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Network Pharmacology of Hedyotis Diffusa in the Treatment of Glioma

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Abstract

In order to study the mechanism of Hedyotis diffusa extract on glioma, we used the methods of network pharmacology and data mining to analyze the target genes of Hedyotis diffusa extract on glioma, constructed the gene expression regulation network, and further determined the key target information of glioma using GEO array data mining, So as to lock in the core gene of Hedyotis diffusa on glioma. We used TCMSP database to predict 7 active components of Hedyotis diffusa, 436 targets predicted by swisstargetprediction, and 5574 targets related to glioma. Finally, we obtained 295 genes of Hedyotis diffusa on glioma, Through GO annotation and KEGG enrichment analysis, it was found that Hedyotis diffusa mainly affected the occurrence and development of glioma through PI3K-Akt and FoxO signaling pathways. Then we found 1115 differentially expressed genes in glioma by using geo data mining. Combined with the previous network pharmacology analysis results, we found 23 key targets of Hedyotis diffusa on glioma. Finally, these key target information can be used as the theoretical basis of traditional Chinese medicine in the treatment of glioma.

Keywords

Glioma; Hedyotis Diffusa; Network Pharmacology; Data Mining.

1. Introduction

Hedyotis diffusa is a plant of Rubiaceae, which is widely used in the treatment of tumor, inflammation, poisoning and other aspects of traditional Chinese medicine [1]. According to the current pharmacological research, Hedyotis diffusa contains anthraquinones, polysaccharides, flavonoids, semi terpenes, polyphenols, organic acids and other components with anticancer activity. A large number of literature studies have shown that Hedyotis diffusa has great potential in the treatment of cancer. Many studies have focused on the chemical, biological and drug mechanisms. At present, the antitumor effect of Hedyotis diffusa on glioblastoma is rarely reported [2]. Zhang et al. Found that Hedyotis diffusa extract can inhibit the proliferation of U87 cells and promote mitochondria mediated apoptosis, and ERKs and Akt signaling proteins are also involved in it [3]. These results indicate that it is feasible to treat glioblastoma with Hedyotis diffusa extract.

As we all know, Chinese herbal medicine has the characteristics of multi-component, multi-target and multi-channel. Network pharmacology is a systematic research method first proposed by Shao Li based on the interaction network of traditional Chinese medicine, compounds, targets, diseases and genes [4]. This method emphasizes the integration of bioinformatics, system biology and pharmacology, which not only explains the complex interaction between traditional Chinese medicine and diseases at the system level, but also conforms to the systematic and holistic perspective of traditional Chinese medicine theory.

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In this study, the oral bioavailability (OB) and drug like properties (DL) of Hedyotis diffusa were evaluated, and the bioactive components were screened to obtain the corresponding targets. Then, the potential target genes of glioma were screened by DisGeNET, OMIM and Genecards databases. The protein-protein interaction (PPI) data were obtained by using STRING database. The GO annotation and KEGG pathway enrichment analysis were carried out by using R language to find the possible mechanism of Hedyotis diffusa in the treatment of glioma. Finally, we used the transcriptome data of glioma in geo database to analyze the differential expression profile, and found the key genes causing glioma. Combined with the previous gene regulatory network of Hedyotis diffusa on glioma, we further revealed the mechanism of Hedyotis diffusa, and provided the basis for clinical targeted therapy of glioma.

2. Materials and methods

2.1. Screening and identification of active constituents from Hedyotis diffusa

TCMSP database was used to screen the active components of Hedyotis diffusa extract. The screening conditions were as follows: oral bioavailability (OB) ≥ 30%, drug like (DL) ≥ 0.18, blood brain barrier (BBB) ≥ -0.3. Download the chemical structure file corresponding to the active ingredient.

2.2. Target prediction of active ingredients

The corresponding information of active components of PubChem Database search for Hedyotis diffusa extract was used. At the same time, swisstargetprediction database was used to predict the target genes of Hedyotis diffusa.

2.3. Analysis of disease-related targets

The key words "glioma" were searched in DisGENET, OMIM and genecards database to obtain the target gene data related to glioma, and the uniprot of the target was improved through the protein database uniprot id and gene name.

2.4. Enrichment analysis of go and kegg

The retrieved target genes were introduced into r language tools, and the go annotation and kegg pathway enrichment analysis of the gliomas related targets of hedyotis diffusa active components were performed by using clusterprofiler package, $p < 0.05$.

