collected through GenCards database, OMIM database and Disgenet database. The action targets of Siwei Xiaoliuyin and glioma disease genes were extracted by R software and 47 cross genes were obtained, mainly AR, PPARG, ESR1, ESR2, EGFR, VEGFA, IL6 and AHR. Venn diagram was drawn (Figure 2).

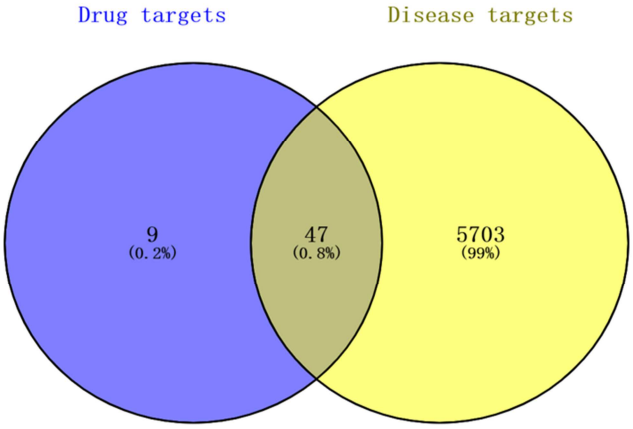

Figure 2. *Venn analysis of component targets versus disease targets. (Blue circles represent Siwei Xiaoliuyin genes and yellow circles represent glioma genes and the intersection of the two is a key target gene).*

3.4. PPI Network Construction and Network Topology Analysis

Key targets were imported into the String database, the study species was selected as human, the setting condition score > 0.4 was considered statistically significant, a protein interaction network was constructed and PPI results were saved (Figure 3). The TSV format was selected to export the results and the data were imported into Cytoscape network topology analysis software. The top 10 targets are IL6, EGFR, ESR1, VEGFA, PPARG, CYP3A4, AHR, mTOR, AR and PGR, which are in a key position in the PPI network map, suggesting that these 10 targets are key targets of Siwei Xiaoliuyin in the treatment of glioma. Topological property plots of degree top-ranked target networks in network topology analysis (Figure 4), key target gene network interaction and network topology analysis data (Table 1).

