Original Article

A network pharmacology-based exploration of the active compounds and potential drug targets of Si-Jun-Zi decoction in the treatment of cutaneous squamous cell carcinoma

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Background: Cutaneous squamous cell carcinoma (cSCC), a kind of skin cancer with high rates of morbidity and mortality, occurs frequently in the clinic. Although early surgical treatment can achieve good results, there is no effective prevention and treatment for the recurrence and metastasis of cSCC. As a useful resource to protect humans from disease, traditional Chinese medicine (TCM) has been adopted by clinicians for thousands of years.

Methods: In this study, we collected a Chinese medicine formula and then employed a data mining method to analyze drug combinations of Si-Jun-Zi (SJZ) decoction. Multiple databases were used in this study to predict various ingredients, compounds, and their targets in the decoction. The potential targets of cSCC were also obtained from the database in the same way. In addition, as bioinformatics analysis methods, Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used in our research as supplementary means to network pharmacology. Finally, we used ultra-performance liquid chromatography (UPLC) fingerprinting to analyze the effective components of the TCM decoction.

Results: We detected 559 active compounds from Ginseng, Largehead Atractylodes, India Bread, and Glycyrrhiza Inflata, and selected 136 molecules under specific conditions. The mechanisms of the TCM formula were illustrated by the network pharmacology, such as compounds-herb network, compounds-target network, disease-target network, and target-target interaction network, as well as characteristics of the TCM. Then, GO analysis and KEGG analysis were performed on the compounds in the network using multiple methods of data mining and bioinformatics, and 10 candidate targets were identified. In addition, the UPLC fingerprinting method was used to analyze the components of SJZ decoction.

Conclusions: Network pharmacology was performed to investigate the characteristics and mechanism of SJZ decoction, and a bioinformatics method was used to analyze the relationship between the effective compounds of the SJZ TCM decoction and cSCC-related specific targets and pathways, to find a variety of candidate compounds with multi-target activity.

Keywords: Cutaneous squamous cell carcinoma (cSCC); Si-Jun-Zi (SJZ) decoction; traditional Chinese medicines (TCMs); network pharmacology; data mining

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Introduction

Cutaneous squamous cell carcinoma (cSCC) is a kind of skin tumor with an incidence second only to that of basal cell carcinoma (1). It arises from keratinocytes in the epidermis or appendages. Currently, management of cSCC involves surgical treatment, radiation therapy, chemotherapy, and targeted therapy. Surgical treatment is the main method for early cSCC. However, the survival problems faced by cSCC patients include postoperative recurrence, lymph node metastasis, and distant metastasis, all of which require clinical solutions (2,3). Although targeted therapy is the main treatment for these cSCC patients, the prognosis remains poor (4).

Network pharmacology is a new research method combining pharmacology and pharmacodynamics based on a variety of network databases, which can help us understand the interactions among traditional Chinese medicines (TCM), compounds, disease, and targets in a more systematic and comprehensive way.

For a long period of time, TCM has successfully treated a variety of complex diseases through the use of multi-compound decoctions aimed at multiple targets. Through online pharmacology, researchers can not only explore the compounds of TCM formulae, but also understand the interaction between active ingredients and their related targets, which provides a new, highly applicable method for clarifying the mechanism of TCM treatment of diseases.

Si Jun Zi (SJZ) decoction is a classical Chinese medicine formula extracted from Ginseng (Ren Shen), Glycyrrhiza inflata (Gan Cao), Largehead Atractylodes (Bai Zhu), and Indian Bread (Fu Ling) in a ratio of 3:3:3:2. It has been used as a main therapeutic method or complementary therapy for the treatment of various diseases, including cancer, for more than 1,000 years. Given that the function of inhibiting tumor growth, improving tumor cachexia, regulating tumor microenvironment, and regulating body immunity, the SJZ decoction is widely used to facilitate the swift recovery of cancer patients, since disease or chemotherapy may lead to poor physical fitness (5). Nowadays, the research of SJZ Decoction has been reported internationally for the treatment of esophageal squamous cell carcinoma and lung squamous cell carcinoma, but there are no reports or studies on the treatment of cSCC. The main compounds and mechanisms of SJZ decoction in the treatment of tumors have not been clarified. As the main active constituent in ginseng, ginsenosides have been widely used in the treatment of photoaging, hair loss, and trauma, among others (6). In recent years, Licochalcone A, Licochalcone B, and Licochalcone D have been reported to inhibit the growth of lung cancer cells through different pathways (7-11). Licochalcone C, Licochalcone H, and GIP1 can induce apoptosis of human oral squamous cell carcinoma cells (HOSCC) (12). Studies have found that Atractylodes I is the main effective ingredient in alleviating the symptoms of gastric cancer, and it can also improve the occurrence and development of melanoma to a certain extent (13,14). Currently, the main active ingredients of SJZ decoction, which is made up of four Chinese herbs, remain to be confirmed.

Therefore, in this study, we used network pharmacology and bioinformatics analysis to try to construct the action network of SJZ decoction and cSCC, with the aim of solving the following questions: (I) what are the active ingredients of SJZ decoction; (II) which cSCC disease targets are related to the active compounds of SJZ decoction; and (III) what new information can network pharmacology yield in the study of the treatment of cSCC by SJZ Decoction.

Methods

Active ingredients and potential targets of TCMs

We used three databases to identify the chemical ingredients in Ginseng, Glycyrrhiza inflata, Largehead Atractylodes, and Indian Bread: (I) Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; available online: https://old.tcmsp-e.com/tcmsp.php); (II) Traditional Chinese Medicines Integrated Database (TCMID; available online: http://47.100.169.139/tcmid/search/); and (III) TCM Database@Taiwan (http://tcm.cmu.edu.tw/). Specified oral bioavailability (OB) and drug-likeness (DL) properties were treated as filter criteria in the TCMSP^{TM}, which may allow the various components of the drug to perform the desired activity. Compounds that met both criteria were considered as candidate molecules.

Network analysis and building

Constructing a 'drug-target-disease-pathway' network allows us to gather a variety of information regarding active ingredients and their corresponding targets and to better understand the anti-tumor mechanism of active ingredients from another perspective. Firstly, we sorted out the screened active ingredients and corresponding potential targets of the four TCM ingredients. Then, we extracted cSCC disease-

related targets from GeneCards online database (https://www.genecards.org/) for sorting and conducted functional enrichment analysis of these common potential targets, including "Gene Ontology (GO)" and "Kyoto Encyclopedia of Genes and Genomes (KEGG)" analyses. Finally, all data were processed by Cytoscape 3.5.1 software (https://cytoscape.org/) to construct a complex network of "drug-component-target-pathway-disease".