2.5. Construction of active component target disease pathway of traditional chinese medicine

According to the established active components and glioma related targets of Hedyotis diffusa, the regulatory network of active components and glioma related disease targets of Hedyotis diffusa was constructed through STRING database. The obtained active components, key targets and main pathways of traditional Chinese medicine were imported into the software of Cytoscape. By using its merge and union functions, the component target main disease pathway related to glioma was constructed.

2.6. Identification of differentially expressed genes in glioma

The transcriptome sequencing data of glioma and its adjacent tissues (GSE136000) in GEO database were used to identify the differentially expressed genes using R language limma package. The screening condition was logFC ≥ 2 and $P < 0.05$.

2.7. Identification of key genes of Hedyotis diffusa on glioma

The results of differential expression profile of glioma in GEO dataset were combined with the gene regulatory network of Hedyotis diffusa on glioma to find the key targets and pathways in the regulatory network.

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3. Results

3.1. Identification of active constituents from Hedyotis diffusa

The active components of Hedyotis diffusa were searched by TCMSP database, and seven active components were obtained by screening conditions, as shown in Table 1. Then, PubChem database was used to find the smile structure formula of each active ingredient as the input of compound target prediction. Finally, swisstargetprediction database was used to predict the target gene of Hedyotis diffusa active ingredient.

Table 1. Statistics of active components in Hedyotis diffusa

Mol ID	Molecule Name	MW	OB (%)	BBB	DL
MOL001646	2,3-dimethoxy-6-methyanthraquinone	282.31	34.86	0.17	0.26
MOL001659	Poriferasterol	412.77	43.83	1.03	0.76
MOL001663	(4aS,6aR,6aS,6bR,8aR,10R,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl -1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene -4a-carboxylic acid	456.78	32.03	0.39	0.76
MOL001670	2-methoxy-3-methyl-9,10-anthraquinone	252.28	37.83	0.13	0.21
MOL000449	Stigmasterol	412.77	43.83	1	0.76
MOL000358	beta-sitosterol	414.79	36.91	0.99	0.75
MOL000098	quercetin	302.25	46.43	0.77	0.28

3.2. Prediction of glioma related targets

5574 glioma related targets were retrieved from DisGeNET, OMIM and Genecards databases, and 436 targets of Hedyotis diffusa were predicted by swisstargetprediction database. According to the analysis of Wayne diagram, 295 targets of the active components of Hedyotis diffusa and glioma were obtained. The results are shown in Figure 1.The regulatory network of Hedyotis diffusa on glioma was constructed by using Cytoscape software. The results are shown in Figure 2.

