ORIGINAL RESEARCH

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Multi-database Based Study of the Pharmacological Mechanisms of Resveratrol in the Treatment of Early Hepatocellular Carcinoma

Erken Hepatoselüler Karsinom Tedavisinde Resveratrolün Farmakolojik Mekanizmalarına İlişkin Çoklu Veritabanı Temelli Çalışma

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Abstract

Objective: To analyze the main target genes, key pathways and their mechanisms of action of resveratrol in the treatment of early hepatocellular carcinoma based on multiple databases using a network pharmacology approach.

Method: The targets of resveratrol was obtained from the Swiss Target Prediction database; the targets of early hepatocellular carcinoma were obtained from the GeneCards, OMIM and DisGent databases. The compound-target-disease network was constructed using Cytoscape software; the protein interaction network was constructed using STRING database; GO function and KEGG pathway enrichment analysis were performed in R language.

Results: Sixty-nine potential targets for resveratrol were obtained through the Swiss Target Prediction database. Searching and de-duplicating the disease database yielded 9682 disease targets for early hepatocellular carcinoma. Seven hundred and fifty four entries were obtained from GO functional enrichment analysis and 99 statistically significant pathways were obtained from KEGG enrichment analysis.

Conclusion: The mechanism of action of resveratrol for early hepatocellular carcinoma is a multi-target, multi-pathway interaction. The receptors may be related to SRC, CYP3A4, PIK3CA, CYP1A1, RELA, ESR1 and other targets, and the major signalling pathways may be related to Ovarian steroidogenesis, steroid hormone biosynthesis, chemical carcinogenesis-receptor activation, endocrine resistance, chemical carcinogenesis-DNA adducts, nitrogen metabolism, hepatocellular carcinoma, etc. It provides a theoretical basis for the next in-depth experimental study.

Keywords: Bioinformatics, early hepatocellular carcinoma, multi-database, pharmacological mechanism, resveratrol

Öz

Amaç: Resveratrolün erken hepatoselüler karsinomun tedavisinde ana hedef genlerini, anahtar yolaklarını ve bunların etki mekanizmalarını bir ağ farmakolojisi yaklaşımı kullanarak çoklu veritabanlarına dayalı olarak analiz etmektir.

Yöntem: Resveratrolün hedefleri, İsviçre Hedef Tahmini veritabanından elde edildi; erken hepatoselüler karsinomun hedefleri GeneCards, OMIM ve DisGent veritabanlarından elde edildi. Bileşik hedef hastalık ağı, Cytoscape yazılımı kullanılarak oluşturuldu; protein etkileşim ağı, STRING veritabanı kullanılarak oluşturuldu; GO işlevi ve KEGG yolağı zenginleştirme analizi, R dilinde gerçekleştirildi.

Bulgular: İsviçre Hedef Tahmini veritabanından resveratrol için altmış dokuz potansiyel hedef elde edildi. Hastalık veritabanının taranması ve kopyalanması, erken hepatoselüler karsinom için 9682 hastalık hedefi ortaya koydu. GO fonksiyonel zenginleştirme analizinden 754 giriş elde edildi ve KEGG zenginleştirme analizinden istatistiksel olarak anlamlı 99 yolak elde edildi.

Sonuç: Resveratrolün erken hepatoselüler karsinom için etki mekanizması, çok hedefli, çok yolaklı bir etkileşimdir. Reseptörler, SRC, CYP3A4, PIK3CA, CYP1A1, RELA, ESR1 ve diğer hedeflerle ilişkili olabilir ve ana sinyal yolakları, hepatoselüler karsinoma yolağı, PD-L1 ekspresyonu ve PD-1 kontrol noktası yolağı ile ilişkili olabilir. Kolin metabolizma yolağı, merkezi cabon metabolizma yolağı ve diğer yolaklar. Bir sonraki derinlemesine deneysel çalışma için teorik bir temel sağlar.

Anahtar kelimeler: Biyoinformatik, çoklu veritabanı, erken hepatoselüler karsinom, farmakolojik mekanizma, resveratrol

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Introduction

Resveratrol is a compound found in grape skins, red wine, peanuts and certain berries. It has antioxidant properties that may help protect against cell damage and disease (1). Resveratrol has also been studied for its potential anti-aging and anti-inflammatory effects. In addition, research suggests that resveratrol may help protect against heart disease, improve cognitive function and physical performance, and have anti-cancer properties (2).