Plant sample preparation

The SJZ decoction is a mixture of Ginseng, Indian Bread, Largehead Atractylodes, and Glycyrrhiza inflata at a mass ratio of 3:3:3:2. The components were purchased from the Chinese Pharmacy of the Guangdong Second Provincial General Hospital (GD2H; Guangzhou, China). A total of 1,650 g of the Chinese herbal medicine (CHM) formula consisting of Ginseng, Indian Bread, Largehead Atractylodes, and Glycyrrhiza inflata was soaked for 30 minutes and extracted with 100 ℃ water twice. The sample was then cooled and concentrated.

Ultra-high performance liquid chromatography for sample

An ultra-high performance liquid chromatography (UPLC) system (Water, Milford, MA, USA) was used for dealing with the filtered sample solutions, combining with the annotation and classification of mass spectrometry database information were completed using precise characterization Instrument ASTAT-DAP. LC-MS (Thermo, Ultimate 3000 LC, HF) fitted with a C18 column [Zorbax Eclipse C18 (1.8 μm × 2.1 mm × 100 mm)] and the separation conditions were as follows: column temperature =30 ℃; flow rate =0.3 mL/min; mobile phase A=water + 0.1% formic acid, mobile phase B = pure acetonitrile; Injection volume =2 μ; and active autokinetic nozzle =4 ℃.

Statistical analysis

In this study, TCMSP, TCMID, TCM Database@Taiwan, and GeneCards database were used for data acquisition, David online tool and Cytoscape software were used for data analysis and image rendering respectively.

Results

cSCC-related gene pathways and networks

A total of 748 human genes with a high score (≥30.0) associated with cSCC were identified in the GeneCard database, and the encoded proteins were assembled into a set of 94 pathways and 25 networks using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; (https://cn.string-db.org/cgi/input.pl) and Cytoscape software. Cluster 1, which has the highest relevant scores among the networks, is shown in *Figure 1A*. The top five pathways involved cytokine-cytokine receptor interaction, lipid and atherosclerosis, rheumatoid, arthritis, malaria, and hepatitis B. The GO enrichment and network analysis showed that "T positive regulation of cytokine production", "response to molecule of bacterial origin", "leukocyte proliferation, positive regulation of mononuclear cell proliferation", and "regulation of mononuclear cell proliferation" covered the top 5 biological processes of cSCC target proteins (*Figure 1B*).

Screening of common targets of TCM compound and cSCC

The targets of the four herbs were predicted using the TCMSP database. A total of 136 candidate compounds were screened from 559 chemicals with DL (≥0.18) and OB (≥30%), including 22 in Ginseng, 7 in Largehead Atractylodes, 15 in Indian Bread, and 92 in Glycyrrhiza inflata. Several compounds have been reported to have more than one biological activity in recent studies. A total of 112 different herb targets obtained from 136 candidate compounds (*Table 1*) were further subjected to the UniProt (https://www.uniprot.org/) for conversing ID name. A total of 54 common candidate targets (*Table 2*) were selected from 748 different candidate cSCC targets filtered from GeneCards and 112 herb targets (*Figure 2*). The STRING database was used to construct the protein-protein interaction (PPI) networks (*Figure 3*).

Network pharmacology of SJZ decoction

The 54 targets of drug active compounds in SJZ which are related to cSCC are mainly enriched in "response to steroid hormone", "response to metal ion", "regulation of apoptotic signaling pathway", "response to lipopolysaccharide", "response to molecule of bacterial origin", "extrinsic apoptotic signaling pathway", "cellular response to oxidative stress", "response to antibiotic", "reproductive structure development", and "regulation of DNA-binding transcription factor activity" by GO analysis for biological process. In this study, we reintegrated "TCMs-compounds", "compounds-targets", "TCMs-targets", "targets-pathways",

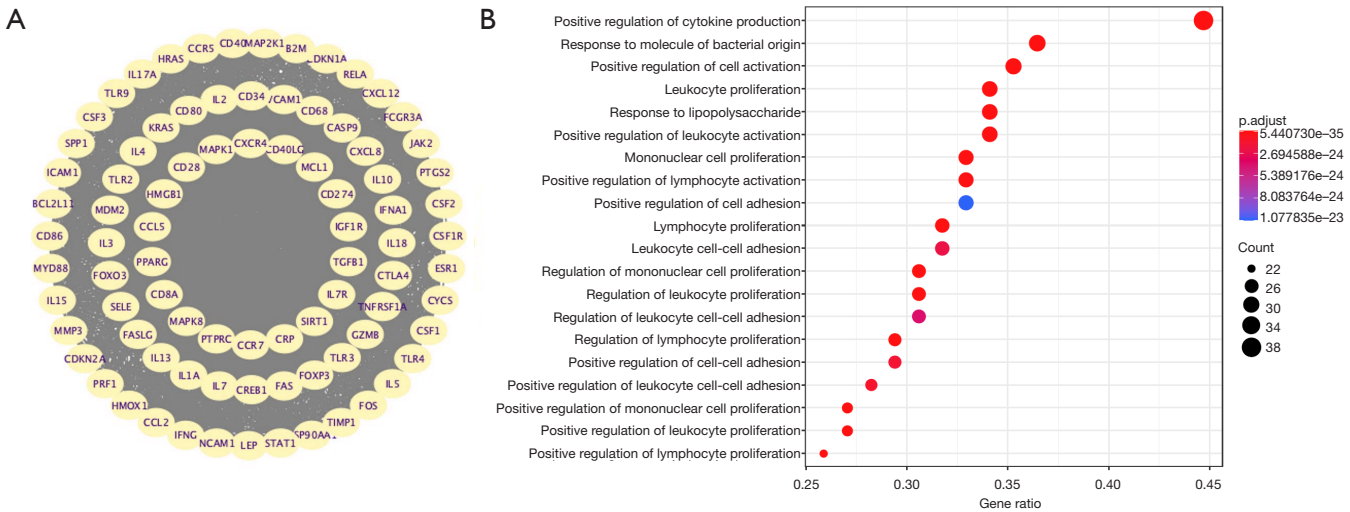

Figure 1 GO enrichment and network analysis of cSCC target genes. (A) Interaction networks between Cluster 1 with the highest score by using MCODE algorithm in Cytoscape software. (B) Top 20 functionally enriched biological processes with corresponding adjusted P values analyzed by cluster Profiler, which are displayed scales indicated the different thresholds of adjusted P values, and the sizes of the dots represented the gene count of each term. GO, Gene Ontology; cSCC, cutaneous squamous cell carcinoma.