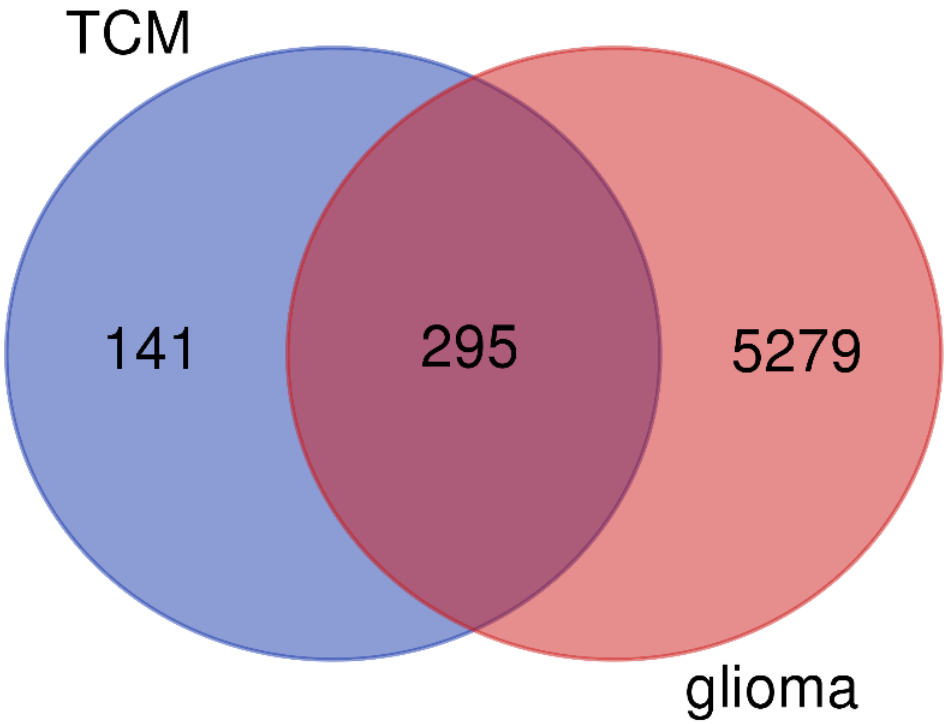

Figure 1. Statistical Analysis of targets of Hedyotis diffusa on glioma

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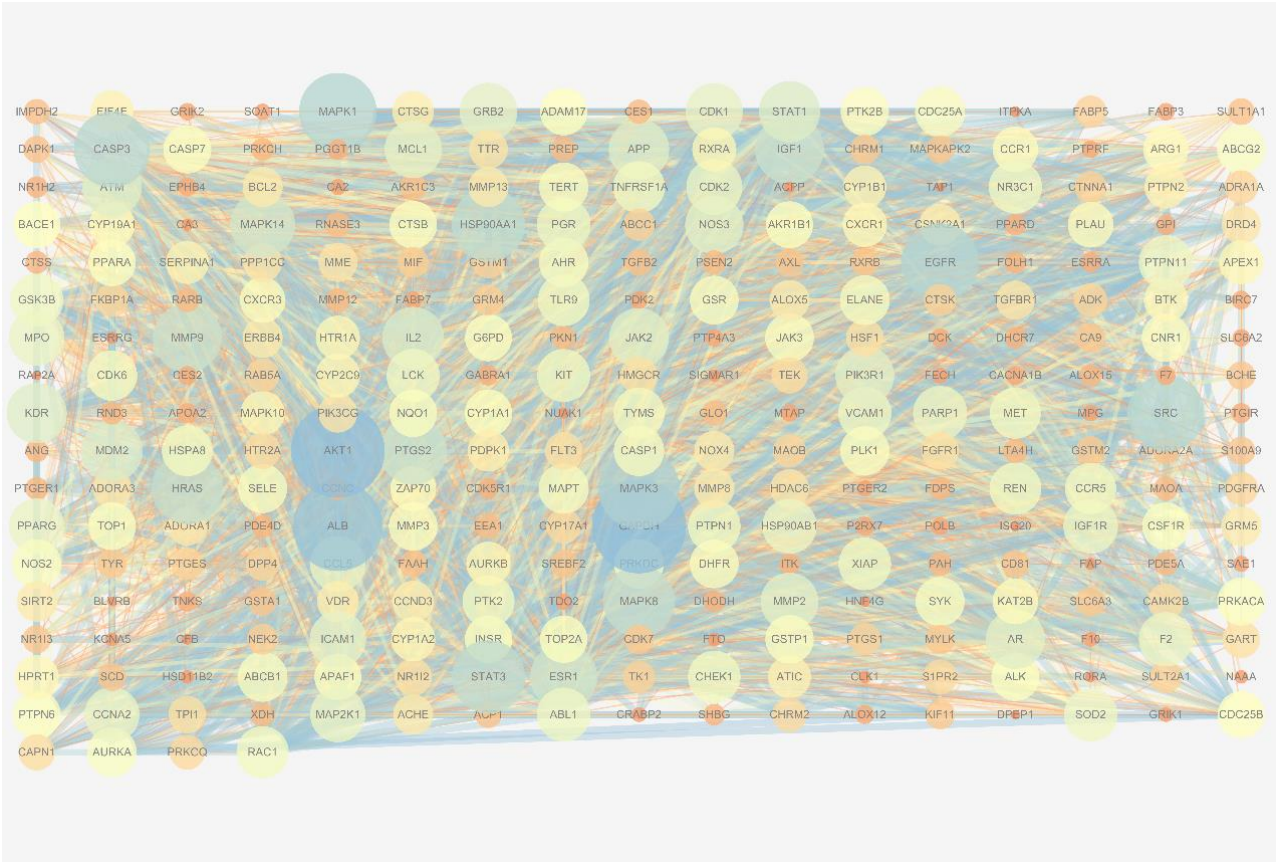