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. It is a fast-growing cancer that starts in the cells of the liver and can spread to other organs. Risk factors for HCC include HBV or C infection, cirrhosis of the liver, excessive alcohol consumption, smoking, obesity and diabetes (3). Symptoms can include abdominal pain, weight loss and jaundice. Diagnosis is made using imaging tests such as computed tomography or magnetic resonance imaging scans, liver function tests and a biopsy. Treatment options include surgery, ablation therapy (destroying tumour cells with heat or cold), radiotherapy (using high-energy X-rays) and chemotherapy (drugs to kill cancer cells). According to statistics, more than 572,000 people are affected each year. The incidence rate has been increasing over time, particularly in parts of Africa, Asia, Eastern Europe and South America (4). In some countries, such as China and Japan, HCC accounts for nearly 40% of all cancers reported each year. The highest incidence rates occur in regions where chronic hepatitis B (HBV) infection is most prevalent. It is estimated that 90-95% of HCC cases are due to HBV or hepatitis C (HCV) (5). The mortality rate for HCC varies widely by geographical region. Worldwide, more than 400,000 people are estimated to die from this form of cancer each year (6). This makes HCC one of the leading causes of cancer death worldwide. In some countries with a high prevalence of HBV/HCV infection, such as China and India, HCC accounts for nearly 80% of all liver cancer deaths (7). Treatment options for HCC depend on several factors, including stage at diagnosis, tumour size and location in the liver, as well as the patient's age and general health. Surgery is often the first-line treatment for early-stage tumours (8,9). However, this option may not always be feasible due to comorbid conditions or other factors. For more advanced stages or tumours that are not suitable for surgery, other treatments such as radiotherapy, systemic chemotherapy or targeted therapies may be used alone or in combination with surgery. Other newer treatments such as transarterial chemoembolization, radiofrequency ablation, stereotactic body radiation therapy, cryoablation and microwave ablation have also been used with varying success in certain cases where surgical resection is not possible or feasible (10). In addition, clinical trials are underway to test new approaches that aim to improve both outcomes and patient safety, while providing a better quality of life after treatment.

In this study, the potential targets of resveratrol for the treatment of early stage HCC were investigated using a network pharmacology approach based on multiple databases and related biological information and pathways. This study has laid the foundation for further research into the pharmacological mechanism of resveratro in the treatment of early HCC.

Materials and Methods

Target Screening of Resveratrol

The structure of resveratrol was searched in PubChem, database (11) (https://pubchem.ncbi.nlm.nih.gov/) using the keyword "resveratrol", and then the obtained structure information was searched in the Swiss Target Prediction database (12) (http://www.swisstargetprediction.ch/) to obtain the target information of resveratrol.

Disease Target Acquisition

The search term "Early Hepatocellular Carcinoma", "human" as the retrieval object, through the Genecards database (13) (https://www.genecards.org/),OMIM database (14) (https://www.omim.org/),DisGeNET database (15) (https://www.disgenet.org) obtain disease targets.

Acquisition of Intersectional Targets

Venny (https: //bioinfogp.cnb.csic.es/tools/venny/) software was used to create VEEN plots of compounds and diseases and to obtain intersection targets. Using intersecting targets as potential targets for resveratrol in early HCC (16).

Compound-target-disease Network Construction

Prepare compound gene "network" files and Type files, import the relevant files using Cytoscape software, and draw a "compound-target-disease" network map.

Protein Interaction (PPI) Network Construction and Network Topology Analysis

Using string (17) (https://string-db.org/) platform, import the intersecting targets, set the object as "homo sapiens"

with the highest confidence level of 0.900, hide the free gene nodes, and obtain the PPI relationship. The results were imported into Cytoscape software, and the network topology parameters were obtained by selecting "network analyzer" and analyzing the degree, betweenness centrality (BC) and closeness centrality (CC) of PPI network nodes. The degree value, BC and CC of the PPI network nodes were calculated. The top ten targets of degree value was used as key targets.

GO and KEGG Enrichment Analysis

Use bioinformatics open source software to install and run clusterProfiler (18), André et al. (19) packages in R language (3.5.1) for GO and KEGG enrichment analysis. And through the microscopic letter platform to visual display (https://www.bioinformatics.com.cn/). The enrichment analysis was performed to annotate resveratrol in terms of biological process (BP), cellular component (CC) and molecular function (MF) for the treatment of early hepatocellular carcinoma. The top 10 GO entries for BP, CCo and MF were filtered according to p-value size and plotted in bubble plots. cnetplot is part of the Gene-Concept Network, which describes the connections between genes and biological pathways as a network, allowing visualisation of the genes involved in these entries and the interacting genes between entries. Cnetplot is used to show the top 10 KEGG pathway interacting genes and Emapplot shows the overlap between the enriched pathways. Finally, the "compound-target-disease-KEGG pathway" network was mapped based on the relevant information.

Results

Natural Drug Active Ingredients, Diseases and Intersecting Targets

The Swiss target prediction database was searched and 69 resveratrol-related targets (Probability >0) were identified. By searching the GeneCards, OMIM and DisGENET databases, 9713 disease targets were screened, and 9451 targets were obtained after removing duplicate items. Using Venny software to intersect the TCM targets with the disease targets, 59 intersecting targets were obtained, which are the potential targets for resveratrol in the treatment of early HCC (Figure 1).

"Compound-component-target" Network Construction

The potential targets for resveratrol in early HCC were summarised and the information was mapped into a network using Cytoscape software. The network diagram had 61 nodes and 118 edges (Figure 2).