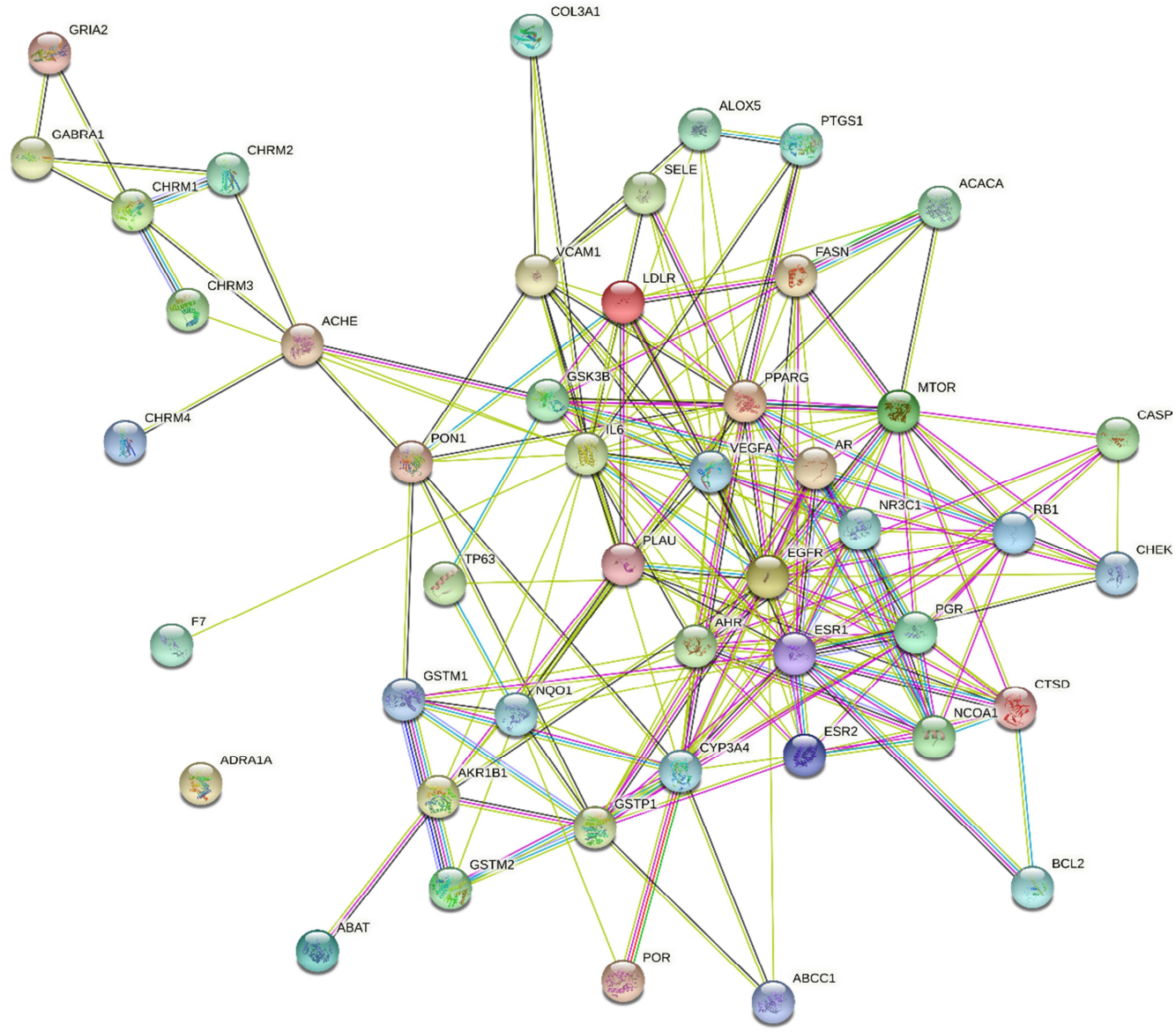

Figure 3. *Gene network interaction of key targets.*

Table 1. *Data of key target gene network interaction and network topology analysis.*

name	Degree	Betweenness Centrality
IL6	52	0.3092448
EGFR	48	0.1000639
ESR1	44	0.0867284
VEGFA	42	0.0559649
PPARG	40	0.0708579
CYP3A4	36	0.072793
AHR	32	0.0130086
MTOR	32	0.0330139
AR	28	0.0088534
PGR	28	0.0086616
GSTP1	26	0.0275758
RB1	24	0.0059879
NR3C1	24	0.0048619
NQO1	22	0.0347604
GSK3B	22	0.0471258
VCAM1	20	0.010506
NCOA1	20	0.0028819
LDLR	20	0.0096971
PLAU	18	0.00579
CTSD	18	0.0085625
ESR2	18	0.0051867
FASN	18	0.0058902
PON1	16	0.0376886
GSTM1	14	0.0040779
CHEK1	12	5.46E-04
ACHE	12	0.165887
AKR1B1	12	0.0454285
ALOX5	10	2.53E-04
CHRM1	10	0.0710611

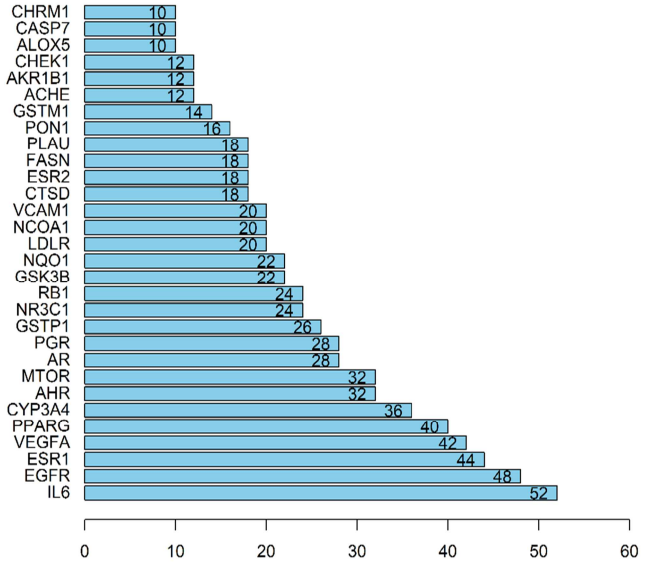

Figure 4. *Topological Attribute Diagram of degree top targets network in network topology Analysis.*

3.5. GO Analysis

Analysis of GO biological processes for key targets yielded a total of 903 GO entries. According to Count value, the top 20 were related to biological processes (Figure 5), the top 20 were related to molecular functions (Figure 6) and the top 20 were related to cellular components (Figure 7). GO entries are mainly involved in fatty acid metabolic processes, response to steroid hormones, amide binding, peptide binding, postsynaptic membranes and cholinergic synapses.