Figure 2. Regulatory network of Hedyotis diffusa on glioma

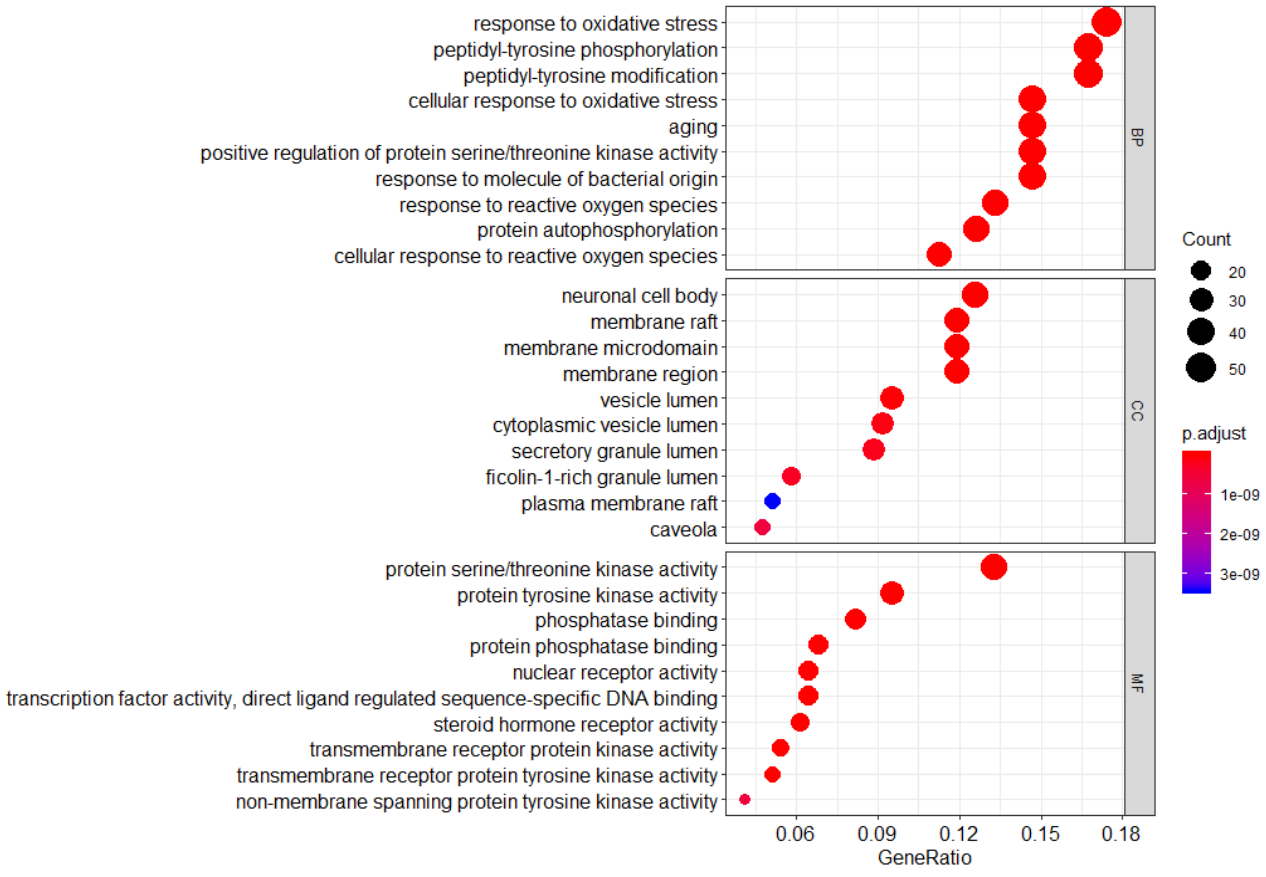

Figure 3. GO annotation