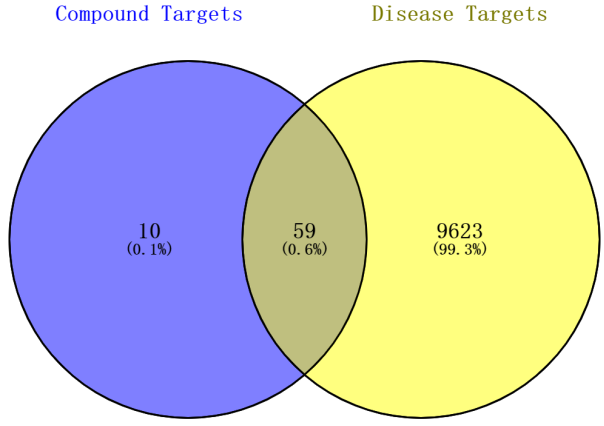


Figure 1. Intersection of drug targets and disease targets

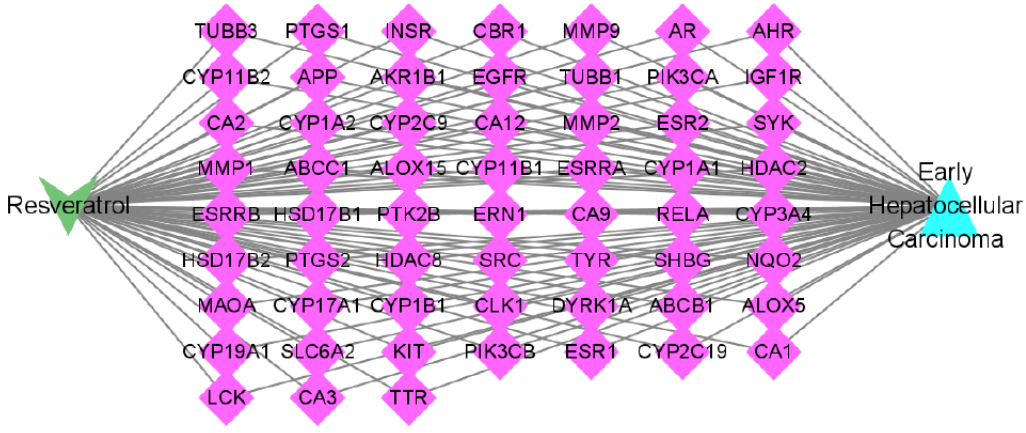


Figure 2. "Chinese herbal medicine-ingredient-target-disease" network diagram
(green represents compounds; purple represents targets: blue represents diseases)

PPI Network Construction and Network Topology Analysis

The topology of the PPI network was analysed using Cytoscape software and consisted of 8 nodes and 11 edges (Figure 3). The degree values of the compounds were analysed using the "network analyzer" plug-in. The key targets were sorted by degree size and listed in a table (Table 1). The SRC target had the highest degree value of 14.

GO and KEGG Enrichment Analysis

A total of 646 statistically significant pathways ($p<0.05$) were involved in the GO enrichment analysis (Figure 4), including 24 statistically significant BP entries, mainly related to olefinic compound metabolic process, cellular hormone metabolic process, Arachidonic acid metabolic process, icosanoid metabolic process, etc.; there were 178 statistically significant CC entries, mainly related to membrane raft, membrane microdomain, membrane region, membrane region, etc. Cytoplasmic side of plasma membrane, etc.; 84 statistically significant MF entries, mainly related to steroid hydroxylase activity, heme binding, tetrapyrrole binding, monooxygenase. KEGG enrichment analysis yielded 99 statistically significant pathways, mainly includes ovarian steroidogenesis, steroid hormone biosynthesis, chemical carcinogenesis-receptor activation, endocrine resistance, chemical carcinogenesis-DNA adducts, nitrogen metabolism, HCC, etc. And the top ten KEGG enrichment analyses are shown in Figure 5. The information on the top ten pathways analysed by KEGG enrichment is shown in Table 2. STAT3, VEGFA, HSP90AA1, FGF2, IL2, MET, BCL2L1, RORC et al. and multiple pathways all have an action relationship with each other, further suggesting the above as key targets. The Cnetplot diagram and Emapplot Diagram are shown in Figure 6.

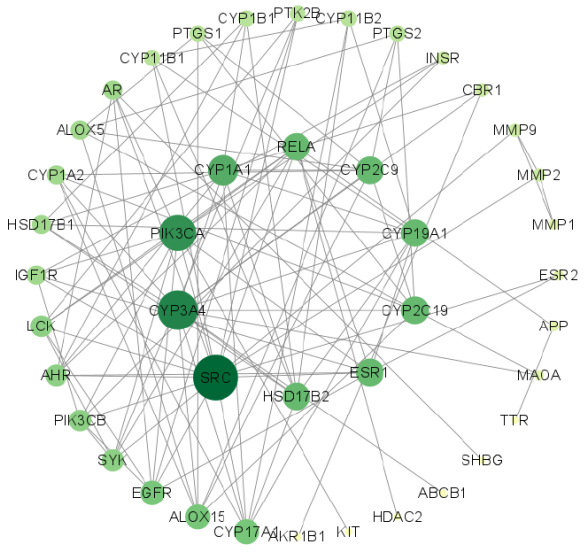


Figure 3. Topology analysis of the PPI network
PPI: Protein interaction

Discussion

The molecular mechanisms by which resveratrol exerts its anticancer effects are complex and involve various pathways. Resveratrol has been found to treat tumours by activating sirtuin family proteins (SIRT1-7), which are involved in regulating cellular stress responses, DNA repair and cell death. Resveratrol has been shown to induce cell cycle arrest, apoptosis and autophagy in cancer cells. In addition, resveratrol has been shown to inhibit angiogenesis, reduce blood supply to tumour cells, inhibit metastasis and regulate several pathways such as NF-kB and PI3K/Akt/mTOR to treat tumours (20).

