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Analysis

Mechanisms of Jisheng-Wumei tablet in laryngeal cancer treatment: a network pharmacology analysis of baicalein's targeting of cytochrome C for laryngeal cancer inhibition

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Abstract

Objectives Jisheng-Wumei Tablet (JSWMT) is traditionally used in Chinese medicine for cancer therapy and shows potential efficacy against laryngeal cancer. This study aimed to elucidate the active components of JSWMT, with a specific focus on the role of baicalein in targeting cytochrome C (CYCS) to inhibit laryngeal cancer.

Methods Utilizing network pharmacology, we identified the active ingredients of JSWMT and their corresponding drug targets by leveraging data from the TCMSP and GeneCards databases to pinpoint laryngeal cancer-related targets. Key targets were identified through survival analysis of TCGA expression data, and molecular docking was employed to evaluate the binding affinity between JSWMT compounds and these targets.

Results Our research identified 35 effective JSWMT ingredients that interact with 132 drug targets, which intersect with 421 targets related to laryngeal cancer. Survival analysis highlighted IL2, CYCS, and CTNNB1 as critical targets significantly correlated with patient survival. Molecular docking demonstrated a strong affinity between CYCS and baicalein, indicating that baicalein is JSWMT's principal active component against laryngeal cancer.

Conclusion Baicalein is identified as the key active ingredient in JSWMT, targeting CYCS to inhibit the growth of laryngeal cancer cells. This investigation highlights baicalein's potential mechanism of action and underscores the importance of integrating traditional Chinese medicine with contemporary cancer treatment strategies, offering a novel approach to laryngeal cancer therapy.

Keywords Laryngeal cancer · Jisheng-Wumei Tablet (JSWMT) · Effective active ingredient · Baicalin · Cytochrome C · Network pharmacology · Dark plums · Zombie silkworm · Curcuma zedoary · Safflower

Abbreviations

BP	Biological process
CC	Cellular components
CYCS	Cytochrome C
DL	Drug-like properties
DHE	Dihydroethidium
EMT	Epithelial-mesenchymal transition
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase

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GO	Gene ontology
IL2	Interleukin 2
KEGG	Kyoto encyclopedia of genes and genomes
MF	Molecular functions
MMP	Mitochondrial membrane potential
NP	Network pharmacology
OB	Oral bioavailability
OS	Overall survival
JSWMT	Jisheng-Wumei Tablets
PDB	Protein Data Bank
PPI	Protein–protein interaction
ROS	Reactive oxygen species
SANI1	Snail family transcriptional repressor 1
SCLC	Small cell lung cancer
TCGA	The Cancer Genome Atlas
TCM	Traditional Chinese medicine
TCMSP	Traditional Chinese Medicine Systems Pharmacology
TWIST1	Twist family BHLH transcription factor 1

1 Introduction

According to the latest estimates from GLOBOCAN 2022, approximately 184,615 new cases and 99,840 deaths due to laryngeal cancer have been reported worldwide. In the United States, the Surveillance, Epidemiology, and End Results (SEER) Program reports an estimated 12,620 new cases and 3,770 deaths in 2024, with a five-year overall survival rate of approximately 60.6%. The global burden of laryngeal cancer remains significant, particularly among males, and indicates the need for improved therapeutic strategies [1]. Smoking and heavy alcohol consumption, particularly in combination, are recognized as key risk factors. This synergistic carcinogenic effect is well documented in head and neck cancer epidemiology literature [2]. Current treatments-including surgery, radiotherapy, and chemotherapy—while effective to some extent, often lead to severe side effects such as bone marrow suppression, gastrointestinal toxicity, and organ dysfunction [3].

In response, traditional Chinese medicine (TCM) has gained recognition for its role in cancer treatment as a primary or adjunct therapy. TCM is applied to alleviate symptoms, reduce recurrence, and enhance survival [4–7]. Several herbal components of the Jisheng-Wumei Tablet (JSWMT), including dark plums, curcuma zedoary, safflower, and Bombyx batryticatus (zombie silkworm), have been reported to exhibit anti-inflammatory and anti-tumor properties in experimental and preclinical studies [4, 8–12].

Pharmacological studies indicate that its components may exert anti-tumor effects by regulating intestinal flora, suppressing oncogene expression, and inducing apoptosis. However, clinical efficacy alone is insufficient without mechanistic validation. Despite growing clinical interest in JSWMT, the bioactive components responsible for its anti-cancer effects and underlying mechanisms remain insufficiently characterized. Due to the complexity of traditional Chinese medicine formulations and their multi-component nature, previous research has rarely addressed the direct interaction between JSWMT compounds and laryngeal cancer-relevant molecular targets. Although JSWMT has demonstrated anti-tumor properties in clinical practice, the specific active compounds and their mechanisms of action remain unclear due to the complexity of traditional Chinese medicine formulations. Previous studies have seldom addressed the interaction between JSWMT compounds and molecular targets relevant to laryngeal cancer. This study aims to fill this gap by applying network pharmacology and molecular docking approaches to identify and validate the core components and molecular pathways involved in JSWMT's therapeutic effect on laryngeal cancer.

