

Molecular mechanism study of cancer treatment based on network pharmacology of lily

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Abstract. OBJECTIVE: To make predictions related to the treatment of cancer by lily. METHODS: A systematic study of the constituents, targets and pathways of lily and cancer treatment was conducted using network pharmacology and molecular docking methods. The active ingredients of lily were screened and selected for investigation using TCMSP, Uniprot and PubChem databases, and the "ingredient-target-pathway" correlation axis was established. PubChem was used to collect the compounds in lily, and the active ingredients and targets with OB $\geq$ 30% and DL $\geq$ 0.18 in lily were obtained using the TCMSP Chinese medicine database. The active ingredients that met the criteria were also screened, and the binding patterns of the core targets and active ingredients were verified using molecular docking techniques before the active ingredients in lily were genetically aligned using Uniprot, and the corresponding genes were collated. The genes of different cancers were collated using CTD. Cytoscape 3.9.0 was used to create a map of the active ingredients and their corresponding targets. Finally, the results obtained were used to make predictions related to the treatment of cancer in lily. Results: The herb-compound-target network was obtained through screening. After cross-matching the active targets of the chemical components in lily with various cancers, 42 intersecting targets were obtained. Conclusion: The rank values (degree) of the intersecting targets were analysed and six targets with a degree greater than 5 were found to be PTGS2 (12), MMP1 (10), PPARG (8), HSP90AA1 (8), TP53 (8) and ESR2 (6); the diseases that were closely linked to the targets were Cancer, unspecified (The findings of this paper may provide a reference for the development of relevant targeted drugs and targeted therapeutic approaches in the future.

Keywords: network pharmacology; lily; cancer; mechanism study.

Cancer is a major public health problem worldwide and one of the deadliest diseases in the world, with 19.3 million new cancer cases and nearly 10 million cancer deaths worldwide in 2020, according to statistics [1]. Despite the emergence of emerging therapies for cancer treatment and the development of various targeted drugs, among others, there is ultimately no complete cure for cancer. Therefore, there is an urgent need to discover early diagnostic tools, prognostic biomarkers and more reliable treatments to improve the cure and survival rates of cancer patients [1].

The Chinese herb *Anemarrhenae Rhizoma* is the root of lily. The rhizome of *Anemarrhenae asphodeloides* Bge, family Anemarrhenaceae. *Anemarrhenae Rhizoma* has a wide range of effects, including clearing heat and fire, promoting the production of body fluid and moistening dryness. In the Chinese Pharmacopoeia (2020 edition), mangostin and saponin B II are used as indicators of the quality of lily [1]. In addition, lily contains a variety of active ingredients such as saponins, flavonoids, lignans, alkaloids, phenylpropanoids and phenols, anthraquinones and other components [4]. In a study on the anti-tumour activity of lily, Pan Huijun et al [2] used the PANC-1 subcutaneous transplantation tumour model to detect the expression of VEGF mRNA in tumour tissues by RT-PCR and VEGF protein in tumour tissues by Western blotting, and found that the tumour growth was effectively inhibited after the injection of lily saponin AIII (1.0 mg·kg⁻¹), which proved that lily saponin AIII has anti-tumour activity. The mechanism was closely related to the inhibition of VEGF. In addition, Huo Zhonghua et al. [3] made nanofiber membranes of *C. sinensis*

saponin BII and polylactic acid and examined the therapeutic effect of the drug-loaded nanomembranes on gastric cancer using the CKK-8 method. [4] However, most of the current studies on cancer-inhibiting substances in *C. sinensis* are only at the molecular level and cannot analyze its anticancer effect at the genetic level, so the explanation related to the mechanism of action is incomplete.

Therefore, this paper predicts the different targets of action of lily for cancer treatment based on network pharmacology and molecular docking, and predicts the mechanism of action for cancer treatment combined with database finding, and predicts drug-target protein interaction by visualization software to determine the binding mode and location, hoping to provide effective target and drug selection for future anti-cancer research.