Table 1 136 candidate compounds in SJZ decoction

Resource	Mol ID	Molecule name	OB (%)	DL
Ginseng	MOL005399	alexandrin_qt	36.91	0.75
Ginseng	MOL005308	Aposiopolamine	66.65	0.22
Ginseng	MOL005320	arachidonate	45.57	0.2
Ginseng	MOL000358	beta-sitosterol	36.91	0.75
Ginseng	MOL005314	Celabenzine	101.88	0.49
Ginseng	MOL004492	Chrysanthemaxanthin	38.72	0.58
Ginseng	MOL005317	Deoxyharringtonine	39.27	0.81
Ginseng	MOL005318	Dianthramine	40.45	0.2
Ginseng	MOL002879	Diop	43.59	0.39
Ginseng	MOL005321	Frutinone A	65.9	0.34
Ginseng	MOL000787	Fumarine	59.26	0.83
Ginseng	MOL005401	ginsenoside Rg5_qt	39.56	0.79
Ginseng	MOL005344	ginsenoside rh2	36.32	0.56
Ginseng	MOL005348	Ginsenoside-Rh4_qt	31.11	0.78
Ginseng	MOL005356	Girinimbin	61.2	0.31
Ginseng	MOL005357	Gomisin B	31.99	0.83
Ginseng	MOL003648	Inermin	65.83	0.54
Ginseng	MOL000422	kaempferol	41.88	0.24
Ginseng	MOL005360	malkangunin	57.71	0.63
Ginseng	MOL005376	Panaxadiol	33.09	0.79

Table 1 (*continued*)

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Resource	Mol ID	Molecule name	OB (%)	DL
Ginseng	MOL000449	Stigmasterol	43.83	0.76
Ginseng	MOL005384	suchilactone	57.52	0.56
Largehead Atractylodes	MOL000020	12-senecioyl-2E,8E,10E-atractylentriol	62.4	0.22
Largehead Atractylodes	MOL000021	14-acetyl-12-senecioyl-2E,8E,10E-atractylentriol	60.31	0.31
Largehead Atractylodes	MOL000022	14-acetyl-12-senecioyl-2E,8Z,10E-atractylentriol	63.37	0.3
Largehead Atractylodes	MOL000028	α-Amyrin	39.51	0.76
Largehead Atractylodes	MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
Largehead Atractylodes	MOL000049	3β-acetoxyatractylone	54.07	0.22
Largehead Atractylodes	MOL000072	8β-ethoxy atractylenolide III	35.95	0.21
Indian Bread	MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81
Indian Bread	MOL000275	Trametenolic acid	38.71	0.8
Indian Bread	MOL000276	7,9(11)-dehydropachymic acid	35.11	0.81
Indian Bread	MOL000279	Cerevisterol	37.96	0.77
Indian Bread	MOL000280	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	31.07	0.82
Indian Bread	MOL000282	ergosta-7,22E-dien-3beta-ol	43.51	0.72
Indian Bread	MOL000283	Ergosterol peroxide	40.36	0.81
Indian Bread	MOL000285	(2R)-2-[(5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,4,10,13,14-pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	38.26	0.82
Indian Bread	MOL000287	3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid	38.7	0.81
Indian Bread	MOL000289	Pachymic acid	33.63	0.81
Indian Bread	MOL000290	Poricoic acid A	30.61	0.76
Indian Bread	MOL000291	Poricoic acid B	30.52	0.75
Indian Bread	MOL000292	Poricoic acid C	38.15	0.75
Indian Bread	MOL000296	Hederagenin	36.91	0.75

Table 1 (*continued*)

Table 1 (*continued*)

Resource	Mol ID	Molecule name	OB (%)	DL
Indian Bread	MOL000300	Dehydroeburicoic acid	44.17	0.83
Glycyrrhiza inflata	MOL000098	Quercetin	46.43	0.28
Glycyrrhiza inflata	MOL000211	Mairin	55.38	0.78
Glycyrrhiza inflata	MOL000239	Jaranol	50.83	0.29
Glycyrrhiza inflata	MOL000354	Isorhamnetin	49.6	0.31
Glycyrrhiza inflata	MOL000359	Sitosterol	36.91	0.75
Glycyrrhiza inflata	MOL000392	Formononetin	69.67	0.21
Glycyrrhiza inflata	MOL000417	Calycosin	47.75	0.24
Glycyrrhiza inflata	MOL000422	Kaempferol	41.88	0.24
Glycyrrhiza inflata	MOL000497	licochalcone a	40.79	0.29
Glycyrrhiza inflata	MOL000500	Vestitol	74.66	0.21
Glycyrrhiza inflata	MOL001484	Inermine	75.18	0.54
Glycyrrhiza inflata	MOL001792	DFV	32.76	0.18
Glycyrrhiza inflata	MOL002311	Glycyrol	90.78	0.67
Glycyrrhiza inflata	MOL002565	Medicarpin	49.22	0.34
Glycyrrhiza inflata	MOL003656	Lupiwighteone	51.64	0.37
Glycyrrhiza inflata	MOL003896	7-Methoxy-2-methyl isoflavone	42.56	0.2
Glycyrrhiza inflata	MOL004328	naringenin	59.29	0.21
Glycyrrhiza inflata	MOL004805	(2S)-2-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano[2,3-f]chromen-4-one	31.79	0.72
Glycyrrhiza inflata	MOL004806	Euchrenone	30.29	0.57
Glycyrrhiza inflata	MOL004808	Glyasperin B	65.22	0.44
Glycyrrhiza inflata	MOL004810	Glyasperin F	75.84	0.54
Glycyrrhiza inflata	MOL004811	Glyasperin C	45.56	0.4
Glycyrrhiza inflata	MOL004814	Isotrifoliol	31.94	0.42
Glycyrrhiza inflata	MOL004815	(E)-1-(2,4-dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one	39.62	0.35
Glycyrrhiza inflata	MOL004820	kanzonols W	50.48	0.52
Glycyrrhiza inflata	MOL004824	(2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one	60.25	0.63
Glycyrrhiza inflata	MOL004827	Semilicoisoflavone B	48.78	0.55
Glycyrrhiza inflata	MOL004828	Glepidotin A	44.72	0.35
Glycyrrhiza inflata	MOL004829	Glepidotin B	64.46	0.34
Glycyrrhiza inflata	MOL004833	Phaseolinisoflavan	32.01	0.45
Glycyrrhiza inflata	MOL004835	Glypallichalcone	61.6	0.19
Glycyrrhiza inflata	MOL004838	8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol	58.44	0.38

Table 1 (*continued*)

Table 1 (*continued*)