To achieve this, we conducted a comprehensive study using network pharmacology to screen active ingredients and predict potential drug–disease target interactions, followed by molecular docking to evaluate binding affinities. Gene expression and survival analysis based on TCGA data were employed to validate the clinical significance of key targets. This integrative strategy allows us to reveal the mechanistic landscape of JSWMT and propose baicalein–cytochrome C (CYCS) interaction as a critical axis in its anti-laryngeal cancer activity.

2 Materials and methods

2.1 Analysis of effective ingredients in JSWMT compound formula

The active ingredients of the main TCM components in JSWMT and its constituent herbs were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) (https://old.tcmsp-e.com/index.php) database. The obtained effective active ingredients are screened per the bioavailability and drug-likeness doorsteps (oral bioavailability (OB) ≥ 30%, drug-like properties (DL) ≥ 0.18). The final active ingredients were identified by eliminating duplicate entries. The corresponding drug targets of these effective ingredients were obtained from the TCMSP.

Screening of laryngeal cancer targets and intersection analysis with drug objectives of the effective ingredients from JSWMTs was conducted. Preliminary relevant laryngeal cancer targets were predicted using the GeneCards (https://www.genecards.org/) database with the keyword "laryngeal squamous cell carcinoma." The final laryngeal cancer targets were obtained by screening on the set of "relevance score ≥ 30." Following, the intersection target analysis of the effective ingredients of JSWMTs and the predicted laryngeal cancer targets was conducted by using the R language VennDiagram package.

2.2 Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

We applied GO and KEGG analyses to investigate the biological functions and pathways associated with JSWMT action on laryngeal cancer. Using the ClusterProfiler R package, we categorized intersected targets into GO's biological processes, cellular components, and molecular functions, highlighting their functional involvement in laryngeal cancer mechanisms. KEGG analysis further mapped these targets to signaling and metabolic pathways, providing insights into the systemic effects of JSWMT's active components. Visualization with enrichplot facilitated the interpretation of enriched GO terms and KEGG pathways, emphasizing their significance in laryngeal cancer pathogenesis with stringent criteria (adjusted p-value < 0.05 & count > 2), thus streamlining our understanding of the therapeutic targets within a broader biological context.

TCM pharmacological regulatory network of the intersected targets was constructed using the intersected targets to develop a TCM pharmacological associative network. This network was visualized using Cytoscape (version 3.8.2) to display the drugs, active ingredients, and possible functional regulatory relationships among the intersected targets within the network.

2.3 Protein–protein interaction (PPI) regulatory network diagram of the intersecting targets

STRING was applied to prepare a PPI network for the intersected targets. By filtering with a confidence level of 0.4, the PPI relationship was obtained and then visualized in a protein network chart using Cytoscape software.

2.4 Laryngeal cancer survival analysis of the key targets

The laryngeal squamous cell carcinoma expression data were downloaded from The Cancer Genome Atlas (TCGA). The samples were classified into high and low-expression groups based on the median gene expression value. The R package 'survival' was used to generate the overall survival (OS) curves for each intersected target, stratified by high and low expression groups. Genes demonstrating significant survival impact ($p < 0.05$) were identified as key targets to pinpoint molecules whose expression levels are significantly associated with survival time.

2.5 Molecular docking of core targets

First, the protein structures of the key target molecules selected from key targets survival analysis were gained from Protein Data Bank (PDB) (https://www.wwpdb.org/). Protein charge and hydrogenation calculation was completed by AutoDock Tools (https://ccsb.scripps.edu/mgltools/downloads/). TCMSP database was applied to download the active ingredient structures of the JSWMT formula. Docking of receptors and ligands was conducted by AutoDock vina. The docking pairs whose docking binding energy was less than -5.0 kcal/moL (for moderate binding) or ≤ -7.0 kcal/mol

(for strong interaction) were selected from the output results. Finally, PyMol (https://pymol.affinitycn.cn/) software was applied to visualize and beautify the selected conformations from the lowest binding energy out of 20 dockings.

3 Results

3.1 Overlapping targets of laryngeal cancer and JSWMT

To elucidate the pharmacologically active ingredients of JSWMT, we initially utilized the TCMSP database. It was determined that the primary components of JSWMT include black plum, zombie silkworm, curcuma zedoary, and safflower. A search in the TCMSP database revealed a total of 40 ingredients for black plum, 81 ingredients for curcuma zedoary, and 189 ingredients for safflower. Zombie silkworm was not included in the TCMSP database. However, 42 components of zombie silkworms were gained from the TCM-ID database and literature [13]. After deduplication, the active ingredients of the compounds were screened according to the set bioavailability and drug-likeness thresholds (oral bioavailability (OB) ≥ 30%, drug-like properties (DL) ≥ 0.18), and then 35 active ingredients were obtained (Table 1). Among them, 24 active ingredients (11 have no targets) had 132 corresponding drug targets based on the TCMSP database analysis (data is not shown).

To analyze disease targets related to laryngeal cancer, we used the GeneCards (https://www.genecards.org/) database with the keyword 'laryngeal squamous cell carcinoma' to identify relevant targets. A total of 1924 targets were predicted, and among these, 421 disease targets were selected based on a relevance score of ≥ 30 (data not shown). To examine the association between laryngeal cancer and JSWMT, we overlapped the 132 drug targets with the 421 disease targets, resulting in 85 overlapped targets (Fig. 1).

