Analysis of Paeoniflorin Treatment of Cancer Based on Bioinformatic Network and Potential Target Prediction

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Abstract

In the synthesis of chemical drugs used to treat various cancers and tumors, more and more scientists are now focusing on the development of natural extracts. Paeoniflorin, as an important compound of the total glucosides of peony, had therapeutic effects on a variety of cancers in vivo and in vitro experiments. The purpose of this study was to use biological information analysis to reveal the basic mechanism and potential molecular mechanism of paeoniflorin treatment. We evaluated the efficacy of paeoniflorin through Traditional Chinese Medicine Systems Pharmacology Database (TCMSP). The potential targets of paeoniflorin were identified and summarized by Comparative Toxicogenomics Database (CTD), and GeneMANIA protein interaction analysis was performed online. Also, we used WebGestalt for Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and functional annotation. Finally, Cytoscape was used to construct the paeoniflorin-target-pathway network to predict the results.

Introduction

Natural products and their extracts are regarded as a very large treasure now used for drug development. Paeoniflorin, a natural product of polyphenols, has shown anticancer activity in a variety of cancers, [1] Paeoniflorin exists in the roots of *Paeonia* genus plants such as Ranunculaceae, Peony, Purple Peony, etc. [2, 3] and have been shown to have a wide range of treatments in nasopharyngeal cancer, [4] non-small cell lung cancer, [5, 6] glioma, [7–9] diabetic nephropathy [10, 11] and other diseases. Pharmacological activity, and especially anti-inflammatory effects, have been demonstrated in a variety of tumors or consumptive disease. The above results showed that paeoniflorin could be used as a therapeutic agent for various diseases. In addition, [7, 8, 12] our previous studies had shown that paeoniflorin could inhibit the migration, invasion, proliferation, and cell cycle of glioma cells. And in the verification of subsequent in vitro experiments, it also has the effect of inhibiting tumor growth.

Biological information analysis brings together a variety of online resources, including artificial intelligence algorithms, database usage, accelerating our research progress, such as using GO, KEGG analysis, PPI network establishment, and using OncoPrint, [13, 14] KEGG database to predict the therapeutic targets of various diseases. These modern methods provided us with a cognitive mechanism and treatment of tumors. Convenient platform, greatly reducing the waste of human resources. And promoting the development of scientific research and medical treatment.

However, the exploration of tumors and cancers was not deep enough. We hoped that through methods such as bioinformatics, we can provide reference for the study of tumor treatment and accelerate the development of drugs.

Materials & Methods

The use of the TCMSP database (<http://lsp.nwu.edu.cn/tcmsp.php>) for pharmacokinetic evaluation of paeoniflorin, which aggregated biological information on drug absorption, distribution, metabolism and excretion. For example, bioavailability (OB), drug similarity (DL), Caco-2 permeability (Caco-2), and blood-brain barrier (BBB). The bioavailability (OB) was considered as an important indicator of an available drug, which indicates the speed and degree of the drug entering the human circulation. The higher the OB value, the more medicine components that can be used by the human body, and the better the treatment effect. In this study, the chemical name "paeoniflorin" was entered into the search box and the pharmacokinetics of the drug was measured from the molecular level.

Drug Target Identification

The Comparative Toxicogenomics Database (<http://ctdbase.org/>) CTD was a powerful public database designed to increase understanding of how environmental exposures affected human health. It provided manual information about chemical gene-protein interactions, chemical disease and genetic disease relationships. This database was integrated

with functional and pathway datas to help develop hypotheses about the underlying mechanisms of environmentally affected diseases, It queried compound-gene interactions by entering the compound chemical formula.We used CTD function to predict the target of paeoniflorin. We chose chemical-gene interaction query, filled in the paeoniflorin in the Chemical column, and choose homo sapiens for the Organism column. Took all the predicted gene targets as the results of our paeoniflorin prediction.

GeneMANIA

GeneMANIA(<http://www.genemania.org>)GenMANIA was a tool for online analysis of protein interactions.By entering the gene list,GeneMANIA analyzed the hypothesis of possible gene functions, displayed the gene associations between the lists, and compared the properties of each gene, including co-expression,co-localization,etc.

GO and KEGG Analysis

WebGestalt(<http://www.webgestalt.org/option.php>) was a set of widely used gene set enrichment analysis tools for functional enrichment analysis in the context of different organisms. It was a powerful integrated data mining system that can manage,retrieve,organize,visualize,and analyze a large number of genes.GO aims to establish a description of gene and protein functions applicable to various species,mainly molecular biology,Biological Process,Cellular Components.KEGG integrated a comprehensive database of genomic,chemical and system function information.and $P \leq 0.05$ was considered statistically significant.