analysis of Hedyotis diffusa on glioma

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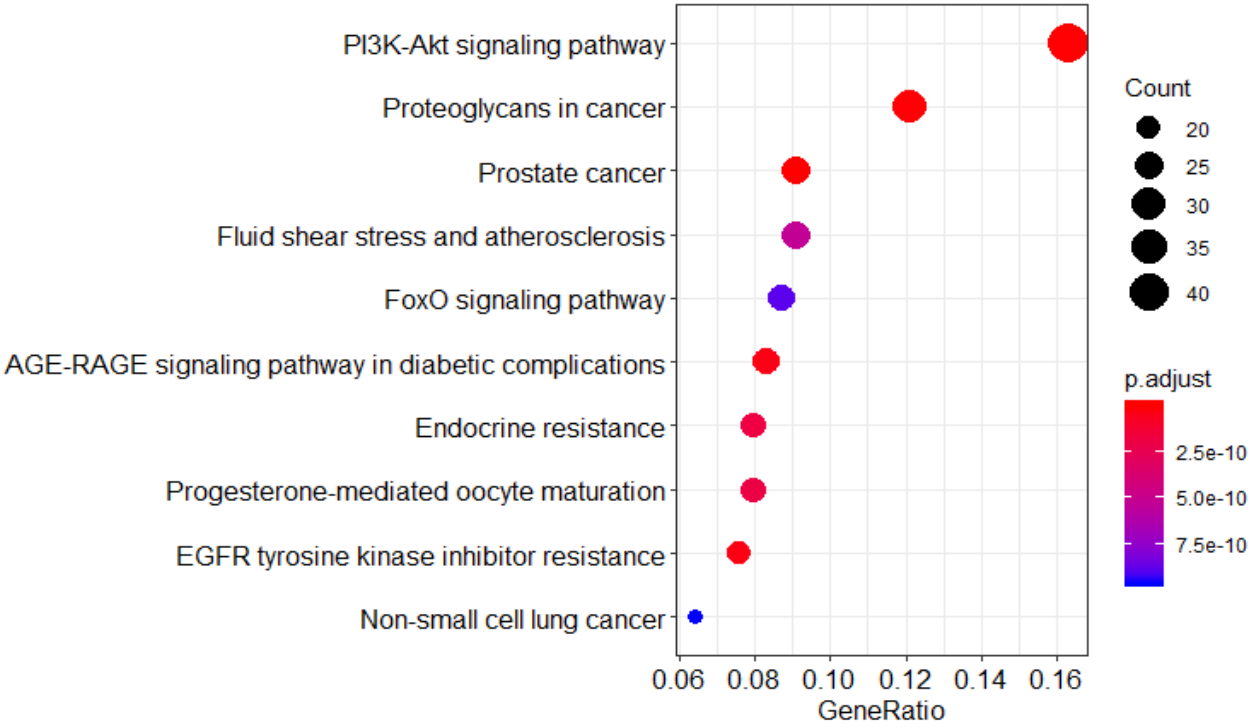