Resource	Mol ID	Molecule name	OB (%)	DL
Glycyrrhiza inflata	MOL004841	Licochalcone B	76.76	0.19
Glycyrrhiza inflata	MOL004848	Licochalcone G	49.25	0.32
Glycyrrhiza inflata	MOL004849	3-(2,4-dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin	59.62	0.43
Glycyrrhiza inflata	MOL004855	Licoricone	63.58	0.47
Glycyrrhiza inflata	MOL004856	Gancaonin A	51.08	0.4
Glycyrrhiza inflata	MOL004857	Gancaonin B	48.79	0.45
Glycyrrhiza inflata	MOL004860	Licorice glycoside E	32.89	0.27
Glycyrrhiza inflata	MOL004863	3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl)chromone	66.37	0.41
Glycyrrhiza inflata	MOL004864	5,7-dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)chromone	30.49	0.41
Glycyrrhiza inflata	MOL004866	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl)chromone	44.15	0.41
Glycyrrhiza inflata	MOL004879	Glycyrin	52.61	0.47
Glycyrrhiza inflata	MOL004882	Licocoumarone	33.21	0.36
Glycyrrhiza inflata	MOL004883	Licoisoflavone	41.61	0.42
Glycyrrhiza inflata	MOL004884	Licoisoflavone B	38.93	0.55
Glycyrrhiza inflata	MOL004885	Licoisoflavanone	52.47	0.54
Glycyrrhiza inflata	MOL004891	Shinpterocarpin	80.3	0.73
Glycyrrhiza inflata	MOL004898	(E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl) prop-2-en-1-one	46.27	0.31
Glycyrrhiza inflata	MOL004903	Liquiritin	65.69	0.74
Glycyrrhiza inflata	MOL004904	Licopyranocoumarin	80.36	0.65
Glycyrrhiza inflata	MOL004905	3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid	34.32	0.55
Glycyrrhiza inflata	MOL004907	Glyzaglabrin	61.07	0.35
Glycyrrhiza inflata	MOL004908	Glabridin	53.25	0.47
Glycyrrhiza inflata	MOL004910	Glabranin	52.9	0.31
Glycyrrhiza inflata	MOL004911	Glabrene	46.27	0.44
Glycyrrhiza inflata	MOL004912	Glabrone	52.51	0.5
Glycyrrhiza inflata	MOL004913	1,3-dihydroxy-9-methoxy-6-benzofurano[3,2-c]chromenone	48.14	0.43
Glycyrrhiza inflata	MOL004914	1,3-dihydroxy-8,9-dimethoxy-6-benzofurano[3,2-c]chromenone	62.9	0.53
Glycyrrhiza inflata	MOL004915	Eurycarpin A	43.28	0.37
Glycyrrhiza inflata	MOL004917	Glycyroside	37.25	0.79

Table 1 (*continued*)

Table 1 (*continued*)

Resource	Mol ID	Molecule name	OB (%)	DL
Glycyrrhiza inflata	MOL004924	(-)-Medicocarpin	40.99	0.95
Glycyrrhiza inflata	MOL004935	Sigmoidin-B	34.88	0.41
Glycyrrhiza inflata	MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl) chroman-4-one	71.12	0.18
Glycyrrhiza inflata	MOL004945	(2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)chroman-4-one	36.57	0.32
Glycyrrhiza inflata	MOL004948	Isoglycyrol	44.7	0.84
Glycyrrhiza inflata	MOL004949	Isolicoflavonol	45.17	0.42
Glycyrrhiza inflata	MOL004957	HMO	38.37	0.21
Glycyrrhiza inflata	MOL004959	1-Methoxyphaseollidin	69.98	0.64
Glycyrrhiza inflata	MOL004961	Quercetin der.	46.45	0.33
Glycyrrhiza inflata	MOL004966	3'-Hydroxy-4'-O-Methylglabridin	43.71	0.57
Glycyrrhiza inflata	MOL004974	3'-Methoxyglabridin	46.16	0.57
Glycyrrhiza inflata	MOL004978	2-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano[6,5-f]chromen-3-yl]-5-methoxyphenol	36.21	0.52
Glycyrrhiza inflata	MOL004980	Inflacoumarin A	39.71	0.33
Glycyrrhiza inflata	MOL004985	icos-5-enoic acid	30.7	0.2
Glycyrrhiza inflata	MOL004988	Kanzonol F	32.47	0.89
Glycyrrhiza inflata	MOL004989	6-prenylated eriodictyol	39.22	0.41
Glycyrrhiza inflata	MOL004990	7,2',4'-trihydroxy-5-methoxy-3-arylcoumarin	83.71	0.27
Glycyrrhiza inflata	MOL004991	7-Acetoxy-2-methylisoflavone	38.92	0.26
Glycyrrhiza inflata	MOL004993	8-prenylated eriodictyol	53.79	0.4
Glycyrrhiza inflata	MOL004996	Gadelaidic acid	30.7	0.2
Glycyrrhiza inflata	MOL005000	Gancaonin G	60.44	0.39
Glycyrrhiza inflata	MOL005001	Gancaonin H	50.1	0.78
Glycyrrhiza inflata	MOL005003	Licoagrocarpin	58.81	0.58
Glycyrrhiza inflata	MOL005007	Glyasperins M	72.67	0.59
Glycyrrhiza inflata	MOL005008	Glycyrrhiza flavonol A	41.28	0.6
Glycyrrhiza inflata	MOL005012	Licoagroisoflavone	57.28	0.49
Glycyrrhiza inflata	MOL005013	18α-hydroxyglycyrrhetic acid	41.16	0.71
Glycyrrhiza inflata	MOL005016	Odoratin	49.95	0.3
Glycyrrhiza inflata	MOL005017	Phaseol	78.77	0.58
Glycyrrhiza inflata	MOL005018	Xambioona	54.85	0.87
Glycyrrhiza inflata	MOL005020	Dehydroglyasperins C	53.82	0.37

SJZ, Si Jun Zi decoction; OB, oral bioavailability DL, drug-likeness.

Table 2 Common genes of herb targets and cSCC targets

	Genes
Herb targets & cSCC targets	*AR, PPARG, RELA, EGFR, VEGFA, CCND1, BCL2, FOS, CASP9, PLAU, RB1, IL6, CASP3, TP63, NFKBIA, CASP8, RAF1, PRKCA, HIF1A, ERBB2, CAV1, MYC, CYP1A1, ICAM1, SELE, VCAM1, BIRC5, NOS3, HSPB1, CYP1B1, CCNB1, GSTP1, NFE2L2, NQO1, PARP1, CHEK2, CRP, RUNX2, RASSF1, CTSD, IGFBP3, IGF2, IRF1, ERBB3, RASA1, GSTM1, PGR, ESR2, CHEK1, ESR1, GSK3B, IKBKB, MAPK8, ABCC1*

cSCC, cutaneous squamous cell carcinoma.

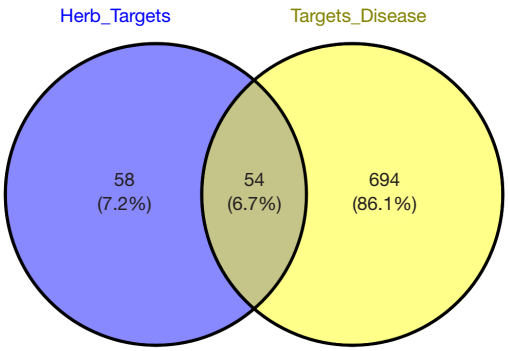

Figure 2 Venn diagram. Among 748 cSCC targets and 112 traditional Chinese medicine targets, 54 co-targets were screened. cSCC, cutaneous squamous cell carcinoma.

and "disease-targets" by using Cytoscape software and obtained a "TCMs-compounds-targets-pathways-disease" network (*Figure 4*).