1. Database and software

Table 1. Databases and software used

No	Name	Website	Uses
1	PubChem	https://pubchem.ncbi.nlm.nih.gov/	Compound composition information gathering
2	TCMSP	http://ibts.hkbu.edu.hk/LSP/tcmsp.php	Find substances and their targets of action in lily
3	SwissTarget Prediction	http://www.swisstargetprediction.ch/	Component target prediction
4	UniProt	http://www.uniprot.org/	Screening for human origin genes, correction of standard gene names
5	Cytoscape		Produce maps of active ingredients and corresponding targets
6	CTD	https://ctdbase.org	Screening for disease targets
7	PDB	https://www.rcsb.org	Obtaining the three-dimensional structure of a specific receptor
8	PubChem	https://pubchem.ncbi.nlm.nih.gov	Download ligand structure

2. Methods

2.1 Screening of disease targets and herbal ingredient targets using databases

With the help of platforms such as the TCMSP (<http://tcmsp.com/tcmsp.php>) database, the compounds in lily were collated and analysed using the keywords of lily. The compounds in lily that met the oral bioavailability (OB) $\geq 30\%$ and drug-like properties (DL) ≥ 0.18 were used as active ingredients, and the binding mode of the core target and the acting component was verified using molecular docking techniques, i.e. the binding affinity between the protein and ligand in a given complex was first predicted using a scoring function, and then the protein was prepared by adding hydrogen ions and removing water molecules with the help of PDB (<https://www.rcsb.org>) to obtain the 3D structure of a specific receptor after preparing the protein by adding hydrogen ions and removing water molecules, finally performing energy minimisation and validating the protein model by Ramachandran plots or other methods, and then using Pub Chem (<https://pubchem.ncbi.nlm.nih.gov>) chemical database to download the ligand structure finally Molecular docking and molecular docking authentication are performed to improve the accuracy of the prediction and thus further identify the target of action of the active ingredient as well as the target site. The CTD (<https://ctdbase.org>) database was used to analyse the target genomic proteins of the cancer to identify valid targets where the drug overlaps with the disease.

2.2 Building component-target-disease networks

The active ingredients and their corresponding targets were imported into Cytoscape 3.9.0 software to construct a "herb-active ingredient-target" network model.

2.3 Performing ppI network construction

The main target genes were screened by subsequent importation using a database of known human genes and proteins in the Uniprot platform (<http://www.uniprot.org/>) to match against the robin targets.

3. Results

3.1 Effective substance screening results

The chemical composition of lily was searched using the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP, <http://tcmspw.com/tcmsp.php>). All chemical components were screened for oral bioavailability (OB) $\geq 30\%$ and drug likeness (DL) ≥ 0.18 , and the active chemical components were collected to establish a dataset of active chemical components, as shown in Figure 1.

MolID	MoleculeName	AlogP	Hdon	Hacc	OB(%)	Caco-2	BBB	DL	FASA-	HL
MOL001677	asperglauclide	4.018	2	6	58.016	0.28393	-0.2151	0.51972	84.5	6.875465
MOL003773	Manziferolicacid	6.466	2	3	36.159	0.66435	-0.06296	0.84358	57.52999878	5.713361
MOL000422	kaempferol	1.771	4	6	41.882	0.26096	-0.55335	0.24066	111.1299973	14.743371
MOL004373	Anhydrocaritin	3.879	3	6	45.411	0.72306	0.00641	0.43786	100.1299973	15.011843
MOL004489	AnemarsaponinF ot	3.917	2	4	60.063	0.42643	-0.33153	0.78538	58.91999817	4.611004
MOL004492	Chrysanthemaxanthin	8.242	2	3	38.723	0.50972	-0.98386	0.58352	49.68999863	17.474598
MOL004497	Hioceastrine	1.174	1	6	51.649	0.02105	-0.49315	0.61953	68.23000336	8.091973
MOL004514	TimosaponinBIII ot	4.773	2	3	35.259	0.4187	-0.4667	0.87013	49.68999863	4.322953
MOL000449	Stigmasterol	7.64	1	1	43.829	1.44458	1.00045	0.75665	20.22999954	5.574595
MOL004528	IcariinI	0.772	8	15	41.583	-2.05633	-3.05546	0.60997	238.1999969	18.015685
MOL004540	AnemarsaponinC ot	4.975	2	3	35.499	0.42981	-0.46344	0.86988	49.68999863	3.983885
MOL004542	AnemarsaponinE ot	4.526	2	4	30.666	0.34431	-0.23305	0.85657	58.91999817	5.142532
MOL000483	(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide	2.859	3	5	118.34	0.50842	-0.26941	0.26399	78.79000092	4.262461
MOL000546	diosgenin	4.634	1	3	80.877	0.81998	0.27383	0.80979	38.68999863	4.137054
MOL000631	coumaroyltyramine	2.876	3	4	112.90	0.60102	-0.22469	0.20234	69.55999756	5.628603

Fig. 1 Active substances obtained from the screening of lily

3.2 Results of effective substance target screening

By using the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP, <http://tcmspw.com/tcmsp.php>), the effective substances in Figure 1 were identified by target searching. The effective substances in Fig. 1 were used for target searching to obtain the individual targets of different effective substances, and were screened for multiple core targets using UniPort human known target proteins and genes using Cytoscape 3.8.0.