Drug-targets-pathways network construction

We used cytoscape 3.7.2 to process our data to build network relationships

Results

Structure And Pharmacokinetics Of Paeoniflorin

We obtained the chemical formula of paeoniflorin, as well as its pharmacokinetic properties and molecular characteristics from the TCMSP database[15].TCMSP's online website provides 12 key properties such as Caco-2,human OB,BBB,and HL for drug evaluation and screening.However, in the search results(Fig. 1),we focused on the bioavailability (OB) value of paeoniflorin was 53.87% (recommended value $\geq 30\%$),intestinal epithelial transmission rate (Caco-2) is $-1.47 \text{ nm} / \text{s}$, and drug half-life (HL) $t^{1/2}$ is 13.88 h,drug similarity (DL) value was 0.79 (recommended value ≥ 0.18),the ability to test the blood-brain barrier (BBB) was -1.86 , and $-0.3 \leq \text{BBB} \leq +0.3$ was considered Medium permeability(Table 1).These were TCMSP's assessments of the molecular structure and pharmacokinetics of paeoniflorin.

Table 1 pharmacokinetic and molecular properties											
MW	AlogP	Hdon	Hacc	OB(%)	Caco-2(nm/s)	BBB	DL	FASA-	TPSA(Iy/s2)	HL(hours)	RBN
480.51	-1.28	5	11	53.87	-1.47	-1.86	0.79	0.34	164.37	13.88	7

Target Genes Prediction Of Paeoniflorin

Further we used CTD[16] to predict potential targets of paeoniflorin, the steps had been mentioned previously. The CTD results showed that there were 28 candidate targets for paeoniflorin (all human genes),plus 6 possible gene targets from our previous research[17–19].A total of 34 identified potential targets were used by us for the next work(Table 2).

Target and Protein Interaction

Table 2CTD prediction targets			
Number	GENE sysmbol	Gene ID	Gene Name
1	TP53	7157	tumor protein p53
2	VCAM1	7412	vascular cell adhesion molecule 1
3	TGFB1	7040	transforming growth factor beta 1
4	TNF	7124	tumor necrosis factor
5	NOS2	4843	nitric oxide synthase 2
6	PIK3R1	7040	phosphoinositide-3-kinase regulatory subunit 1
7	MTOR	2475	mechanistic target of rapamycin kinase
8	NFE2L2	4780	nuclear factor, erythroid 2 like 2
9	MAPK1	5594	mitogen-activated protein kinase 1
10	MAPK3	5595	mitogen-activated protein kinase 3
11	IL1B	3553	interleukin 1 beta
12	IL6	3569	interleukin 6
13	IFNG	3458	interferon gamma
14	IL10	3586	interleukin 10
15	HMOX1	3162	heme oxygenase 1
16	ICAM1	3383	intercellular adhesion molecule 1
17	CASP9	842	caspase 9
18	CD68	968	CD68 molecule
19	CNR2	1269	cannabinoid receptor 2
20	HIF1A	3091	hypoxia inducible factor 1 subunit alpha
21	BNIP3	664	BCL2 interacting protein 3
22	CASP3	836	caspase 3
23	BAX	581	BCL2 associated X, apoptosis regulator
24	BCL2	596	BCL2, apoptosis regulator
25	APAF1	317	apoptotic peptidase activating factor 1
26	ARG1	383	arginase 1
27	HGF	3082	hepatocyte growth factor
28	ROCK1	6093	Rho associated coiled-coil containing protein kinase 1
29	RHOA	387	ras homolog family member A
30	MET	4233	MET proto-oncogene, receptor tyrosine kinase
31	SKP2	6502	S-phase kinase associated protein 2
32	STAT3	6774	signal transducer and activator of transcription 3

33	MRC1	4360	mannose receptor C-type 1
34	NFKBIA	4792	NFKB inhibitor alpha

In order to explore the predicted 34 genes and their protein effects, we used the GeneMANIA[20] online analysis website to enter the above genes into the search box. From the results, we found that 45.30% had co-expression effects, 14.52% had physical interactions, and 14.98% played Co-localization, pathway, shared protein domains and genetic interactions also depicted in the results below(Fig. 2).20 genes associate with 34 targets genes also be selected.