Table 1. Table of key target information

Name	Degree	BC	CC
SRC	14	0.249149377	0.431578947
CYP3A4	12	0.217415796	0.39047619
PIK3CA	11	0.10011896	0.38317757
CYP1A1	9	0.267417115	0.436170213
RELA	8	0.168627213	0.401960784
ESR1	8	0.246560377	0.445652174
CYP2C9	8	0.116902386	0.344537815
CYP2C19	8	0.020897213	0.303703704
HSD17B2	8	0.018156214	0.362831858
CYP19A1	8	0.229654384	0.431578947

BC: Betweenness centrality, CC: Closeness centrality

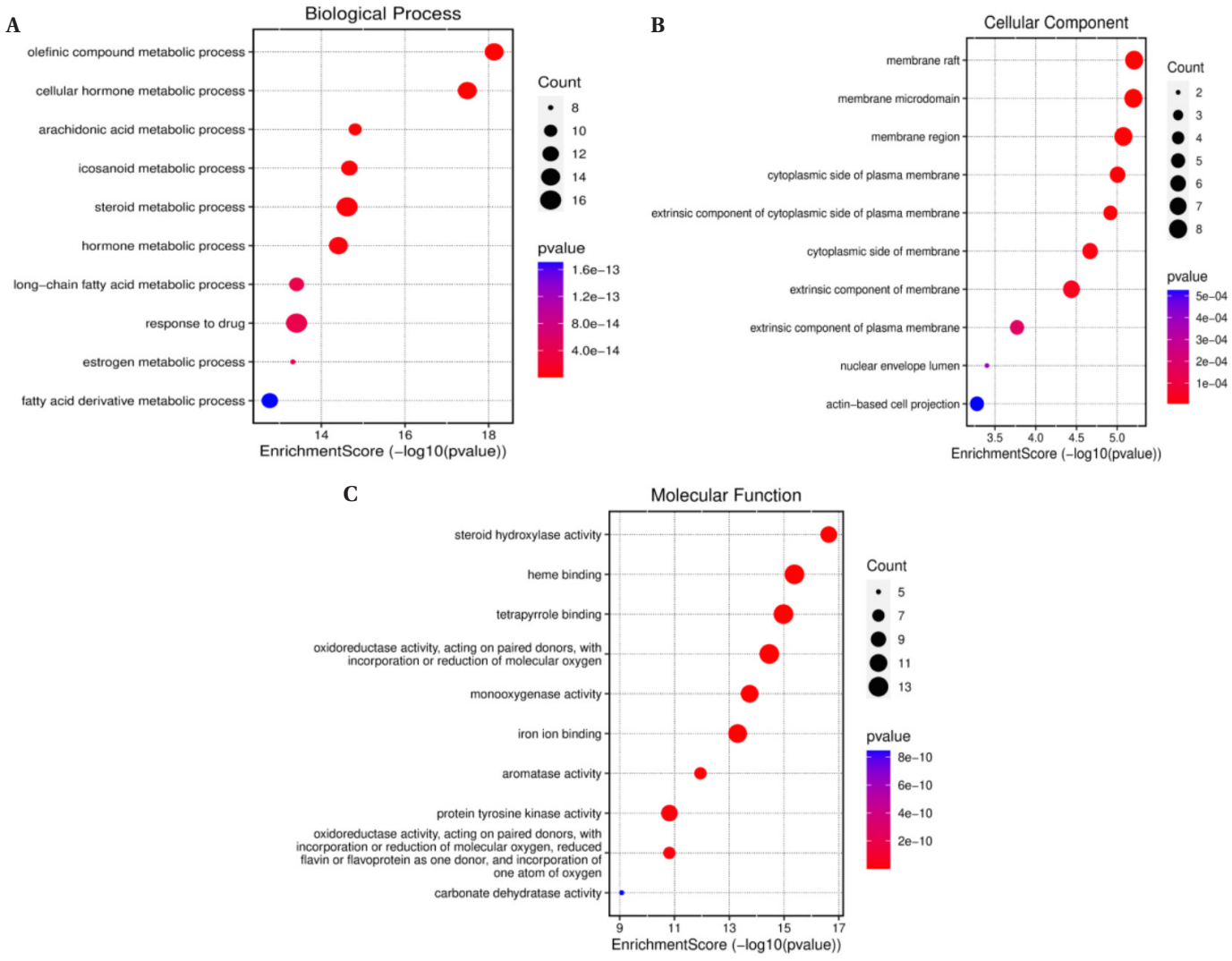


Figure 4. A) Bubble diagram of GO-BP enrichment analysis; **B)** Bubble diagram of GO-CC enrichment analysis; **C)** bubble diagram of GO-MF enrichment analysis