The herb-compound-target network has a total of 12 nodes, including 12 compound nodes and 150 target nodes, see Figure 9, which shows the relationship between the compounds and their targets in lily.

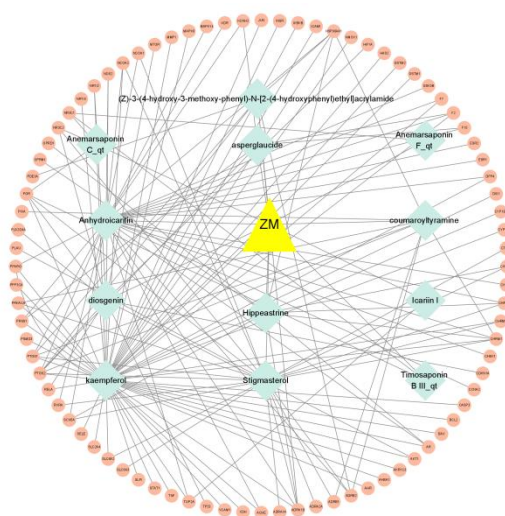


Fig. 2 Proteins and genes corresponding to the targets of the active substances in lily

3.3 Cross-matching saprophytes with cancer for gene targets

After collating the genes related to the active ingredients of lily using TCMSP and Uniport, the chemical targets in lily were cross-matched with a variety of cancers, and after matching, 42 intersecting targets were obtained between the chemical targets in lily and cancers, which are important targets for the treatment of cancer in lily, and were integrated to obtain Figure 10.

3.4 Target-Disease Interaction Networks

The 42 intersecting targets of the chemical composition action in lily in Figure 10 and the presence of cancer were mapped by Cytosape 3.9.0 to obtain Figure 11.

By analysing the rank values (degree) of the intersecting targets, six targets with a degree greater than 5 were found to be PTGS2 (12), MMP1 (10), PPARG (8), HSP90AA1 (8), TP53 (8) and ESR2 (6); the diseases that were closely linked to the targets were Cancer, unspecified (non-specific), Breast cancer (breast cancer), Prostate cancer (prostate cancer).

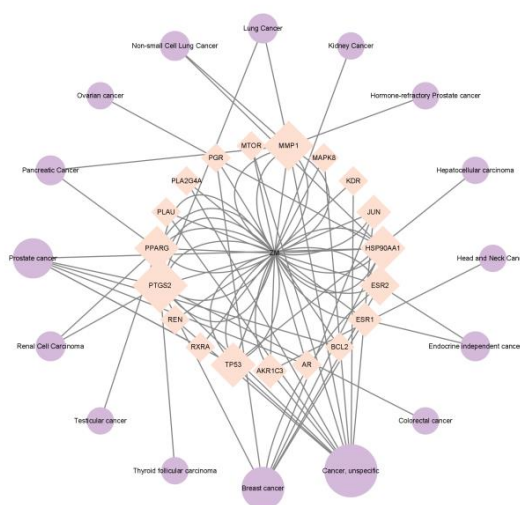


Fig. 3 Disease and target network diagram

3.5 Key target pathway analysis

In order to explore the function of common targets of drugs and diseases, enrichment analysis was performed by RStudio online tool, screened at $P < 0.05$ & $Q < 0.05$, and the genes associated with ≥ 4 (proportion $> 20\%$) genes were screened and the results are shown in Figure 20. pathways with the current number of enriched genes 20 were selected for visualisation, the colour indicates the pathway. The number of enriched pathways was visualised, with the larger the number, the more red the colour, and the smaller the number, the more blue the colour. The results are shown in Figure 21.