3.2 Analysis of regulatory network for intersection targets and function

Through Gene Ontology (GO) enrichment analysis, the top 20 terms from each gene ontology category, biological process (BP), cellular component (CC), and molecular function (MF) were obtained. A regulatory network diagram of the intersection targets-function was drawn using Cytoscape (Fig. 2A). It contains 60 GO Terms, 82 intersection targets, and 866 relationship pairs.

Furthermore, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were used to enrich and analyze the relationship between drug intersection targets and functional pathways. Arranging the top 50 pathways and their corresponding intersection targets in ascending order of p-values, a network diagram of intersection targets and functional pathways was constructed using Cytoscape (Fig. 2B). The network contains 50 KEGG pathways, 82 intersecting targets, and 985 relationship pairs.

The pharmacological regulation network of the intersecting targets was constructed using the effective ingredients related to the intersection goals. This network was prepared in Cytoscape and included the four TCM herbs, 22 active ingredients, 85 overlapped targets, and 385 relationship pairs (Fig. 3A).

3.3 Protein–protein interaction (PPI) regulatory network chart of the overlapped objectives

A PPI network diagram was created by Cytoscape using the physical contact relationships as lines and the proteins as nodes, which participate in the same metabolic pathways, biological processes, structural complexes, and functional associations. Proteins interact to regulate signaling, metabolism, and disease progression. Proteins form networks through interactions to engage in many life processes, including biological signal transduction, energy and substance metabolism, gene regulation, and cell cycle control. Therefore, PPI networks are valuable for understanding the cellular network function and structure. Such networks are essential for understanding the mechanisms of disease development. We used STRING to build a PPI network to investigate if there is any cooperative relationship between the 85 overlapped targets. Screening with a confidence level of 0.4, 85 protein interaction networks resulted, including 85 nodes and 555 edges. (Fig. 3B).

Table 1 Active ingredients of Jisheng-Wumei Tablets

Molecule name	OB	DL
(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36332114	0.21141
β-sitosterol	36.91390583	0.75123
Kaempferol	41.88224954	0.24066
Stigmasterol	43.82985158	0.75665
Campest-5-en-3beta-ol	37.57681789	0.71481
Methyl arachidonate	46.89969301	0.23381
CLR	37.87389754	0.67677
Quercetin	46.43334812	0.27525
Cholesterol	37.87	0.68
β-carotene	37.18	0.58
Hispidulin	30.97	0.27
Folic Acid	68.96	0.71
Quercetin	46.43	0.28
Kaempferol	41.88	0.24
β-sitosterol	33.94	0.7
Hederagenin	36.91390583	0.75072
Wenjine	47.92807652	0.272
Bisdemethoxycurcumin	77.38200887	0.26088
Poriferast-5-en-3beta-ol	36.91390583	0.75034
Flavoxanthin	60.412944	0.55609
4-[(E)-4-(3,5-dimethoxy-4-oxo-1-cyclohexa-2,5-dienylidene)but-2-enylidene]-2,6-dimethoxycyclohexa-2,5-dien-1-one	48.46631493	0.36494
Lignan	43.31815989	0.65067
Lupeol-palmitate	33.98364826	0.31954
Phytoene	39.56307142	0.50463
phytofluene	43.18172626	0.50316
Pyrethrin II	48.35707237	0.35025
6-Hydroxykaempferol	62.1326676	0.27266
Baicalein	33.51891869	0.20888
Qt_carthamone	51.02582086	0.20055
6-Hydroxynaringenin	33.22920875	0.24203
Quercetagetin	45.00699322	0.30991
7,8-dimethyl-1H-pyrimido[5,6-g]quinoxaline-2,4-dione	45.75093773	0.18605
β-carotene	37.18433337	0.58358
Baicalin	40.12360996	0.75264
Luteolin	36.16262934	0.24552

3.4 OS curves analysis of the key PPI targets in laryngeal cancer

Gene expression data of laryngeal squamous cell carcinoma was obtained from TCGA to identify the essential genes that deeply affect laryngeal cancer growth. After excluding 11 standard samples and one duplicate sample, data from 109 laryngeal squamous cell carcinoma samples were analyzed (data not shown). These samples were classified into high and low-expression groups based on the median gene expression values. The R package 'survival' was utilized to generate the OS curves for each intersected target, distinguishing between the high and low expression groups. Among the 85 PPI target genes in OS curve analysis, only the expression levels of interleukin 2 (IL2, $p < 0.011$), cytochrome C (CYCS, $p < 0.0064$), and catenin β1 (CTNNB1, $p < 0.016$) genes were significantly correlated with survival time of laryngeal cancer (Fig. 4). They were the possible key target molecules for laryngeal cancer treatment.