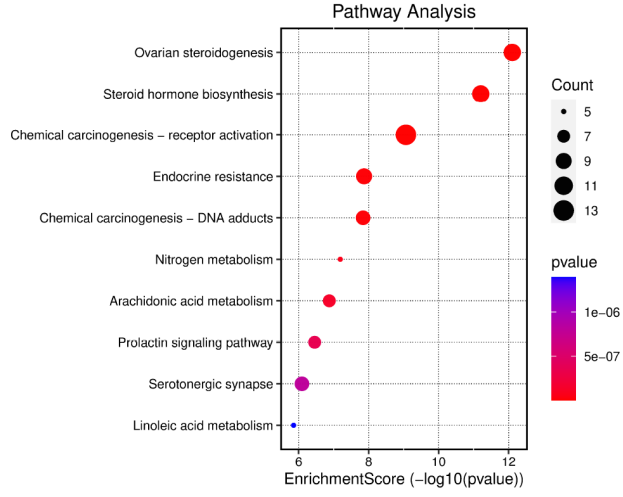


Figure 5. Bubble diagram of top ten KEGG enrichment analysis

Table 2. Top ten KEGG enrichment analysis data table

ID	Description	GeneRatio	p-value	Count
hsa04913	Ovarian steroidogenesis	10/55	7.85885E-13	10
hsa00140	Steroid hormone biosynthesis	10/55	6.25835E-12	10
hsa05207	Chemical carcinogenesis-receptor activation	13/55	8.60283E-10	13
hsa01522	Endocrine resistance	9/55	1.35159E-08	9
hsa05204	Chemical carcinogenesis-DNA adducts	8/55	1.44305E-08	8
hsa00910	Nitrogen metabolism	5/55	6.47215E-08	5
hsa00590	Arachidonic acid metabolism	7/55	1.33399E-07	7
hsa04917	Prolactin signaling pathway	7/55	3.50005E-07	7
hsa04726	Serotonergic synapse	8/55	8.06724E-07	8
hsa00591	Linoleic acid metabolism	5/55	1.39526E-06	5