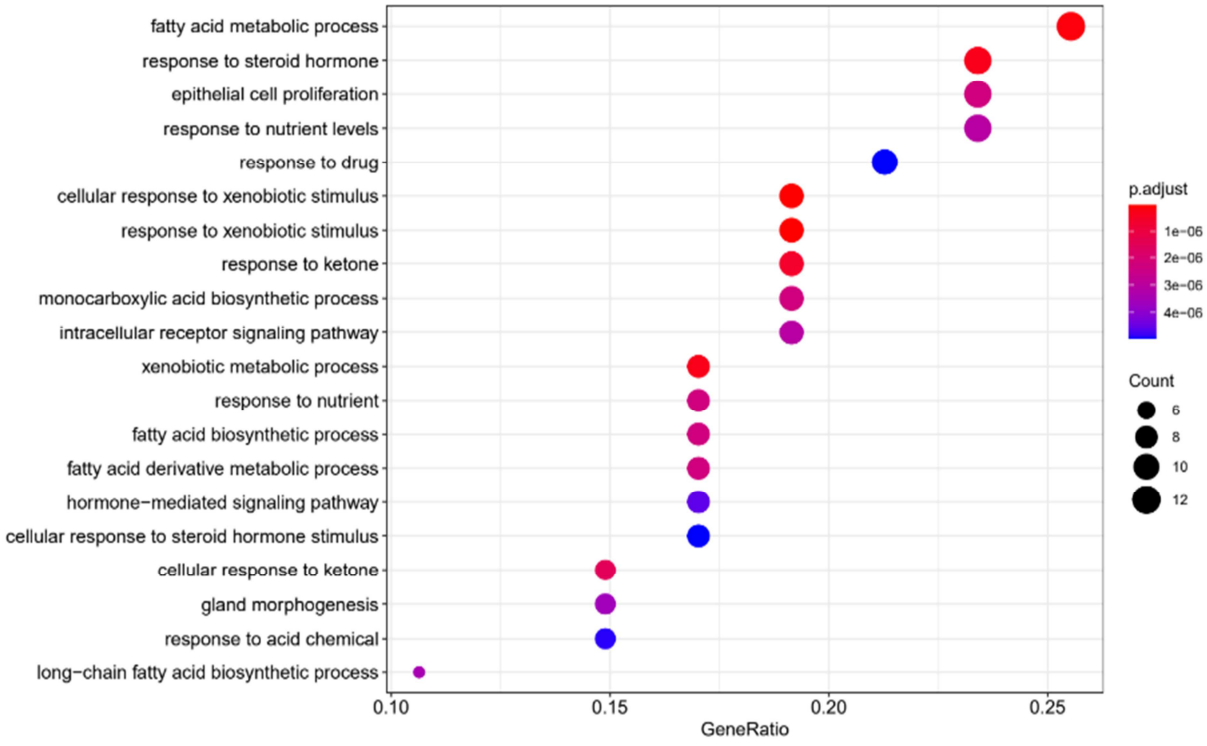

Figure 5. *Biological Process.*

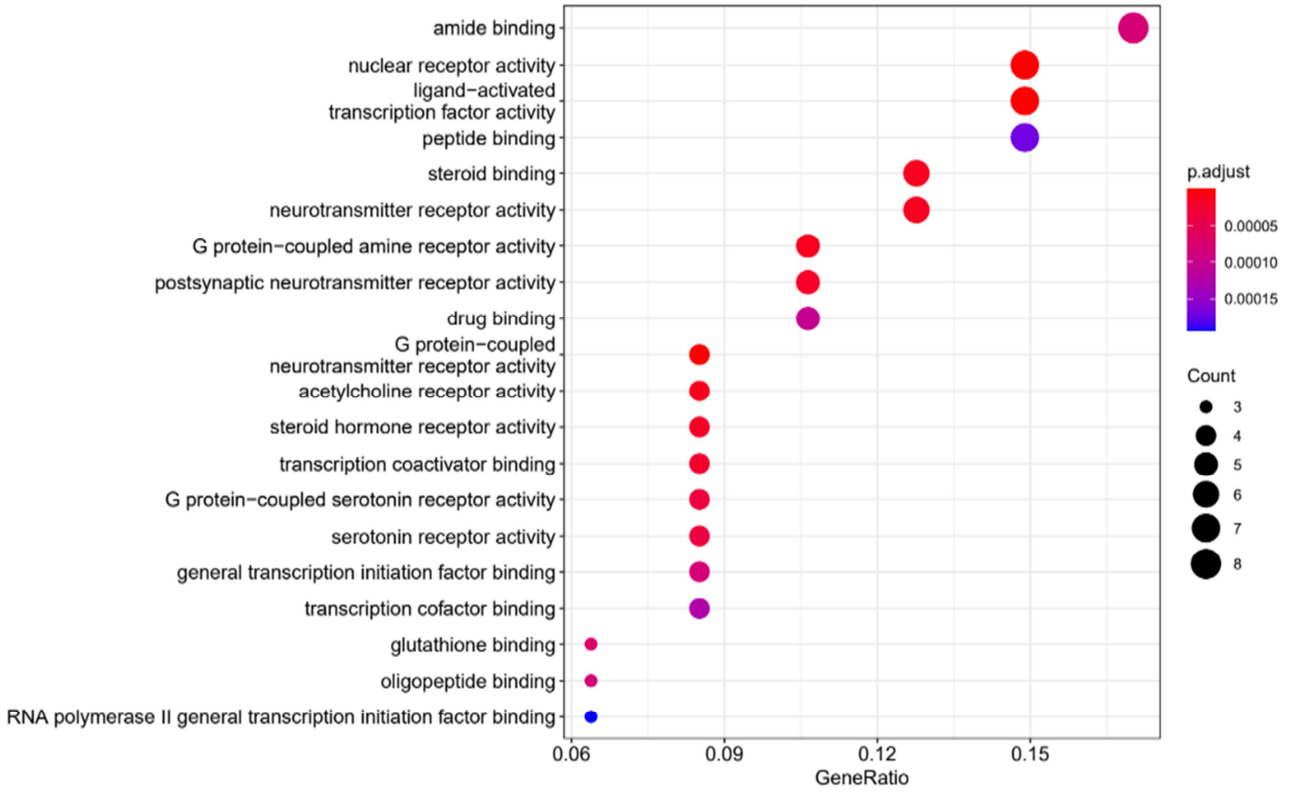

Figure 6. *Molecular Function.*

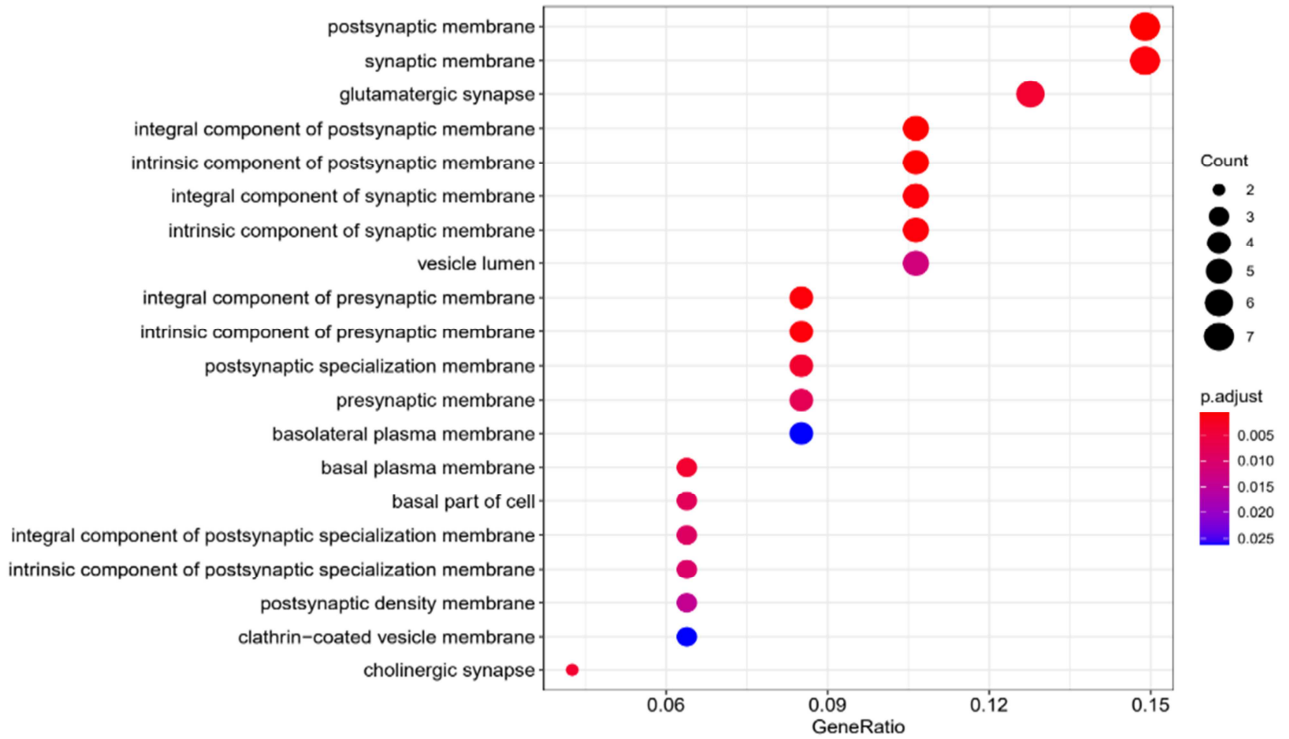

Figure 7. *Cell composition.*

3.6. KEGG Pathway Analysis

Key targets were analyzed using R software, mainly focusing on PI3K-Akt signaling pathway, estrogen signaling pathway, HIF-1 signaling pathway, AMP signaling pathway and EGFR tyrosine kinase inhibitor resistance (Figure 8).