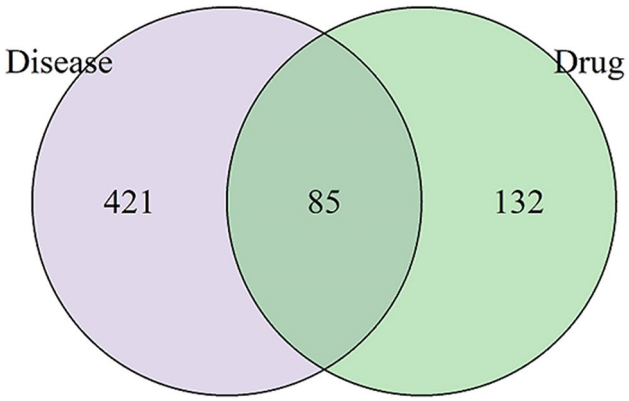

Fig. 1 Overlay of the JSWMTs' ingredients and disease targets of laryngeal cancer. The main ingredients of JSWMTs are dark plums, silkworms, curcuma zedoary, and safflower. From the TCMSP database, 352 active ingredients were obtained. According to OB ≥ 30% and DL ≥ 0.18 setting, 35 effective ingredients were screened in the TCMSP database. Among them, 24 active ingredients act on 132 drug targets (light green). Using the Genecards (https://www.genecards.org/) database, 421 disease targets were predicted related to the keyword "laryngeal squamous cell carcinoma" from the GeneCards database. By R language VennDiagram package, intersection analysis was performed on the drug and disease targets, resulting in 85 intersection objects

3.5 Molecular docking of the effective ingredients of JSWMT on the vital target molecules of laryngeal cancer

Molecular docking primarily evaluates spatial complementarity and binding energy between the ligand and the receptor. Spatial compatibility ensures structural fit, whereas energy minimization reflects the stability of the ligand–receptor complex. To explore the docking of the active ingredients of JSWMT on the key molecules, we selected IL2, CYCS, and CTNNB1, which had significant intersection target survivals (Fig. 5), and the 35 active ingredients of JSWMT (Table 1) for molecular docking. Corresponding crystal structures of IL2 (PDB ID: 4NEJ), CYCS (PDB ID: 6DUJ), and CTNNB1 (PDB ID: 1QZ7) were obtained from the PDB database. The 3-D configuration of the active ingredients was downloaded from the TCMSP database, and AutoDock vina was applied for molecular docking. The docking pairs were considered to be the molecules with docking binding energy less than − 1.2 kcal/moL. The conformation with minimum docking coupling energy among 20 analyses was used to draw the graph. The molecular docking analysis found four docking pairs (IL2-quercetin, IL2-luteolin, CYCS-baicalein, and CTNNB1-carotene) (Table 2). The CYCS-baicalein docking pair had the highest energy matching (− 8.3 kcal/mL).

4 Discussion

Due to the lack of comprehension of the herbal formulas and mechanisms of therapeutic action, TCM is only considered an alternative therapeutic strategy [14]. Unlike synthetic drugs, TCM is characterized by multi-targets, multi-components, and multi-pathways. The intricate network comprising herbal medicines, ingredients, targets, pathways, diseases, metabolites, and gut microbiota necessitates thorough exploration to elucidate the mechanisms of TCM. With the advent of the big data era, comprehensive research on TCM can now be approached in novel ways. This extensive analysis of vast data volumes has also facilitated the emergence of systems biology in TCM, based on NP [15]. NP uses pharmacology and systems biology big data and computers to integrate system data to analyze the overall process of reciprocity between compounds and the human body and predict the possible mechanisms of the therapeutic effects of TCM [16]. TCMSP's TCM framework based on systems pharmacology proposed to build a component-target-disease (CTD) network and try to apply CTD in TCM [17]. In this study, using the TCMSP database, we discovered 35 active ingredients (Table 1) and their 132 action targets in JSWMT. Overlap analysis of these targets with the laryngeal cancer-related targets found 85 overlaps (Fig. 1A). Those 85 overlapped proteins were then applied to do OS curves analysis in the gene expression data of laryngeal squamous cell carcinoma downloaded from TCGA. The expression levels of IL2, CYCS, and CTNNB1 genes were significantly correlated with survival time in