Screening and analysis of cluster and hub genes

We selected the module with the highest score from 54 common targets through the MCODE plug-in in Cytoscape. This cluster contains the 25 most correlated targets (*Figure 5A*). The 25 targets are mainly enriched in "response to steroid hormone", "response to metal ion", "myeloid cell differentiation", "epithelial cell proliferation", and "regulation of epithelial cell proliferation" by GO analysis for biological process (*Figure 5B*). The CytoHubba plug-in was used to sort degree value as a standard from high to low, and ten genes with the highest degree value were selected (*Figure 6*). Through GO analysis of hub genes, we found that "mammary gland alveolus development", "mammary gland lobule development", "positive regulation of epithelial cell proliferation", "epithelial cell proliferation", and "response to light stimulus" were the most closely related biological processes, in which "positive regulation of epithelial cell proliferation" and "epithelial cell proliferation" and Cluster 1 had the same closest relationship. These two processes included the *MYC*, *ESR1*, *EGFR*, *HIF1A*, *CCND1*, *VEGFA*, and *ERBB2* genes. The pathways which had the top five highest relevant scores the candidate genes in Cluster 1 were mainly enriched in: "Kaposi sarcoma-associated herpesvirus infection", "hepatitis B", "human cytomegalovirus infection", "chemical carcinogenesis-receptor activation", and "lipid and atherosclerosis". The common target-related main signaling pathways might have included: the JNK/NF-κB signaling pathway, the NF-κB/VEFG signaling pathway, the NF-κB/ICAM-1 signaling pathway, the JNK/AP-1 signaling pathway, the AP-1/ICAM-1 signaling pathway, the AP-1/VEFG signaling pathway, the HIF1A/VEFG signaling pathway, and the Caspase-9/Caspase-3 signaling pathway.

UPLC fingerprints

Through comparison with the "Similarity Evaluation System for Chromatographic Fingerprint of TCMs (Version 2012)", the fingerprints of chromatograms in SJZ decoction showed 13 common peaks, which were identified as Atractylon, Liquiritin, 25-hydroxyporicoic acid H, Isoliquiritin apioside, ginsenoside Rg1, Ginsenoside Rg3, Ginsenoside Re, Kanzonol H, Ginsenoside Ro, Poricoic acid, Licoricesaponin G2, Glycyrrhizic acid, and Liquiritigenin, respectively (*Figure 7*). These ingredients may provide important laboratory evidence for antitumor therapy of SJZ.

Discussion

As a kind of non-melanoma skin tumor, cSCC has a high incidence and metastasis rate. In the treatment of this disease, surgery is suitable for the early, non-metastatic type, but is not the best choice for metastatic or recurrent cSCC (15). Formulae of TCM have been applied and

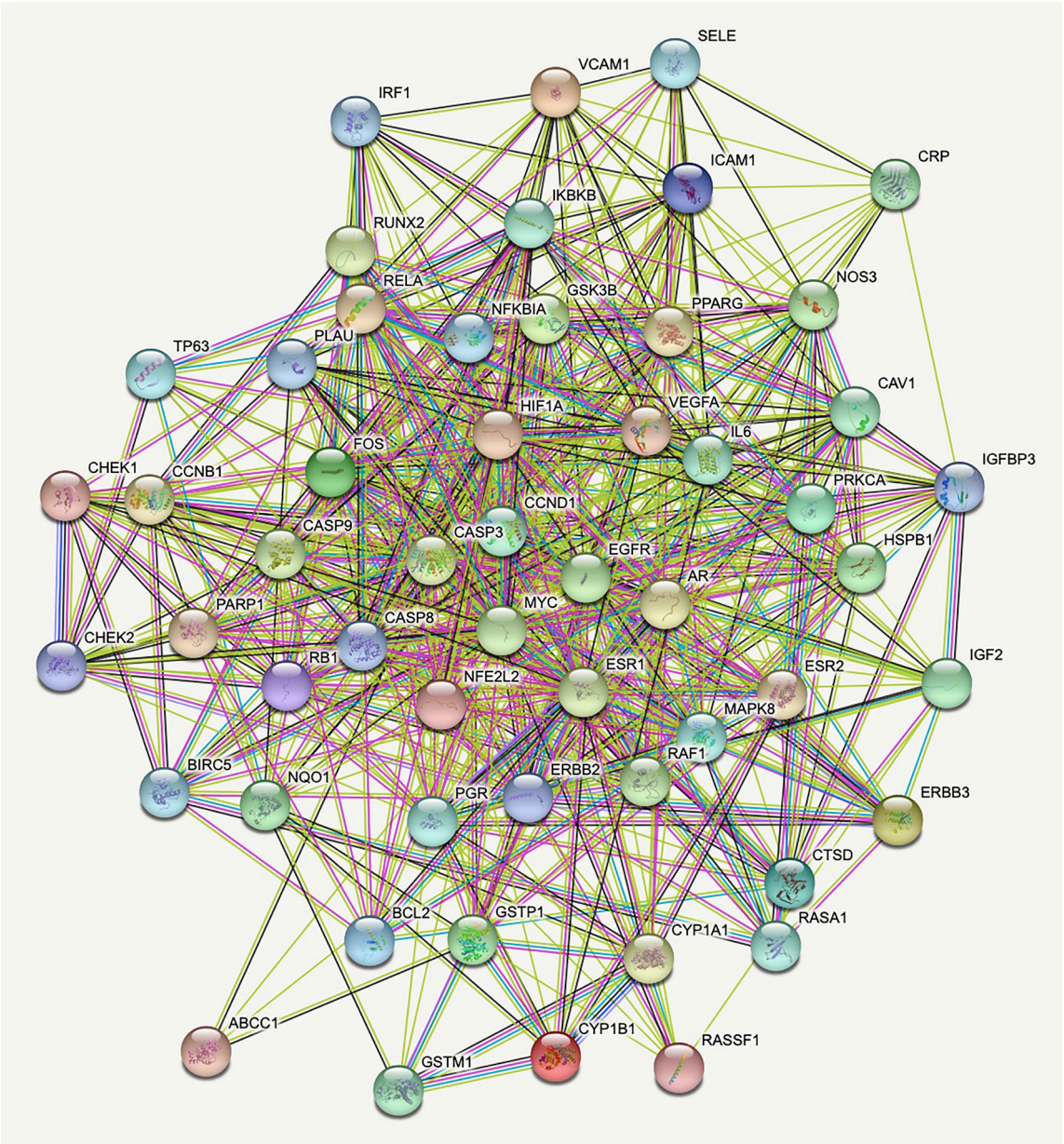

Figure 3 PPI network from 54 co-candidate targets. The PPI network is built through the STRING website. Network nodes represent proteins, each of them represents all the proteins produced by a single, protein-coding gene locus. Edges represent protein-protein associations, which are meant to be specific and meaningful. PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interaction Genes/Proteins.