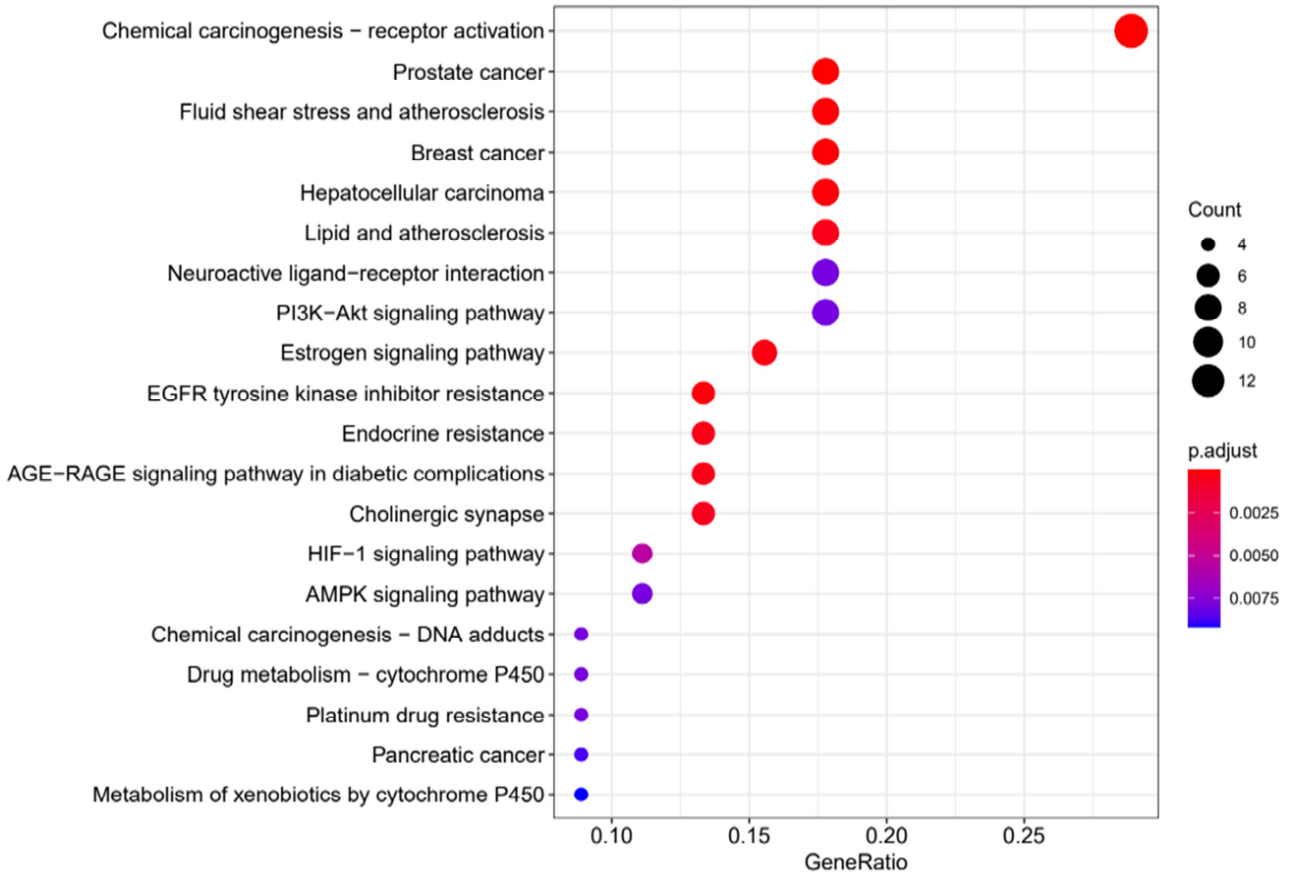

Figure 8. *KEGG Enrichment.*

3.7. Molecular Docking

To further validate potential targets, we performed molecular docking of small molecules to target proteins. Docking analysis successfully predicted Vina scores between small molecules and Hub genes, which were all negative and less than −6, the result are shown in Table 2. In particular, the molecular docking between Diosgenin and AHR has a higher cavity size and the lowest Vina score, as shown in figure 9, but the docking score can only be said to indicate a better combination from a structural point of view and the specific case should be verified according to the experimental results. Overall, the results of molecular docking indicate that the small molecules have good binding activity for target proteins (Figure 10).