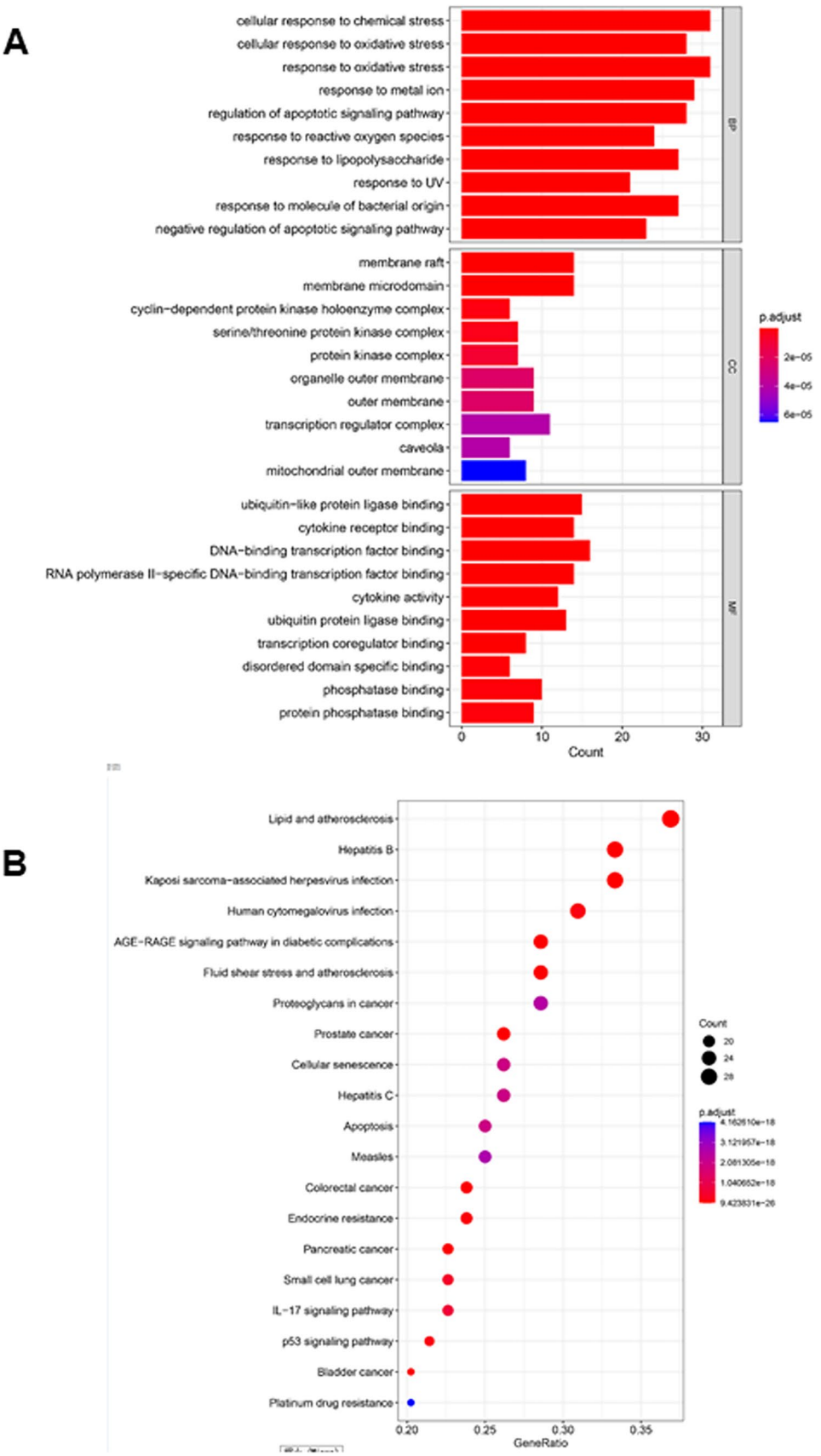

Fig. 2 Functional and Pathway Enrichment Analysis of Intersected Targets. **A** GO enrichment analysis of the intersected targets. The default background gene set from the ClusterProfiler package was applied to analyze the intersection between drug targets and disease targets (see Fig. 1A). The enrichment results were visualized by drawing a bar chart using the enrichplot package (version 1.10.2). It enriched 2164 GO processes (p.adjust < 0.05&count > 2), including 2012 biological processes (BP) directories, 43 cellular components (CC) directories, and 108 Molecular Function (MF) directories. The figure shows each of the top 10 BP (up panel), MF (middle panel), and CC (lower panel). **B** KEGG enrichment analysis of the intersected targets. The default background gene set from the ClusterProfiler package was applied to analyze the intersection between drug targets and disease targets (see Fig. 1A). The enrichment results were visualized by drawing a bar chart using the enrichplot package (version 1.10.2). KEGG was enriched in 160 pathways (p.adjust < 0.05 & count > 2). The top 20 pathways are shown in the figure