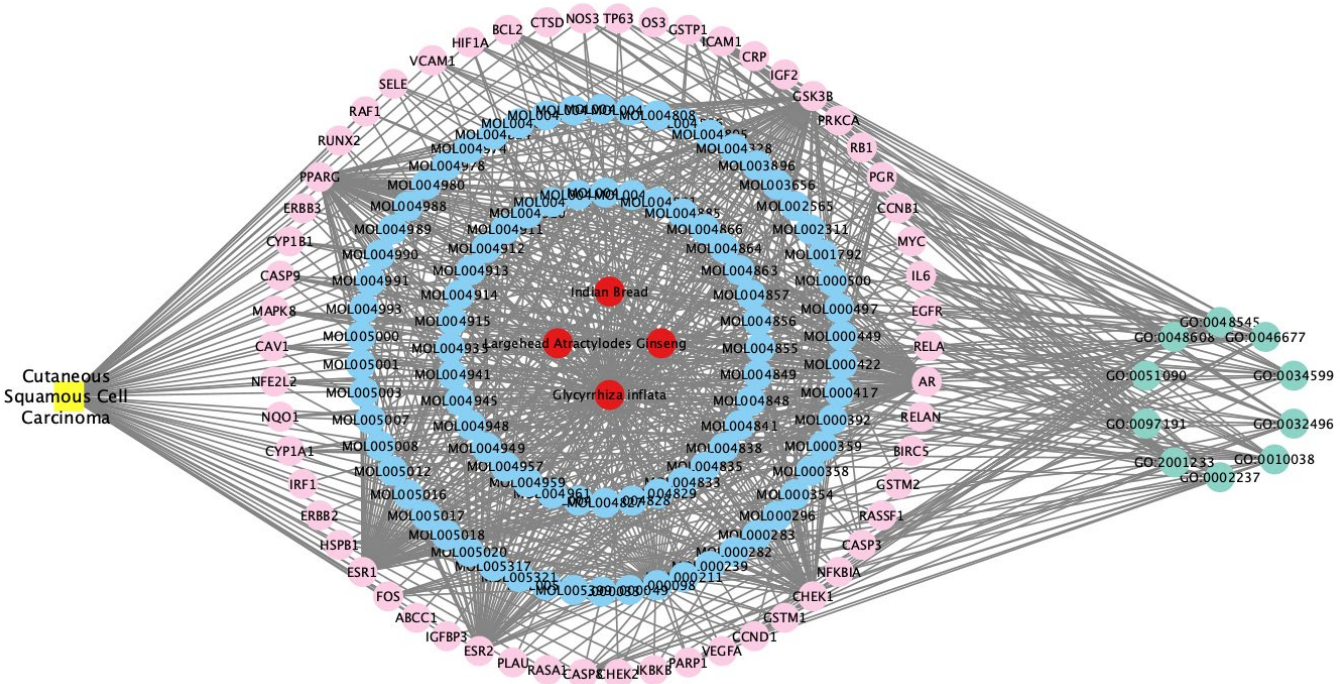

Figure 4 Network pharmacology of 'TCMs-compounds-targets-pathways-disease'. Blue dot represents cSCC, green dots represent key pathways related to biological processes obtained by GO analysis, red dots represent the nine kinds of TCMs, pink dots represent active compounds related to those TCMs, and yellow dots represent common targets related to diseases and active compounds of TCMs. TCM, traditional Chinese medicine; GO, Gene Ontology; cSCC, cutaneous squamous cell carcinoma.

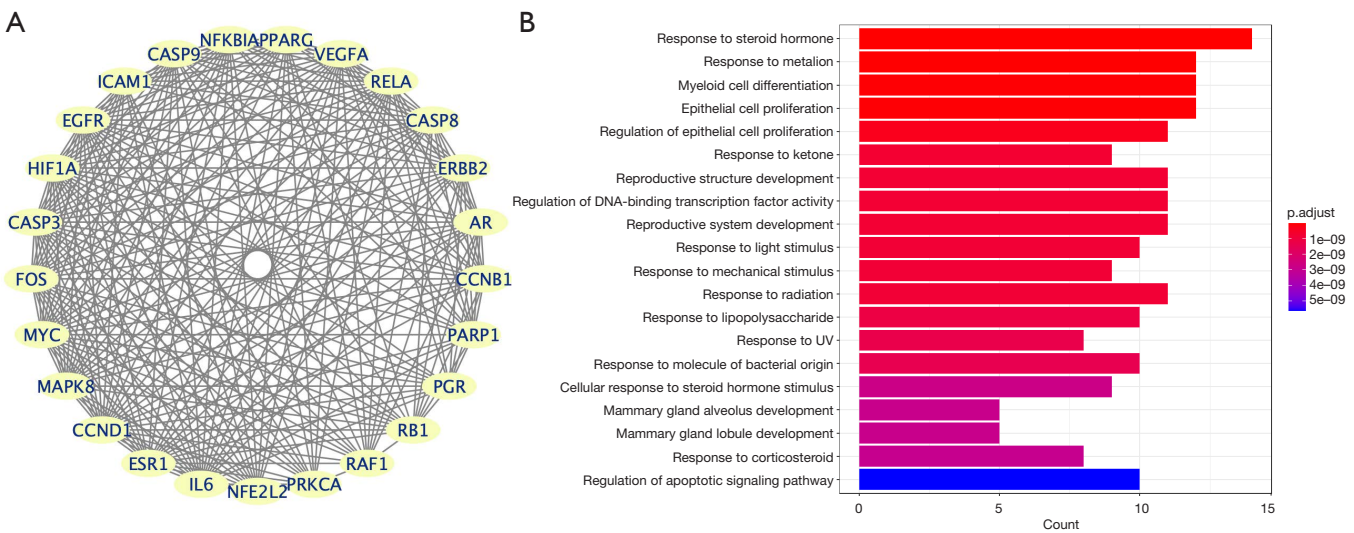

Figure 5 Cluster 1 of interacted proteins in SJZ against cSCC by use of MCODE algorithm. (A) 25 targets in cluster1; (B) GO enriched analysis with biological processes of functionally. SJZ, Si Jun Zi decoction; cSCC, cutaneous squamous cell carcinoma; GO, Geno Ontology.