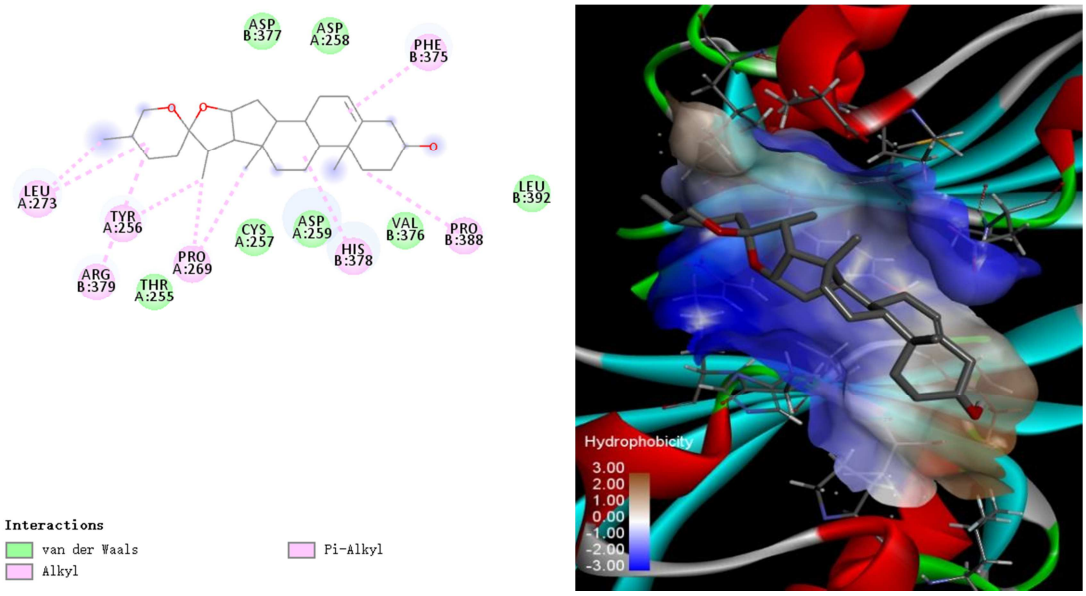

Figure 9. *2D and 3D molecular docking between Diosgenin and AHR.*

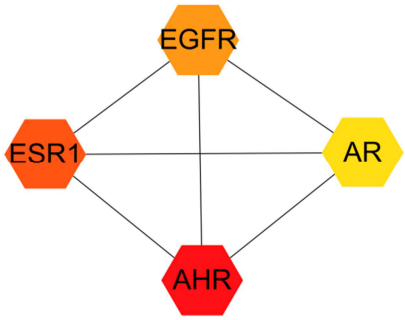

Figure 10. *Top 4 Hub genes in PPI network.*

Table 2. *The results of cavity-detection guided blind docking.*

Ligand	Protein	PDB ID	Vina score	Cavity size
beta-sitosterol	AHR	3F1O	-8.0	156
beta-sitosterol	AR	1E3G	-8.1	830
beta-sitosterol	EGFR	1VIO	-7.7	1150
beta-sitosterol	ESR1	1A52	-7.1	901
Diosgenin	AHR	3F1O	-9.2	221
Diosgenin	AR	1E3G	-7.6	830
Diosgenin	EGFR	1VIO	-8.4	730
Diosgenin	ESR1	1A52	-8.1	601
Quercetin	AHR	3F1O	-6.8	156
Quercetin	AR	1E3G	-9.0	436
Quercetin	EGFR	1VIO	-8.2	730
Quercetin	ESR1	1A52	-8.3	2073
Stigmasterol	AHR	3F1O	-8.1	219
Stigmasterol	AR	1E3G	-8.5	830
Stigmasterol	EGFR	1VIO	-8.0	1150
Stigmasterol	ESR1	1A52	-7.2	601

4. Discussion

Although studies have shown that Siwei Xiaoliuyin can promote the volume reduction of implanted glioma and directly kill glioma cells [16], the specific active ingredients, targets and mechanism of action are not clear. In this paper, network pharmacology technology, combined with molecular docking to test the binding characteristics of the active components and core targets of Siwei Xiaoliuyin, so as to explore the potential targets and molecular mechanisms of Siwei Xiaoliuyin in the treatment of glioma.