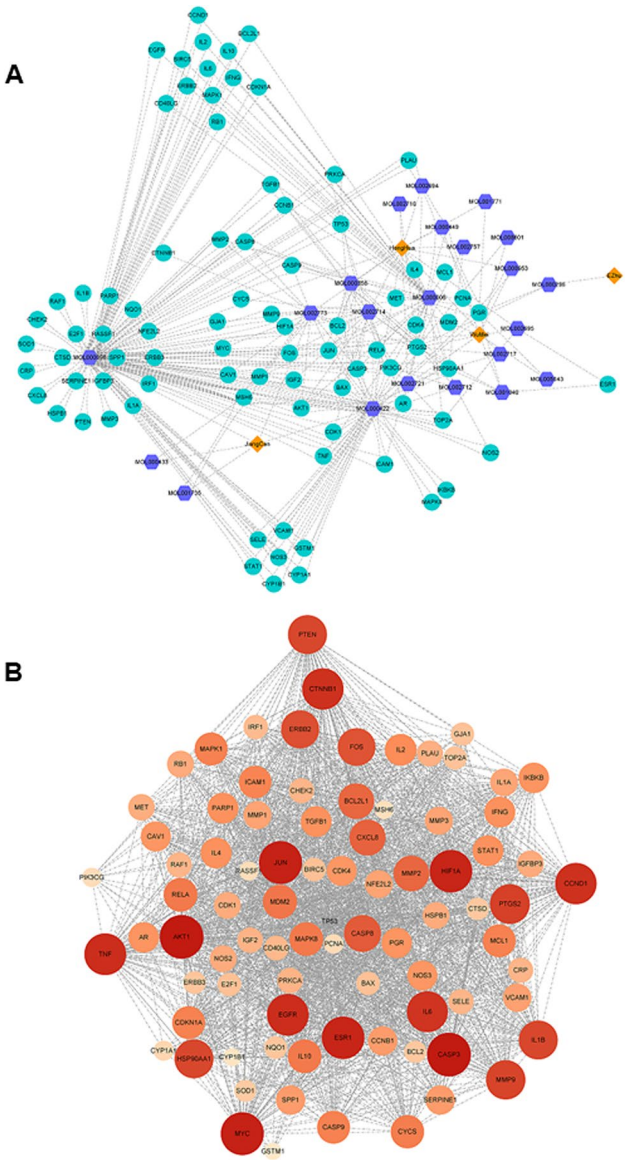

Fig. 3 Network pharmacology (NP) analysis of Jiwu-Shengmei tablets (JSWMT) in treating laryngeal cancer. **A** A pharmacological regulatory network was constructed for TCM using active ingredients corresponding to intersecting targets, which were visualized by Cytoscape. The network contains four drugs (orange), 22 active ingredients (blue), 85 intersecting targets (light platinum omitted), and a total of 385 relationship pairs. **B** Utilizing STRING, a PPI network was created for the 85 intersecting targets. With confidence = 0.4, 85 protein interaction networks were screened out, including 85 nodes and 555 edges. The protein network diagram was beautified using Cytoscape. The dimension and color depth of nodes reflect their grade of connectivity

laryngeal cancers (Fig. 4). They subsequently underwent a molecular docking analysis with the 35 active ingredients in JSWMT (Table 1). The results indicated that four pairs, IL2-quercetin, IL2-luteolin, CYCS-baicalein, and CTNNB1-carotene, which have passed the filtering threshold and could be the active ingredients-target proteins of JSWMT on laryngeal cancer.

In the active ingredient analysis of the four primary TCM medicinal materials of JSWMT, 35 ingredients with thresholds OB ≥ 30% and DL ≥ 0.18 were found (Table 1). In addition to baicalein, which was identified and verified in this study, a review of the literature indicates that the primary ingredients of JSWMT exhibit a range of therapeutic effects, including antioxidant, anti-inflammatory, anti-cancer, antibacterial, anti-thrombotic, cholesterol-lowering, and protective effects on cardiovascular and nervous tissues. Specifically, dark plums, curcuma zedoary, and safflower ingredients demonstrate antioxidant and antibacterial properties, whereas dark plums and safflower additionally show anti-cancer, cholesterol-lowering, and cardiovascular protective effects. The main constituents of zombie silkworms are various vitamins [18–25]. These data are consistent with JSWMT's clinical application in treating inflammation and tumors [26, 27]. However, their detailed pharmacological mechanisms remain unclear and warrant further investigation.

Among these ingredients, baicalein, a flavonoid compound, is particularly notable for its anti-tumor activity. Previous studies have shown that baicalein can initiate apoptosis by upregulating Bax, downregulating Bcl-2, disrupting mitochondrial membrane potential, and promoting the release of cytochrome C (CYCS) into the cytoplasm, which then activates caspase-3 and triggers apoptosis [24]. Our docking analysis (– 8.3 kcal/mol) supports this apoptotic mechanism,