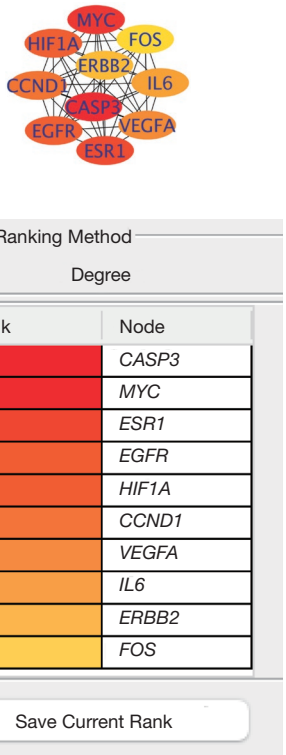

Figure 6 Hub targets of SJZ. As results, the 10 regulator targets were finally identified, showing *CASP3*, *MYC*, *ESR1*, *EGFR*, *HIF1A*, *CCND1*, *VEGFA*, *IL-6*, *ERBB2*, and *FOS*. SJZ, Si Jun Zi decoction.

studied by medical and scientific researchers in many countries worldwide for many years, which have been reported to have the characteristics of high absorption, complex ingredients, and multi-target (16). The different combinations and proportions of various CHMs in the prescription make the drugs have different mechanisms and effects, which has also become a research hotspot in clinical treatment.

The SJZ decoction, as a classical prescription, has been used by many patients to improve the weakness caused by malignant tumors, and is an auxiliary treatment method. In recent years, there are a number of reported results show that SJZ decoction or SJZ based Chinese traditional medicine in the bladder cancer mice, lung cancer mice that accept chemotherapy have enhanced the effect of chemotherapy drugs, reduce the effect of chemotherapy drugs toxic side effects through inhibiting tumor growth and can prolong the survival of mice (17,18). In addition to gastric cancer (19-22), it has also been studied at an animal level or cellular level in the clinical treatment of HOSCC and multiple types of lung cancer (23), but we did not find any previous studies related to SJZ in cSCC.

In this study, the constituents of Ginseng, Glycyrrhiza inflata, Largehead Atractylodes, and Indian Bread in SJZ decoction were screened by network pharmacology, and the targets of these candidate constituents were further analyzed and categorized. At the same time, comprehensive comparison and intersection were made between these potential targets above and potential targets related to cSCC, and candidate targets were screened out for functional enrichment analysis. Meanwhile, a network diagram of "drug-component-target-pathway-disease" was made for future reference and use.

The study found that the potential targets of the more prominent active ingredients of SJZ decoction were mainly concentrated in the biological processes: response to steroid hormone and may be most closely related to the pathways: Kaposi sarcoma-associated herpesvirus infection. At the same time, these targets, pathways, and biological processes are related to the occurrence and development of cSCC.

The results of UPLC analysis showed that the top ten active ingredients extracted from SJZ decoction were Atractylon, Liquiritin, 25-hydroxyporicoic acid H, Isoliquiritin apioside, ginsenoside Rg1, Ginsenoside Rg3, Ginsenoside Re, Kanzonol H, Ginsenoside Ro, Poricoic acid, Licoricesaponin G2, Glycyrrhizic acid, and Liquiritigenin. The ingredients come from the four different Chinese herbs.

In short, the ten main active components of SJZ decoction analyzed by UPLC may act on cSCC-related targets through specific pathways, and then play a role in the treatment or adjuvant treatment of cSCC.

Conclusions

This study conducted correlation analysis on the active components of SJZ decoction and the disease target of cSCC through network pharmacology, and constructed a network diagram with software combining TCM-component-target-disease-pathway. The analysis of the effective components of SJZ decoction by UPLC is helpful to provide a reference direction and theoretical basis for omgoing research on the treatment of cSCC by SJZ decoction.