The components, targets and related signaling pathways of Siwei Xiaoliuyin were analyzed by network pharmacology to investigate the correlation between Siwei Xiaoliuyin and glioma. The results showed that the active ingredients of Siwei Xiaoliuyin in the treatment of glioma are diosgenin, stigmasterol, enhydrin and beta-carotene and many studies have shown the anticancer effects of the above ingredients. Diosgenin, a natural product extracted from medicinal plants, has lipid-lowering, anticancer and hepatoprotective effects and shows significant anticancer activity against glioma cells by promoting ROS accumulation, inducing DNA damage and activating mitochondrial signaling pathways [23] and diosgenin inhibits the growth of glioma cells, inhibits cell migration and decreases matrix metalloproteinase 2 (MMP2) and MMP9 expression and reduces the protein levels of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2), thereby inhibiting the growth, migration, invasion and angiogenesis of glioma cells [24]. Plant sterol promotes the apoptosis of cancer cells mainly by inhibiting the production of carcinogens, angiogenesis, cancer cell growth, invasion and metastasis [25]. In vitro cytotoxicity test (MTT) showed that Enhydrin had no significant cytotoxic effect on peripheral blood mononuclear cells of healthy human subjects, while significant cytotoxic effect was observed in leukemia and pancreatic cancer cells [26]. B-carotene inhibits the growth of C-6 glioma cells and the mechanism is mainly through enhancing anti-tumor immune effects [27].

Siwei Xiaoliuyin may initiate anti-tumor therapeutic effects on gliomas by acting on targets such as IL6, EGFR, mTOR, AHR and AR. IL6 is an inflammatory autocrine and paracrine cytokine that is overexpressed in glioblastoma and it is a biomarker of poor prognosis [28], other studies have also shown that glioma samples contain significantly high levels of IL6 protein compared with controls [29], IL6 signaling contributes to glioma malignancy by promoting glioma stem cell growth, survival and targeting IL6 may benefit glioma patients [30]. Epidermal growth factor receptor (EGFR) was one of the first proto-oncogenes to be considered to play a potential role in the pathogenesis of glioblastoma because of its high expression in most patients (up to 90%), genomic alterations in EGFR in 57% of patients and wide variation, including gene amplification, rearrangements and point mutations. Receptor signaling occurs through a variety of pathways, but the most studied is the recruitment and activation of the phosphatidylinositol-3-kinase (PI3K) signaling network, which ultimately activates AKT and mTOR proteins downstream to maintain tumor growth [31]. AHR controls the recruitment of peripheral macrophages to gliomas and the up-regulation of AHR expression in the tumor microenvironment [32]. RTK/PI3K/mTOR is one of the most critical pathways regulating cell growth and survival in cancer biology, so its targeting remains a strong reason for the development of strategies against glioma [33] and concomitant mTOR inhibition in glioma stem cells also enhances the anti-tumor effect of PI3Kα inhibition [34] and Atsushi Sato et al similarly showed that simultaneous inhibition of PI3K and mTOR is effectively reducing the self-renewal ability of neural stem/progenitor cells [35]. Tzu-Chi Chen et al confirmed a drug targeting direct degradation of AR by in vitro and in vivo experiments and effectively inhibited glioma cell survival [36]. It is well-known that HSP27 is a chaperone protein that can stabilize AR and targeting HSP27 to induce AR degradation emerged as a novel approach to treat AR overexpression in gliomas [37-39] and these molecular targets are highly relevant to the development of gliomas.