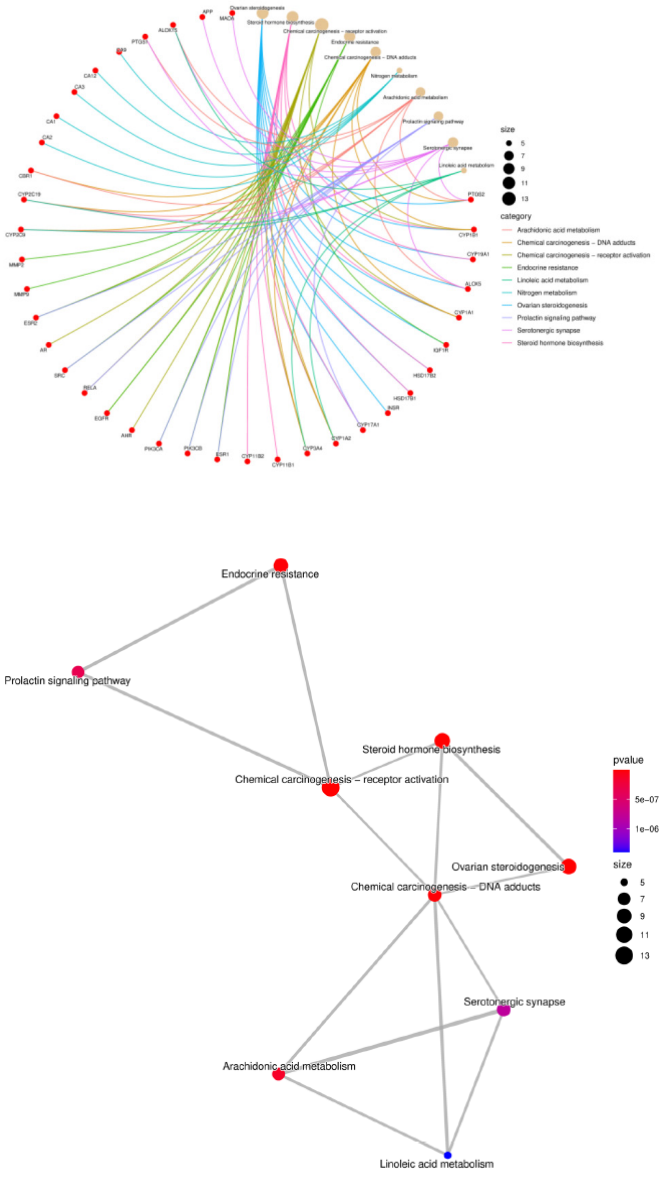


Figure 6. Cnetplot diagram and Emapplot diagram

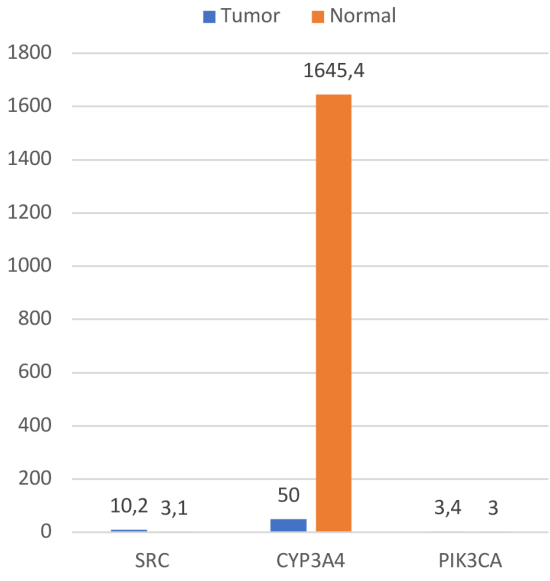
In this study, a network pharmacology approach was used to analyse 59 potential targets of resveratrol for the treatment of early hepatocellular carcinoma. The PPI analysis of the intersecting targets identified 10 key targets, namely SRC, CYP3A4, PIK3CA, CYP1A1, RELA, ESR1, CYP2C9, CYP2C19, HSD17B2 and CYP19A1. Three of these targets had a degree of more than ten, namely SRC, CYP3A4 and PIK3CA. PIK3CA.

SRC is a protein-coding gene. Diseases associated with SRC include thrombocytopenia, colorectal cancer and hepatocellular carcinoma (21). Pathways related to it include signaling downstream of RAS mutants and negative regulation of FGFR1 signaling. Gene ontology (GO) annotations associated with this gene include transferase activity, transfer of phosphorus-containing motifs and protein tyrosine kinase activity (22,23).

CYP3A4 is a protein coding gene. Diseases associated with CYP3A4 include vitamin D-dependent rickets, type 3 and acetaminophen metabolism. Among its related pathways are Imipramine/desipramine pathway, pharmacokinetics and metapathway biotransformation phase I and II (24,25). GO annotations related to this gene include enzyme binding and iron ion binding (26).

PIK3CA is a protein coding gene. Diseases associated with PIK3CA include megalencephaly-capillary malformation-polymicrogyria syndrome and congenital lipomatous overgrowth, vascular malformations, and epidermal nevi (27). Among its related pathways are Downstream signaling of activated FGFR2 and translation insulin regulation of translation. GO annotations related to this gene include transferase activity, transferring phosphorus-containing groups and protein serine/threonine kinase activity (28). The expression of the three genes *SRC*, *CYP3A4* and *PIK3CA* in *HCC* is shown in Figure 9.

Resveratrol can have a direct therapeutic effect on early HCC (Figure 8) as well as a therapeutic effect on early HCC through the regulation of related gene expression and related metabolism (Figures 9-11).



	SRC	CYP3A4	PIK3CA
Tumor	10,2	50	3,4
Normal	3,1	1645,4	3

Figure 7. The expression of the three genes SRC, CYP3A4 and PIK3CA in hepatocellular carcinoma

Conclusion

This study predicted the key targets and pathways of action of resveratrol in the treatment of early HCC based on multiple databases and using a network pharmacology approach. The mechanism of action of resveratrol for Early HCC was found to be a multi-target, multi-pathway interaction. The key targets of resveratrol for Early HCC were found to be SRC, CYP3A4, PIK3CA, CYP1A1, RELA and others. Related KEGG pathways are HCC pathway, PD-L1 expression and PD-1 checkpoint pathway, choline metabolism pathway, central carbon metabolism pathway, etc. It provides a theoretical basis for the next in-depth experimental study.

Ethics

Ethics Committee Approval: This is a description of the article. The data in my article comes from multiple network databases (such as BioGPS Database, Oncomine database, Kaplan-Meier Plotter, Database, etc.). And the article does not involve any real patients or experimental animals. There is therefore no need to submit an application to the Ethics Committee.

Informed Consent: N/A.

Peer-review: Internally and externally peer-reviewed.

Financial Disclosure: The author declared that this study received no financial support.