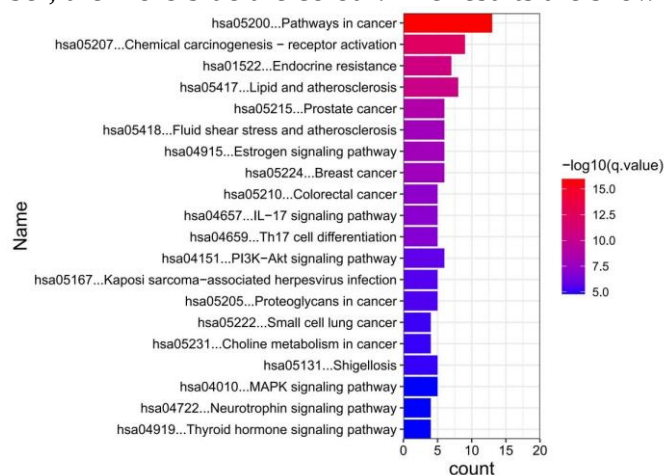


Fig. 4 Enrichment analysis graph

4. Modern pharmacological studies on cancer-inhibiting substances in lily

(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide (n-cis feruloyl tyramine, PubChem CID: 6440659)

According to Tang [5] et al. showed that n-cis feruloyl tyramine has clear oncogenic effects, and in the experiment by applying 0.8 mg-L-1 of n-cis feruloyl tyramine to MDA-MB-231 could exhibit 10.20% growth inhibition and 31.6% chemotaxis inhibition, and when the applied amount reached 4 mg-L-1 the growth inhibition and chemotaxis inhibition rates could reach 12.33% and 37.9%, respectively.

The anti-inflammatory activity of n-cis feruloyl tyramine in mouse BV2 cells was assessed in the Pubchem database as an inhibition of LPS-induced NO production by treatment 30 min prior to LPS stimulation, with activity measured as positive by Griss response measurements 24 h later.

Coumaroyltyramine (p-coumarolamine PubChem CID: 5372945)

In a study by Park JB et al [6] it was shown that there is a novel activity against coumarin luramine that can inhibit the growth of human tumour cells such as U937 and Jurkat cells.

Asperglaucide (orlanyl acetate PubChem CID: 10026486)

In a study by Wang Hongwei et al [7] olanyl acetate could act as a tumour necrosis factor to inhibit tumour cells.

Kaempferol (kaempferol PubChem CID:5280863)

Wu A clear inhibitory effect of kaempferol on breast cancer cells can be obtained from a study by [8].

Anhydroicaritin (anhydrous rhodamine protein 3-rhamnosyl-(1->2)-rhamnoside PubChem CID:44259058)

Stigmasterol (stigmasterol PubChem CID:5280794)

A study by Chen Yu et al [9] showed that stigmasterol has a definite therapeutic effect on human colorectal cancer.

Diosgenin (Saponin PubChem CID:99474)

Experiments by Li Xiaoli [10] showed that saponin could inhibit the proliferation of human endometrial cancer, and studies by Ma Yilong [11] epitomised that saponin could inhibit the proliferation of human cervical cancer cells.

Hippeastrine (Allicine minor PubChem CID: 441594)

In a study by Chen Guilin et al [12], it was shown that *S. cerevisiae* strongly inhibited the proliferation of HT-29 and Hep G2 cells in an intuitive dose-dependent manner. 50 was 3.98 ± 0.29 $\mu\text{g/mL}$ and 11.85 ± 0.20 $\mu\text{g/mL}$, respectively, and also induced significant cell morphological changes with clear and effective anti-tumour effects.

5. Summary and outlook

Lily it is a perennial herb with the properties of clearing heat and fire, promoting the production of body fluid and moistening dryness, and entering the lung, stomach and kidney meridians. It is used for external fever, high fever and thirst, lung fever and dry cough, bone fever and dampness, internal fever and thirst, constipation and dryness of the intestines.

In this paper, we analyse the pharmacological effects of lily from both the cancer-inhibiting substances and cancer targets in lily using network pharmacology.

Eight core substances were screened for significant cancer inhibitory effects of *C. chinensis*. (n-cis feruloyl tyramine, p-coumarylamine, orantheline acetate, kaempferol, anhydrous rhodopsin 3-rhamnosyl-(1->2)-rhamnoside, stigmasterol, saponin, and minor allicin) and these eight substances have a total of six intersecting targets with cancer. (PTGS2, MMP1, PPARG, HSP90AA1, TP53, ESR2).

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