Figure 4. Pathway enrichment analysis of Hedyotis diffusa on glioma

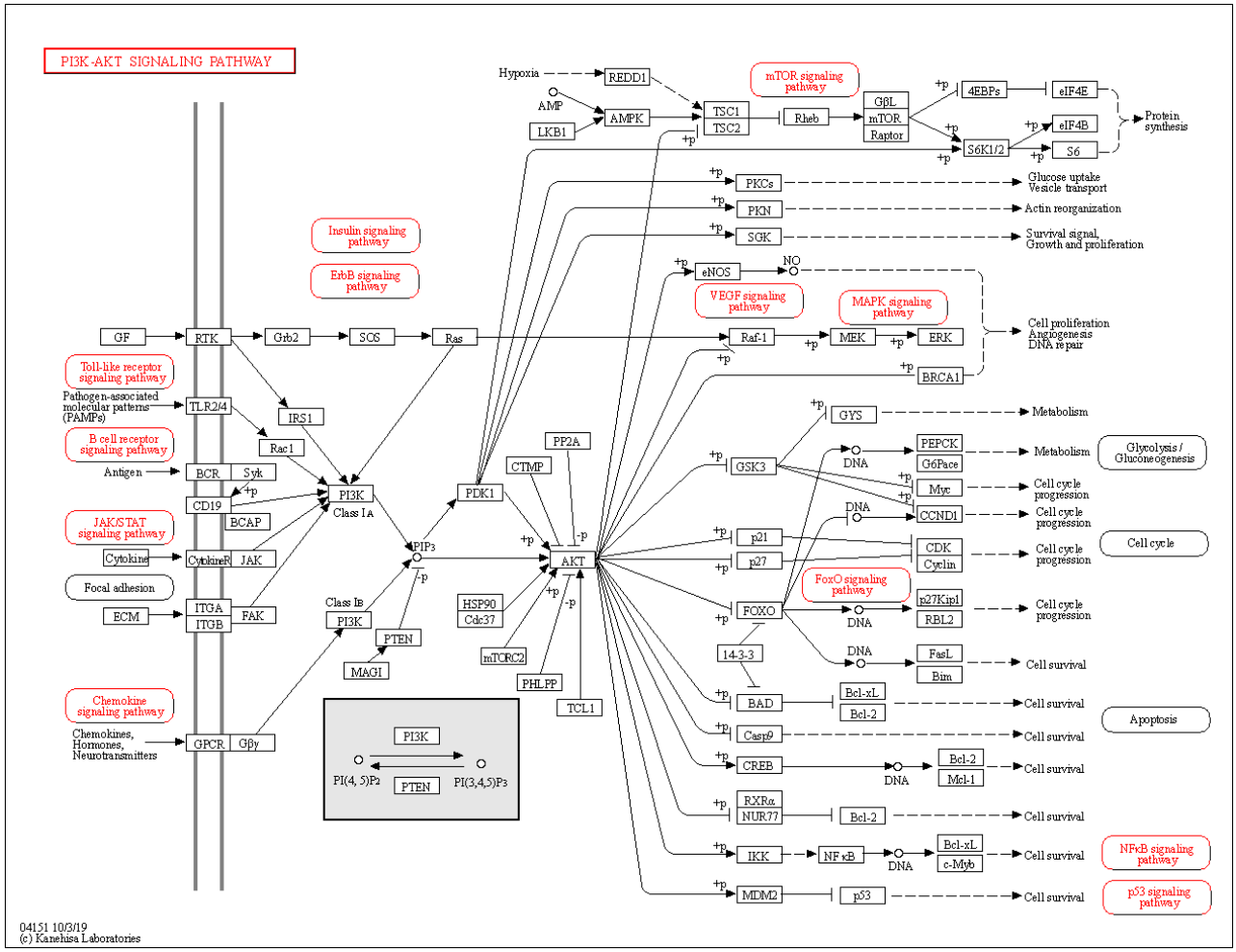

Figure 5. PI3K-Akt signaling pathway

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3.3. GO annotation and KEGG pathway analysis of Hedyotis diffusa on glioma target genes

We used R language cluster profiler package to annotate 295 target genes of Hedyotis diffusa on glioma and analyze KEGG pathway. Go functional annotation analysis showed that the biological process involved in steroid hormone response, regulation of lipid metabolism and lipid localization(Fig. 3) according to KEGG analysis, the regulatory pathway involves PI3K-Akt signal pathway, FoxO signal pathway, EGFR tyrosine kinase inhibition, etc. These results suggest that Hedyotis diffusa may achieve the goal of glioma treatment through the above regulatory pathways(Fig. 4) further analysis shows that the important target genes are mainly distributed in PI3K-Akt signaling pathway and FoxO signaling pathway. The results are shown in Fig. 5 and Fig. 6. PI3K-Akt signaling pathway is an important pathway of tumor cell proliferation, and the FoxO signaling pathway is related to apoptosis and cell cycle. We can infer that Hedyotis diffusa can inhibit the development of glioma by mediating the above signaling pathways, so as to achieve the purpose of treatment.