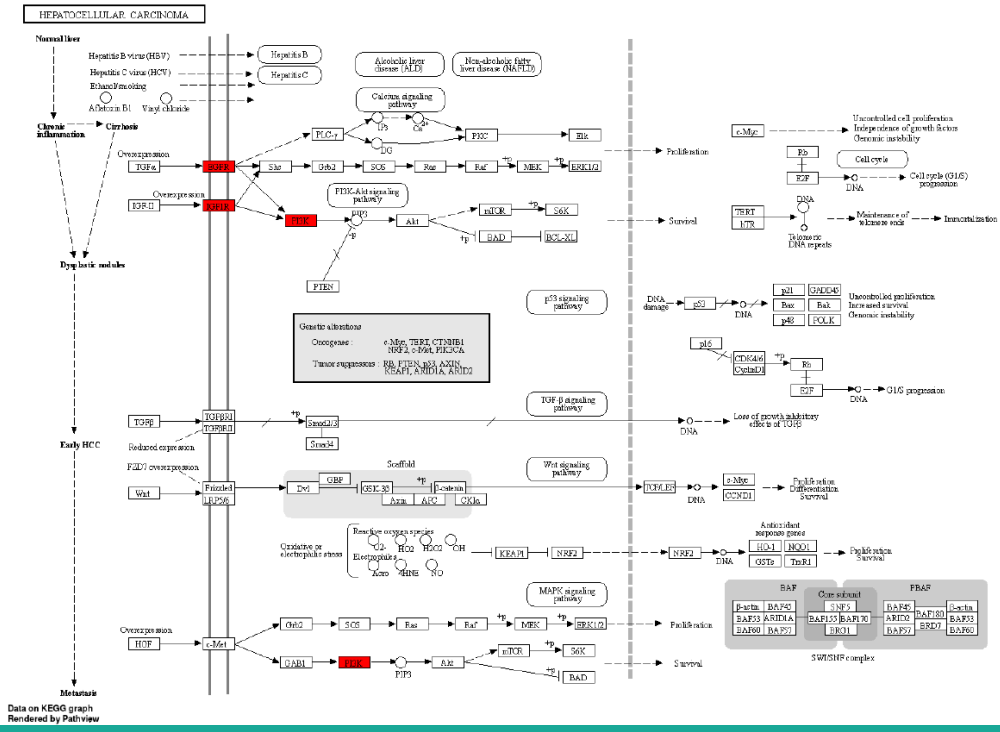


Figure 8. Resveratrol pathway for early-stage hepatocellular carcinoma

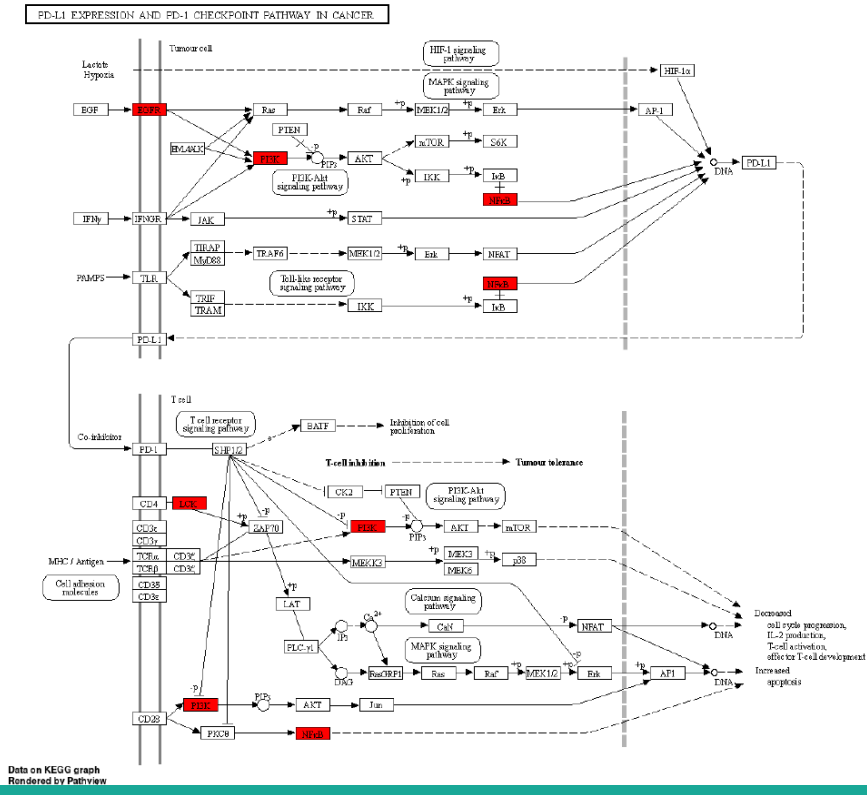


Figure 9. PD-L1 expression and PD-1 checkpoint pathway in cancer