Limitation

Whether SJZ decoction has the effect of preventing and

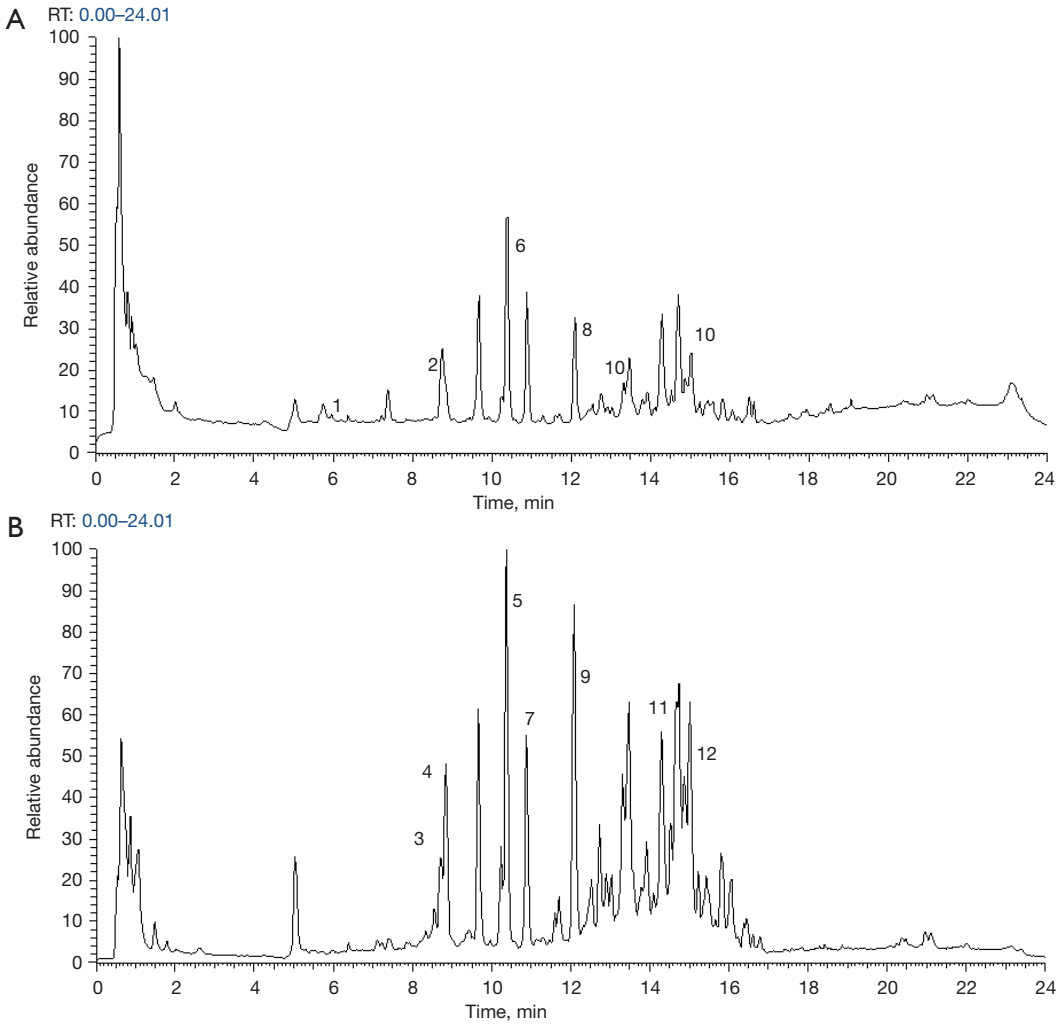

Figure 7 UHPLC-ESI-MS/MS analysis of SJZ: Total ion chromatograms of water extraction of SJZ decoction by negative mode (A) and positive mode (B). The numbers in the chromatograms showed the constituent peaks. Peak 1 was identified as Atractylon; Peak 2 was identified as Liquiritin; Peak 3 was identified as 25-hydroxyporicoic acid H; Peak 4 was identified as Isoliquiritin apioside; Peak 5 was identified as Ginsenoside Rg1; Peak 6 was identified as Ginsenoside Rg3; Peak 7 was identified as Ginsenoside Re; Peak 8 was identified as Kanzonol H; Peak 9 was identified as Ginsenoside Ro; Peak 10 was identified as Poricoic acid; Peak 11 was identified as Licoricesaponin G2; Peak 12 was identified as Glycyrrhizic acid; Peak 13 was identified as Liquiritigenin. UHPLC-ESI-MS/MS, ultra-high performance liquid chromatography-electrospray ionization tandem mass spectrometry; SJZ, Si Jun Zi decoction.

treating cancer and its mechanism of action still need to be further explored.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1716/coif). The authors

have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Fania L, Didona D, Di Pietro FR, et al. Cutaneous Squamous Cell Carcinoma: From Pathophysiology to Novel Therapeutic Approaches. Biomedicines 2021;9:171.
2. Keeping S, Xu Y, Chen CI, et al. Comparative efficacy of cemiplimab versus other systemic treatments for advanced cutaneous squamous cell carcinoma. Future Oncol 2021;17:611-27.
3. Hughes BGM, Munoz-Couselo E, Mortier L, et al. Pembrolizumab for locally advanced and recurrent/metastatic cutaneous squamous cell carcinoma (KEYNOTE-629 study): an open-label, nonrandomized, multicenter, phase II trial. Ann Oncol 2021;32:1276-85.
4. Keohane SG, Botting J, Budny PG, et al. British Association of Dermatologists guidelines for the management of people with cutaneous squamous cell carcinoma 2020. Br J Dermatol 2021;184:401-14.
5. Unlu A, Nayir E, Kirca O, et al. Ginseng and cancer. J BUON 2016;21:1383-7.
6. Sabouri-Rad S, Sabouri-Rad S, Sahebkar A, et al. Ginseng in Dermatology: A Review. Curr Pharm Des 2017;23:1649-66.
7. Huang HC, Tsai LL, Tsai JP, et al. Licochalcone A inhibits the migration and invasion of human lung cancer cells via inactivation of the Akt signaling pathway with downregulation of MMP-1/-3 expression. Tumour Biol 2014;35:12139-49.
8. Kang TH, Seo JH, Oh H, et al. Licochalcone A Suppresses Specificity Protein 1 as a Novel Target in Human Breast Cancer Cells. J Cell Biochem 2017;118:4652-63.
9. Kim KH, Yoon G, Cho JJ, et al. Licochalcone A induces apoptosis in malignant pleural mesothelioma through downregulation of Sp1 and subsequent activation of mitochondria-related apoptotic pathway. Int J Oncol 2015;46:1385-92.
10. Wu CP, Lusvarghi S, Hsiao SH, et al. Licochalcone A Selectively Resensitizes ABCG2-Overexpressing Multidrug-Resistant Cancer Cells to Chemotherapeutic Drugs. J Nat Prod 2020;83:1461-72.
11. Yuan LW, Jiang XM, Xu YL, et al. Licochalcone A inhibits interferon-gamma-induced programmed death-ligand 1 in lung cancer cells. Phytomedicine 2021;80:153394.
12. Oh HN, Lee MH, Kim E, et al. Licochalcone B inhibits growth and induces apoptosis of human non-small-cell lung cancer cells by dual targeting of EGFR and MET. Phytomedicine 2019;63:153014.
13. Oh HN, Lee MH, Kim E, et al. Licochalcone D Induces ROS-Dependent Apoptosis in Gefitinib-Sensitive or Resistant Lung Cancer Cells by Targeting EGFR and MET. Biomolecules 2020;10:297.
14. Oh HN, Seo JH, Lee MH, et al. Licochalcone C induced apoptosis in human oral squamous cell carcinoma cells by regulation of the JAK2/STAT3 signaling pathway. J Cell Biochem 2018;119:10118-30.
15. Kwak AW, Cho SS, Yoon G, et al. Licochalcone H Synthesized by Modifying Structure of Licochalcone C Extracted from Glycyrrhiza inflata Induces Apoptosis of Esophageal Squamous Cell Carcinoma Cells. Cell Biochem Biophys 2020;78:65-76.
16. Nho SH, Yoon G, Seo JH, et al. Licochalcone H induces the apoptosis of human oral squamous cell carcinoma cells via regulation of matrin 3. Oncol Rep 2019;41:333-40.
17. Liu Y, Jia Z, Dong L, et al. A randomized pilot study of atractylenolide I on gastric cancer cachexia patients. Evid Based Complement Alternat Med 2008;5:337-44.
18. Yan Ye, Chou GX, Hui Wang, et al. Effects of sesquiterpenes isolated from largehead atractylodes rhizome on growth, migration, and differentiation of B16 melanoma cells. Integr Cancer Ther 2011;10:92-100.
19. Knackstedt TJ, Knackstedt RW, Djohan M, et al. New Developments in the Management of Cutaneous Squamous Cell Carcinoma. Plast Reconstr Surg 2021;147:492-504.
20. Wang L, Xu L, Wang Y. Huaier Inhibits Proliferation, Migration, and Invasion of Cutaneous Squamous Cell Carcinoma Cells by Inhibiting the Methylation Levels of CDKN2A and TP53. Integr Cancer Ther 2021;20:15347354211031646.
21. Li YJ, Liao LL, Liu P, et al. Sijunzi Decoction Inhibits

Stemness by Suppressing beta-Catenin Transcriptional Activity in Gastric Cancer Cells. Chin J Integr Med 2021. doi: 10.1007/s11655-021-3314-9.
22. Ding P, Guo Y, Wang C, et al. A Network Pharmacology Approach for Uncovering the Antitumor Effects and Potential Mechanisms of the Sijunzi Decoction for the Treatment of Gastric Cancer. Evid Based Complement Alternat Med 2022;2022:9364313.
23. Shao N, Xiao Y, Zhang J, et al. Modified Sijunzi Decoction Inhibits Epithelial-Mesenchymal Transition of Non-Small Cell Lung Cancer by Attenuating AKT/GSK3beta Pathway in vitro and in vivo. Front Pharmacol 2022;12:821567.

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