Identification and functional annotation of 34 targets with GO and KEGG analysis

The above summarizes the functional relationships of 34 target genes and their proteins. Further study of these genes involved pathways, metabolic pathways, and protein and gene functions. We used WebGestalt[21] to perform GO and KEGG analysis[22] on 34 target genes (Organism is hsapiens)(Fig. 3, Table 3), and functional annotation of by GO and KEGG results. more detailed annotation information can be found in Table S1. GO analysis comprehensive analysis from three aspects of Biological Process, Cellular Component and Molecular Function (Fig. 3A), immune response (26/34), whole membrane (14/34), identical protein binding (17/34). The relationship between genes and diseases was determined through analysis of the KEGG pathway. KEGG analysis was used to speculate that a important function during malaria and AGE-RAGE signaling pathway in diabetes complications(Fig. 3B). The following listed the top 10 genes and false discovery rate(FDR) ≤ 0.05 (Table 3). The relationship between the gene's enrichment ratio in diseases and pathways and FDR was represented by a volcano plot(Fig. 3C), It turned out that cancer and tuberculosis were significant. so the results indicated that paeoniflorin may play an critical role in cancers.

Table 3.KEGG analysis results						
Gene Set	Description	Size	Expect	Ratio	P Value	FDR
hsa05200	Pathways in cancer	526	2.3944	10.023	0	0
hsa05152	Tuberculosis	179	0.81483	19.636	0	0
hsa04933	AGE-RAGE signaling pathway in diabetic complications	99	0.45066	28.846	1.11E-16	1.21E-14
hsa05161	Hepatitis B	144	0.65551	21.357	4.44E-16	3.62E-14
hsa05144	Malaria	49	0.22306	44.832	6.44E-15	4.20E-13
hsa05163	Human cytomegalovirus infection	225	1.0242	14.645	1.07E-14	5.79E-13
hsa05145	Toxoplasmosis	113	0.51439	23.328	3.29E-14	1.53E-12
hsa05210	Colorectal cancer	86	0.39148	28.098	5.64E-14	2.09E-12
hsa05205	Proteoglycans in cancer	201	0.91498	15.301	5.77E-14	2.09E-12
hsa04066	HIF-1 signaling pathway	100	0.45521	24.164	3.14E-13	1.02E-11

Network Construction

Through the aforementioned prediction of paeoniflorin targets, functional analysis, and pathway analysis. We further administrated Cytoscape3.7.2[23] to build a complete network diagram (drug-target-pathway). As showed in the Fig. 4, red indicated compounds, yellow indicated predicted targets, and the outermost circle indicated possible disease pathways. They were connected by grey lines, indicating the connection between the three.

Discussion

The application of modern data, such as the use of databases and artificial intelligence, can quickly process information and integrate accuracy. Now many online resource websites have become an indispensable tool for our scientific research and speed up our work needs. Databases like TCGA[24] and oncomine[25] are the two major databases that we commonly use. But in the end it still depends on the repeated demonstration of experimental data.

Plant extracts are now an important source for our treatment of diseases[26, 27], and many countries have invested huge amounts of money in the development of extracts to treat cancer. Paeoniflorin is used as an extract of Ranunculaceae, Peony, and Purple Peony[28]. The compound structure was shown in Fig. 1, and many factors should be considered, such as half-life, indicating the continuous effect of the drug, and oral bioavailability to prove the biological activity of the molecule[29]. The blood-brain barrier should be considered first in brain diseases treatments[30]. According to Lipinski's five-point rule to determine the choice of drugs[31], the recommended choice for oral drugs was molecular weight < 500, Logp < 5. The number of hydrogen-bonded donors and acceptors was less than 5 and 10, respectively (Table 1). Among the drugs we selected, paeoniflorin's Hacc values > 10, but other parameters meet the pharmacokinetics of oral drugs. Therefore, we still consider paeoniflorin as a new drug option and a new target need to be explored deeply.

After determining the pharmacokinetic properties of a drug, we needed to find the target genes for drug action. Many articles had shown that compounds extracted from plants were inextricably linked to genes or proteins[32–34]. To this end, we used CTD to predict 28 genes online (Table 2). Since 6 genes had been reported in our previous research, a total of 34 target genes were in our study. To further explore the function of these 34 genes, we used the GeneMANIA online tool to prove their co-expression, physical communication, co-localization and other functional information (Fig. 2). In addition, through the establishment of protein interactions, we also found 20 interesting genes related to the query genes. For example, the key role of RPTOR in breast cancer[35], the low expression of VDAC1 in cholangiocarcinoma is closely related to the overall survival rate[36]. CREBBP inactivation is associated with HDAC3-dependent lymphoma[37]. So above results suggested that strong relationship and identical functions between above targets genes and further more related targets genes need to be explored.