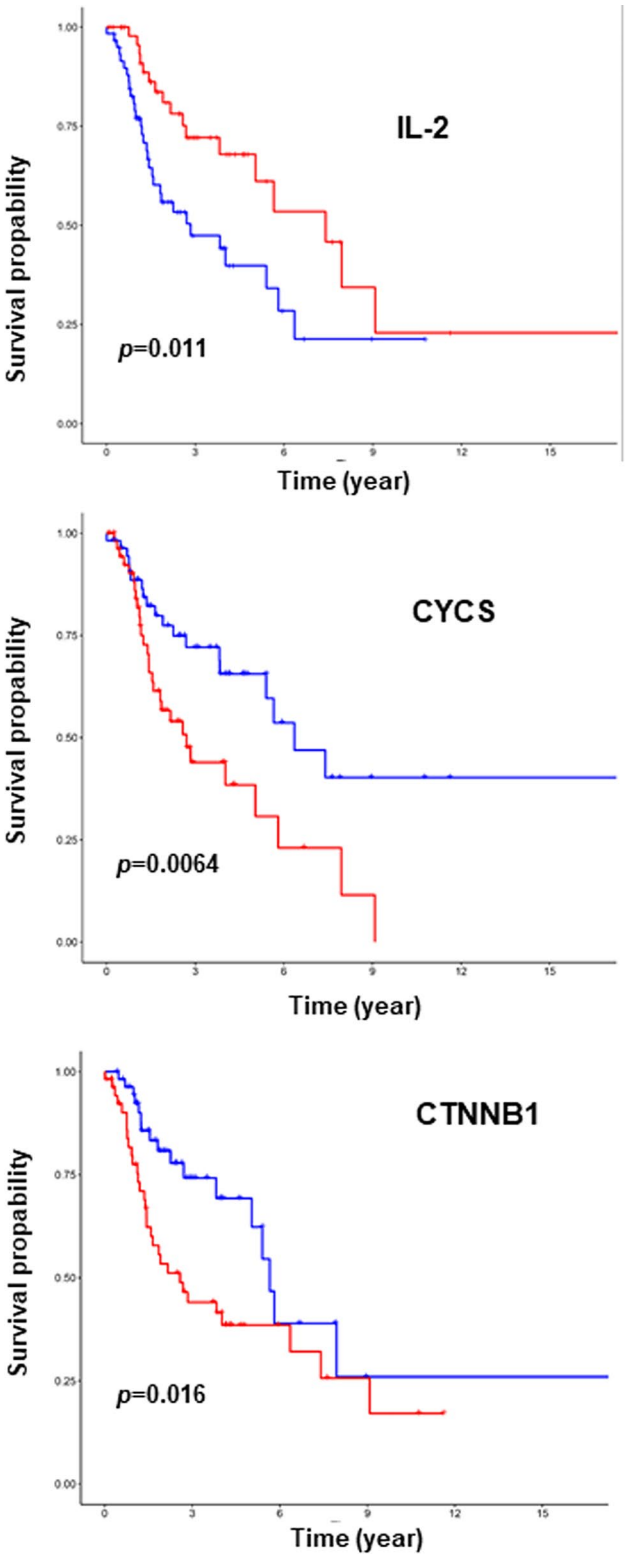

Fig. 4 Survival analysis of key targets in laryngeal squamous cell carcinoma. The expressed crucial protein data for laryngeal squamous cell carcinoma were downloaded from TCGA. The samples were classified into high (red line) and low (blue) expressive groups based on the median of the gene expression. Kaplan–Meier curves were prepared using R package survival for the overall survival of the interesting targets according to low (blue line) and high (red line) expression groups. Genes with significant survival ($p < 0.05$) were used as key targets. Among the 85 intersection targets, the expression levels of three target genes, IL2 (up panel), CYCS (middle panel), and CTNNB1 (lower panel), were significantly correlated with survival time

suggesting that baicalein may contribute to JSWMT's anti-laryngeal cancer effect by directly engaging CYCS to initiate mitochondrial-mediated cell death. Furthermore, recent studies have demonstrated that nano-curcumin and its analogs, such as BDMC-A, can induce apoptosis through intrinsic and extrinsic pathways. A key mechanism involves the mitochondrial release of cytochrome C (CYCS) into the cytoplasm, subsequently triggering downstream caspase activation. Notably, BDMC-A has been shown to exert a more potent apoptotic effect in laryngeal carcinoma (Hep-2) cells than

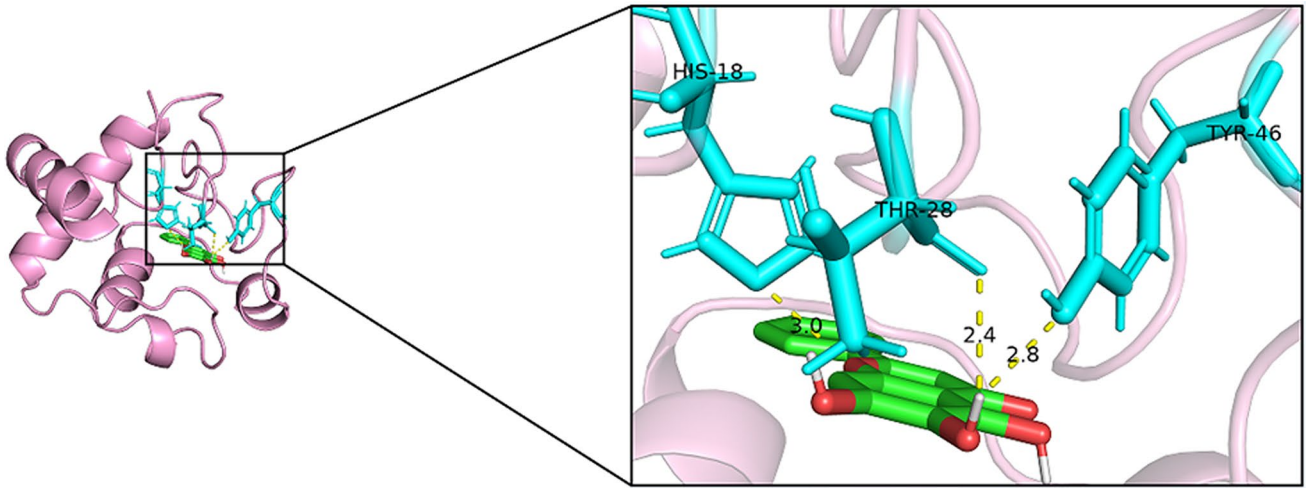

Fig. 5 Molecular docking of cytochrome C (CYCS) with baicalein. Molecular docking was performed between the CYCS with significant intersection target survival and active ingredients (quercetin, luteolin, baicalein, and beta-carotene). The crystal structure corresponding to CYCS was obtained from the PDB database. The 3-D structure of the active ingredient was downloaded from the TCMSP database. AutoDock vina was used for molecular docking. The docking affinity between CYCS and baicalein is -8.3 kcal/mol. The green stick model in the picture is the effective molecule baicalein, and the cyan stick structures closed to baicalein are amino acid residues that have hydrogen bond interactions with the effective molecule. Yellow dashed lines are the hydrogen bonds between the amino acid residues and active molecules. Among them, HIS-18 residue, THR-28 residue, and TYR-46 residue have hydrogen bonds associated with the baicalein molecule