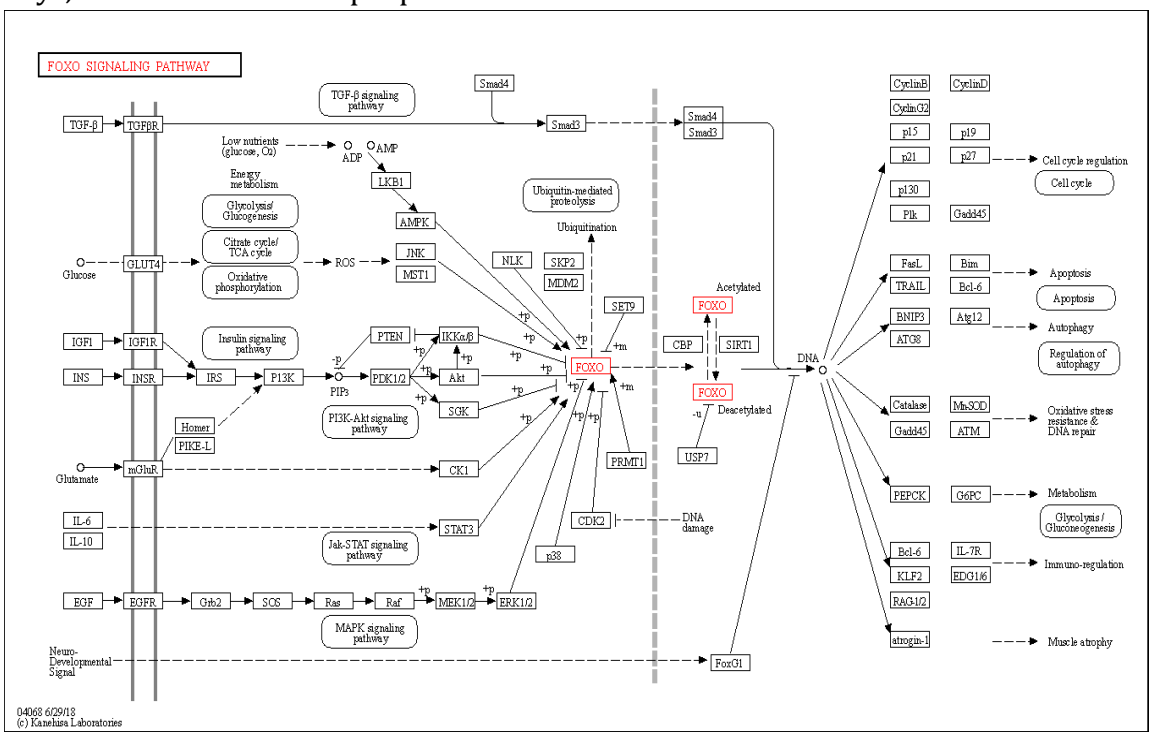

Figure 6. FoxO signaling pathway

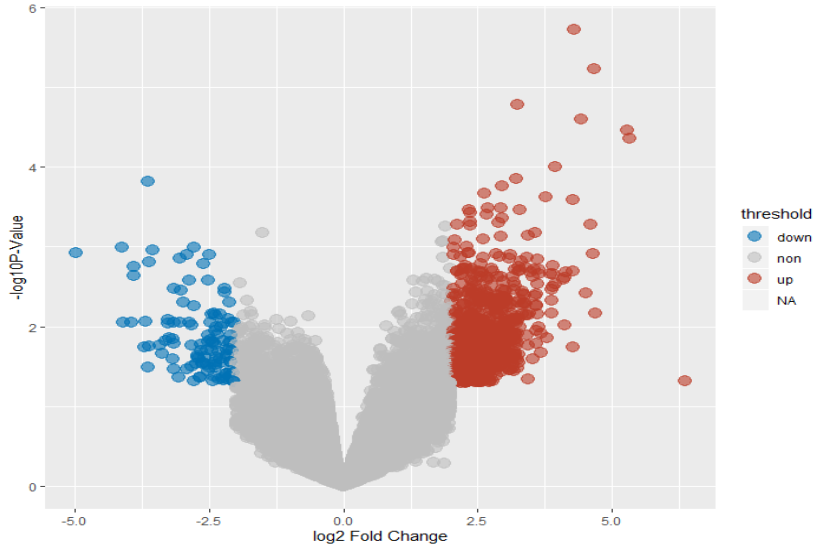

Figure 7. Volcanic map of differential expression profile of glioma

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3.4. Analysis of differential expression profile in glioma

By searching the transcriptome data of glioma in GEO database, we obtained GSE136000 data set, which contains 10 sample information, including glioma tissue and adjacent tissue, with 5 duplicate samples in each. Then, R language tool was used to analyze the differential expression profile. Using FPKM as the standardized method, 1115 differentially expressed genes were obtained according to the screening conditions, including 1003 up-regulated genes and 112 down regulated genes (Figure 7).