From the network topology analysis, it can be seen that the main key signaling pathways for the treatment of glioma focus on PI3K-Akt signaling pathway, estrogen signaling pathway, HIF-1 signaling pathway, AMP signaling pathway and EGFR tyrosine kinase inhibitor resistance. The

RTK/PI3K/Akt/mTOR signaling pathway is a key signaling pathway in the initiation and progression of glioma [33]. Through the establishment of intracranial tumor models, all of them were found to contain PI3K/AKT/mTOR pathway gene activating mutations, including PIK3CA and H1047L hotspot mutations of E542K. Progressive tumor cells with mutants, PIK3CA, are susceptible to alkylating agents and PI3K/AKT/mTOR pathway inhibitors in vitro and in vivo, thus concluding that the activation of PI3K/AKT/mTOR pathway is an oncogenic driver [40]. Key pathways control multiple downstream effectors, which are involved in the development of a variety of tumors. PI3K pathway is affected by the changes of several signaling proteins, such as phosphatase and tension protein homolog (PTEN) loss of function and EGFR amplification/mutation. Two characteristics of glioma pathogenesis [41]. EGFR is highly amplified, mutated and overexpressed in human malignant glioma cells. PI3K/mTOR inhibitors and gene EGFR inhibitors can significantly delay recurrence and prolong survival time. Relevant literatures indicate that combined inhibition of EGFR and PI3K/mTOR may synergize in the treatment of malignant glioma driven by clinically abnormal EGFR signaling [42], suggesting that EGFR tyrosine kinase inhibitor resistance and PI3K-Akt signaling pathway act together on the proliferation of glioma cells. The PI3K/Akt-activated transcription factor HIF-1 regulates the expression of several glycolytic genes and plays a key regulatory role in apoptosis, thereby promoting chemoresistance [43]. Hypoxic levels in tumors stabilize the transcription factor HIF-1, which translocates to the nucleus and activates VEGF gene transcription, thereby increasing angiogenesis. The increased levels of VEGF and VEGF receptors in gliomas contribute to the high vascularization of gliomas [33]. HIF1α and HIF2α -KO induce cell cycle entry into G2/M+S phase, thereby promoting glioma cell growth while reducing stemness, resulting in increased sensitivity of glioma cells to chemotherapy and HIF1α and HIF2α regulate glioma malignant progression with positive feedback through the EGFR-PI3K/AKT pathway [44]. Estrogen receptor β (Erβ) signaling has a tumor suppressor function in gliomas, functional activation of the ERβ pathway is a potential therapeutic target in gliomas [45] and ERβ overexpression or agonist treatment reduces the glutamate receptor signaling pathway and induces the apoptosis pathway. In orthotopic models, ERβ overexpression or ERβ agonist treatment significantly reduced glioma stem cell-mediated tumor growth and improved the overall survival of mice [46]. AMP-activated protein kinase (AMPK), an evolutionarily conserved serine/threonine kinase, is a major modulator of energy homeostasis and plays a key role in metabolic disorders and cancer [47]. Deliang Guo et al. showed in vitro cell experiments that AICAR, a metabolic stressor that activates AMPK, inhibits the growth of U87MG, while preferentially inhibiting the growth and lipogenesis of EGFR-activated glioma cells [48]. AMPK supports the mechanism of tumor bioenergetics, growth and survival in human glioblastoma and oncogenic stress activates AMPK in glial maternal stem cells in the long term by phosphorylating CREB1, which appears abundantly in glioblastoma, using the AMPK-CREB1 pathway to coordinate tumor bioenergetics through the transcription factors HIF1α and GABPA and adult mice tolerate systemic deletion of AMPK in vitro, supporting the effect of AMPK inhibitors in glioma treatment [49].

5. Conclusion

In this study, network pharmacology and molecular docking were used to verify the interaction between Siwei Xiaoliuyin and key target moleculesy and to explore the main role of Siwei Xiaoliuyin treatment glioma. The above results were discussed that IL6, EGFR, mTOR, AHR and AR and PI3K-Akt signaling pathway, estrogen signaling pathway, HIF-1 signaling pathway and AMP signaling pathway play an important role in the inhibitory process of glioma. The results of this study provide basic research data for further analysis of Siwei Xiaoliuyin adjuvant therapy of glioma and provide some theoretical basis for improving the efficacy of chemotherapeutic drugs, reducing toxic and side effects and other adjuvant therapy, new drug research and development in the future. However, there are still some shortcomings. First, the database is continuously updated, only revealing part of the mechanism of action to a certain extent. Second, no cell or animal experiments were performed to validate the results of this paper. In summary, the pathways and complex mechanisms of TCM in fighting tumors require more in-depth clinical experimental studies to provide supporting data.

Data Availability

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

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Biaogang Han and Aixia Sui wrote the article and designed the study. Xiaohong Wu and Yongqing Shen were responsible for data collection and analysis. Xiaohong Wu, Xiaopei Zhang and Shihua Liu were responsible for data analysis and mapping. Biaogang Han and Aixia Sui revised and critically reviewed the article. All authors have agreed on the journal to which the article has been submitted, agreed to be accountable for all aspects of the work criteria and made a significant contribution to the work reported.

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