Table 2 Molecular docking of the active ingredients of Jisheng-Wumei Tablets on the target molecules of laryngeal cancer

Target	PDB ID	Mol_id	Molecule_name	Binding energy (kcal/moL)
IL2	4NEJ	MOL000098	quercetin	– 6.3
IL2	4NEJ	MOL000006	luteolin	– 6.3
CYCS	6DUJ	MOL002714	baicalein	– 8.3
CTNNB1	1QZ7	MOL002773	beta-carotene	– 7.4

curcumin, likely due to its enhanced structural stability and cellular uptake [28]. Baicalein induces apoptosis by activating the p53 signaling pathways in various cancer types, including hepatocellular carcinoma [29]. Additionally, baicalein activates the MAPK/ERK pathway to inhibit cancer cell growth and induce apoptosis, as reported in breast cancer cells [30].

CYCS is crucial in the apoptosis pathways, including in cancer cells. Previous studies suggest its potential as a therapeutic target for laryngeal cancer. For instance, treatments involving oridonin and cetuximab have shown synergistic effects against laryngeal squamous cell carcinoma by influencing pathways that may involve CYCS. Combining our findings from this study underscores the significance of targeting CYCS-mediated pathways in developing novel treatments for laryngeal cancer using JSWMT.

Interleukin-2 (IL-2) has long been recognized for its pivotal role in regulating immune responses, including the activation and proliferation of T cells. This cytokine's potential in cancer immunotherapy, particularly for laryngeal cancer, stems from its ability to stimulate both effector immune cells and regulatory T cells, enhancing the body's natural defense mechanisms against tumors. Research into IL-2-based therapies has evolved to focus on developing biologic agents that leverage IL-2's immunostimulatory effects while minimizing the adverse effects associated with its systemic administration. Despite the promise shown in preclinical studies, IL-2-based compounds have not yet received regulatory approval for the treatment of cancer, including laryngeal cancer. However, ongoing clinical trials continue to explore the efficacy of these compounds, with some reaching phase 3 trials [31, 32]. Parallel to these developments, studies employing dendritic cell-based vaccination strategies against laryngeal cancer have incorporated IL-2 to enhance the immunogenicity of tumor-derived antigens. Such approaches aim to prime the immune system more effectively against tumor cells, highlighting the versatile role of IL-2 in cancer immunotherapy [33]. Collectively, these efforts underscore the potential of IL-2 as a component of immunotherapeutic strategies against laryngeal cancer, albeit with the acknowledgment that

more research is needed to refine these treatments for clinical application. These data support our finding that IL-2 could be the target for laryngeal cancer therapy by JSWMT.

Research has increasingly recognized Catenin Beta 1 (CTNNB1) as a promising therapeutic target in laryngeal squamous cell carcinoma (LSCC). Greco et al. (2016) linked E-cadherin and β-catenin overexpression with LSCC outcomes, suggesting β-catenin's tumor-suppressing potential [34]. Psyrri et al. highlighted Wnt pathway's significance in LSCC, advocating for the use of Wnt inhibitors based on β-catenin protein expression [35]. Further, Wang et al. showed how microRNA-protein interactions enhance the β-catenin pathway, influencing LSCC progression [36]. Sun et al. demonstrated lncRNA UCA1's role in activating the Wnt/β-catenin pathway, promoting cell proliferation and invasion, which points to lncRNAs as potential LSCC targets [37]. Additionally, Galera-Ruiz et al. and Tang et al. discussed the cadherin-catenin complex and YAP's involvement in LSCC, underscoring the therapeutic potential of modulating specific molecular pathways, including Wnt/β-catenin signaling [38, 39]. These studies elucidate the role of CTNNB1 in the cellular dynamics of LSCC. Our study employs network pharmacology methods and theoretically proves the interference function of CTNNB1 in laryngeal cancer cells, providing a theoretical basis for using JSWMT to treat laryngeal cancer.

The study's limitations include the lack of experimental verification that CYCS is the core target of baicalein in its anti-laryngeal cancer efficacy. Indeed, this will be the focus of our next research plan.

5 Conclusion

This study identifies baicalein as the primary active ingredient in JSWMT against laryngeal cancer, acting through the upregulation of CYCS to induce cell death. Utilizing network pharmacology, we discovered 35 active ingredients and 132 action targets, with IL2, CYCS, and CTNNB1 showing significant associations with survival. Molecular docking studies revealed baicalein's high affinity for CYCS. These findings elucidate baicalein's anti-cancer mechanism and present a novel approach to treating laryngeal cancer, demonstrating a significant methodology for TCM research.

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Data availability The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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