Previously, we have obtained 295 genes of Hedyotis diffusa on glioma, combined with 1115 differentially expressed genes of glioma in GEO dataset, and 23 overlapping genes were obtained by Venn diagram analysis. In conclusion, Hedyotis diffusa has 23 key genes which can be used as a key target for the treatment of glioma in the future. [5-17]

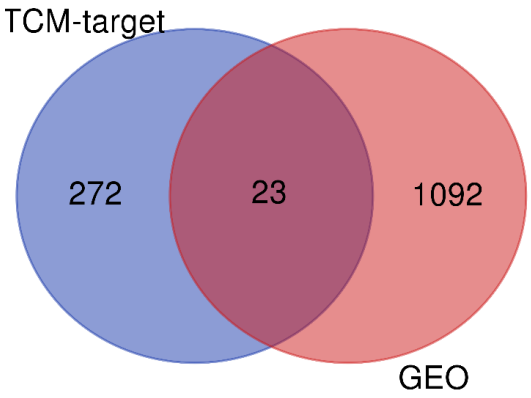

Figure 8. Target information statistics of network pharmacology and GEO datasets

4. Discussion

Glioma is the most common malignant tumor in the central nervous system, which originates from glial cells. It accounts for more than half of all intracranial tumors. High grade gliomas grow rapidly, recur quickly and have high mortality. At present, the treatment of glioma includes surgery, chemotherapy and radiotherapy, gene therapy and immunotherapy are also under active research. However, the overall effect is still not ideal, and the biological mechanism of the occurrence and development of glioma is still unclear. With the progress of scientific research, several traditional Chinese medicines have been found to inhibit the development of glioma. The combination of traditional medicine and modern scientific methods provides a new research direction for the treatment of glioma.

Hedyotis diffusa is a kind of annual herb, which is derived from Hedyotis diffusa. Its leaf shape is like the tongue, the flower is white, therefore the name. It is also known as qiandahui, yangxucao, Hedyotis and Salix tenuifolia. It is distributed in Yunnan, Guangxi, Zhejiang, Guangdong, Fujian, Jiangsu, Anhui and other places, and grows on hillsides, roadsides and streams. It is bitter, sweet, cold and non-toxic. There are more than 20 terpenoids in the extract of Hedyotis diffusa, among which iridoids are the main terpenoids with antitumor activity; There are about 10 kinds of acid compounds, among which ursolic acid and oleanolic acid have antitumor activities; There are 19 kinds of flavonoids, 21 kinds of anthraquinones, about 29 kinds of volatile components, 4 kinds of phenylpropanoids and coumarins, as well as trace elements Mo, Mg, Al, Se, Mn, Ti, Zn, Fe, Ca and polysaccharides. Polysaccharide is also an important active ingredient of Hedyotis diffusa, which can inhibit the growth of tumor cells, improve immunity and anti-aging. Hedyotis diffusa has anticancer effect. The anticancer effect of Hedyotis diffusa is related to the inhibition of cell cycle and the expression of apoptosis

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related genes. Zhang et al. Prepared different concentrations of drug serum of Hedyotis diffusa and acted on cervical cancer cells at different times, and concluded that with the increase of time and concentration of Hedyotis diffusa, the inhibition rate of Hedyotis diffusa on cervical cancer cells also increased significantly, and it can inhibit the gene expression of cancer cells. It has been reported that Hedyotis diffusa can induce apoptosis of drug-resistant (5-FU) colorectal cancer cells in transplanted tumor tissues, and has no obvious effect on the general living conditions of mice. Hu et al. Found that Hedyotis diffusa has a significant inhibitory effect on the proliferation of human prostate cancer cells and can induce their apoptosis.

In this study, we found that Hedyotis diffusa extract acts on glioma through PI3K-Akt and FoxO signaling pathways. In recent years, PI3K-Akt signaling pathway has been found to be closely related to human cancer. This pathway regulates the proliferation and survival of tumor cells, and its abnormal activity can not only lead to malignant transformation of cells, but also be related to the migration, adhesion and apoptosis of tumor cells and tumor angiogenesis and degradation of extracellular matrix. FoxO, as a tumor suppressor, plays an important role in a variety of tumors. Therefore, the use of Hedyotis diffusa in the treatment of glioma has become the focus of current research. We used the network Pharmacology Method to predict the active components of Hedyotis diffusa and construct the regulatory network of active components, targets and diseases. At the same time, GEO serves as a public database of various high-throughput experimental data, including experiments based on single channel and dual channel microarrays, detection of mRNA, genomic DNA and protein abundance, as well as non-array technologies, such as gene expression series analysis (SAGE), mass spectrometry proteomics data and high-throughput sequencing data. We used the transcriptome data of glioma in GEO to identify the differentially expressed genes between glioma and normal tissues. By comparing the expression profile data of glioma with the regulatory network data of Hedyotis diffusa, the key gene information of Hedyotis diffusa on glioma was summarized.

Acknowledgments

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