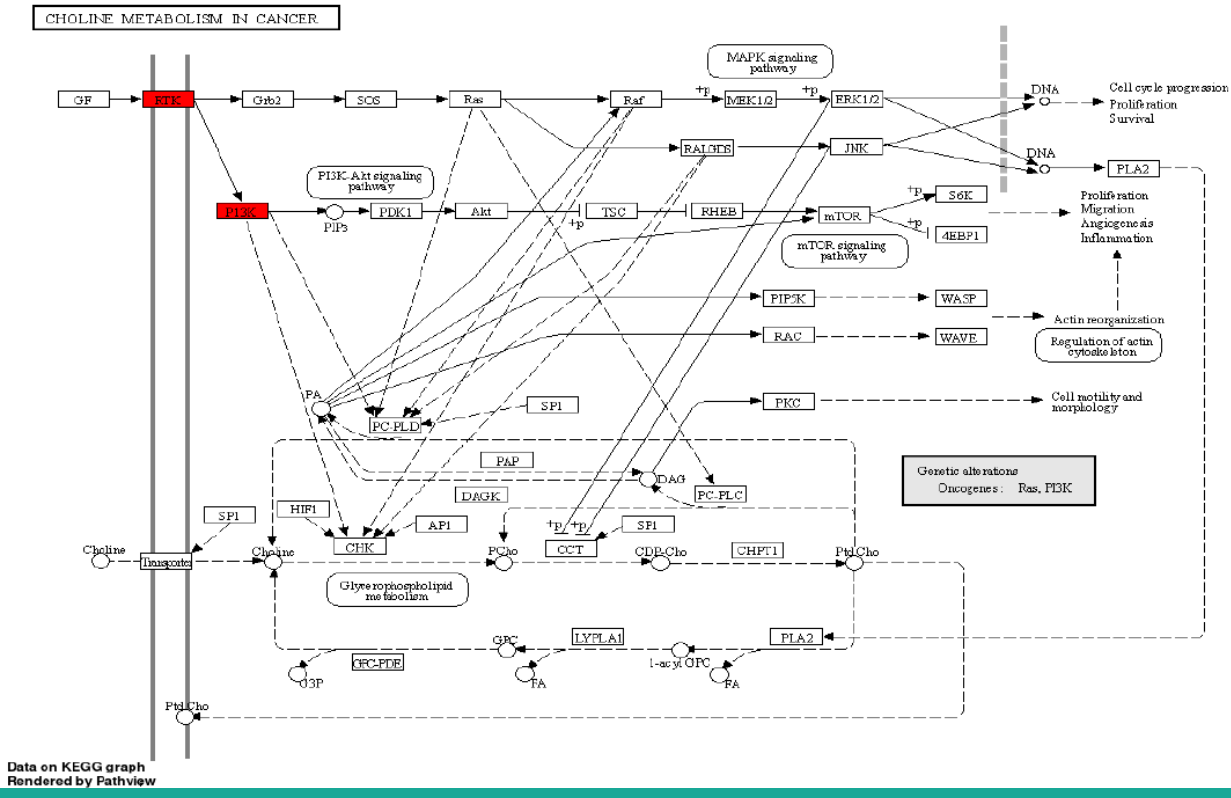


Figure 10. Choline metabolism in cancer

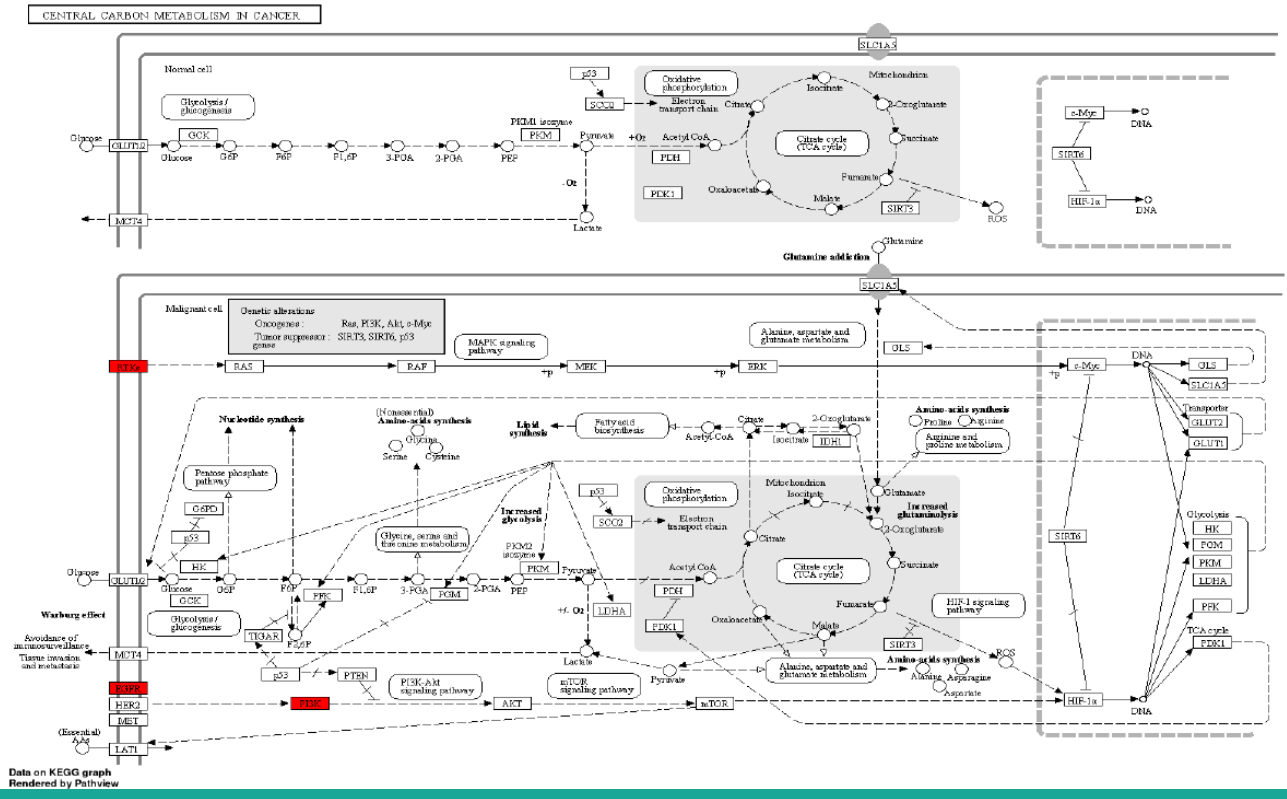


Figure 11. Central cabon metabolism in cancer

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