Then we performed GO analysis and KEGG analysis on 34 target genes (Fig. 3A,B). We found that most of genes were related to the response to immune, Cancer immunotherapy is a breakthrough in cancer, such as cytokine therapy[34]. Previous articles have confirmed the inflammation and immunity of paeoniflorin in diseases such as rheumatoid[38], allergic asthma[39] and arthritis[40]. In addition, the GO analysis results also explain that paeoniflorin is also involved in cell migration[17, 41], cell activation, and cell activity regulation. and KEGG analysis indicated that multiple pathway correlated to paeoniflorin, especially HIF-1 signaling pathway. Especially HIF-1 signaling pathway actively participates in the progress of various cancers[42, 43], These results are very consistent with our results from GO and KEGG analysis, suggesting that paeoniflorin has full potential as a cancer treatment.

In order to express paeoniflorin, 34 target genes, and their respective disease pathways more intuitively, we used Cytoscape to construct a drug-gene-pathway network (Fig. 4). paeoniflorin links 34 target genes, and 34 target genes link 1064 disease pathways. Figure 4 shows that paeoniflorin is a multi-target compound. in the treatment of cancer, multi-target

therapy is usually the only choice, effectively improving the efficiency of treatment. Establishing a drug-targets-pathway network to visualize the pharmacological activity of paeoniflorin. For example, breast cancer[44], colorectal cancer[45], glioma[17], pancreatic cancer[46], multi-target potential mechanisms are usually our exploration targets for cancer research. Therefore, paeoniflorin may be a promising resource for use as a chemical moiety or active ingredient for future drug discovery.

Conclusions

In summary, we defined the potential chemical properties of paeoniflorin, searched for multiple targets and corresponding different pathways, and provided safe and effective multi-target anti-cancer drug options for the development of cancer treatment. In addition, by analyzing the target of paeoniflorin, we have also discovered new targets, which require further experiments to verify, and also provide opportunities for further research on the physical and chemical properties of paeoniflorin and subsequent clinical applications.

Declarations

Availability of data and materials

The datasets analyzed in this study are available in Gene Expression Omnibus (GEO) public repository.

Ethics approval and consent to participate

Not applicable

Competing interests

All authors declared that there are no competing interests

Table 1

pharmacokinetic and molecular properties. Molecule Weight (MW). AlogP value represented the partition coefficient between octanol and water. The Hdon and Hacc are measures of hydrogen-bond donors and acceptors. Oral bioavailability (OB). blood-brain barrier (BBB), ≥ 0.3 were considered as non-penetrating, ≥ 0.3 were referred to strong penetrating. Drug-likeness (DL). Fractional water Accessible Surface Area (FASA). Total Polar Surface Area (TPSA). RBN was considered bonds which allowed free rotation around themselves. Drug Half-Life (HL).

Table 2

CTD prediction targets.

Table 3

Table 3. KEGG analysis results. KEGG analyzed the relationship of gene-pathway, and the results were expressed in P value and FDR, and the top ten were listed.

Table S1

Annotation of gene targets with GO and KEGG.GO(Gene Ontology),BP (biological process), CC (Cellular Component), MF(Molecular Function),KEGG(Kyoto Encyclopedia of Genes and Genomes).

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Authors' contributions

LRE designed the experiment, JYA collected data and wrote the manuscript. LJY performed and prepared data and graphs. HWW reviewed data. LDD revised manuscript.

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Not applicable

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Figures

Paeoniflorin

Figure 1

Chemical structure formula of paeoniflorin obtained from TCMSP database (Molecule ID:MOL001924).

Figure 2

GeneMANIA predicts the relationship between paeoniflorin targets. Black nodes represent the target protein, while Co-expression is represented by light purple, Physical interactions (red), Co-localization (blue), pathway (light blue), Predicted (orange), Shared protein domains (yellow), and Genetic interactions (green).

Figure 3

GO analysis.(A)GO analysis had summarized the Biological Process,Cellular Component, and Molecular Function.(B)GO analysis conducted disease types and pathways,and listed enrichment ratios of top 10.(C)The volcano plot showed the results, the X-axis represented the enrichment ratio, and the Y-axis referred to the FDR.

Figure 4

Targets network construction. Constructing a drug-target-path map. Cytoscape constructed the relationship between paeoniflorin, 34 targeted genes and the pathway relationship between these targeted genes and diseases.

Supplementary Files

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