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Research Article

Mechanisms of Ranunculus Ternatus against Thyroid Carcinoma Based on Network Pharmacology and Molecular Docking

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Abstract

Purpose: Thyroid Carcinoma (THCA) is a type of endocrine cancer. The roots of Ranunculus Ternatus (RT) are widely used because of their anti-inflammatory activity and ability to antivirus and treatment for multi-drug resistant diseases, especially in the treatment of lymphatic tuberculosis in China, indicating that RT may have potential therapeutic value in THCA treatment. Therefore, this study aimed to clarify the efficacy and possible mechanisms of RT in THCA treatment.

Methods: TCMSP, PubChem, Swiss Target Prediction, DrugBank, GeneCards, DAVID, and other databases were used to identify the active compounds and target proteins of RT. The putative targets of RT and THCA were collected from multiple databases. Network topology and enrichment analyses were performed to screen for key targets and mechanisms. Finally, molecular docking tools were used to evaluate the drug and target binding.

Results: Six compounds (7-O-Methyleriodictyol, Beta-sitosterol, Mandenol, Stigmasterol, CLR, and Truflex OBP) were identified after network analysis and virtual screening based on molecular docking. Nine targets (SRC, PTGS2, PPARG, MMP9, MAPK1, HIF1A, ESR, ERBB2, and EGFR) were considered vital therapeutic targets with excellent binding affinity. Enrichment analysis revealed that the underlying mechanisms were related to cell proliferation, apoptosis, immunity, and adhesion, especially through the SRC and EGFR signaling pathways. Molecular docking results revealed that all six components of RT had good binding ability to their respective targets.

Conclusion: In brief, we elucidated the potential mechanism of RT in the treatment of THCA. This study provides a basis for future experimental research, and serves as a reference for clinical treatment.

Keywords: Molecular docking; Network pharmacology; Ranunculus ternatus; Thyroid carcinoma

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Abbreviations

ADME: Absorption, Distribution, Metabolism and Excretion

ATC: Anaplastic Thyroid Carcinoma

CLR: 10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol;

COX-2: Cyclooxygenase-2

DAVID: Database for Annotation, Visualization and Integrated Discovery

DFS: Disease-Free Survival

EGF: Epidermal Growth Factor

EGFR: Epidermal Growth Factor Receptor

ER: Estrogen Receptor

ERBB2: Receptor Tyrosine-Protein Kinase erbB-2

ESR1: Estrogen Receptor Alpha

ESR1: Estrogen Receptor Alpha Gene

GO: Gene Ontology

FTC: Follicular Thyroid Carcinoma

HCC: Hurthle Cell Carcinoma

HIF1A: Hypoxia-Inducible Factor 1-alpha

ICGC: International Cancer Genome Consortium

KEGG: Kyoto Encyclopedia of Genes and Genomes

MAPK1: Mitogen-Activated Protein Kinase 1

MMP9: Matrix Metallopeptidase 9

OMIM: Online Mendelian Inheritance in Man

PDTC: Poorly Differentiated Thyroid Carcinoma

PPARG: Peroxisome Proliferator- Activated Receptor Gamma

PPI: Protein-Protein Interaction

PTC: Papillary Thyroid Carcinoma

PTGS2: Prostaglandin-Endoperoxide Synthase 2

ROS: Reactive Oxygen Species

RT: the Roots of Ranunculus Ternatus

SRC: Proto-oncogene tyrosine-protein kinase SRC

TCGA: The Cancer Genome Atlas

TCM: Traditional Chinese Medicine

TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform

THCA: Thyroid Carcinoma

Truflex-OBP: Butyl Octyl Phthalate

TSH: Thyroid-Stimulating Hormone

UCSC: University of California Santa Cruz

Introduction

Thyroid Cancer (THCA) is one of the most common endocrine neoplasms, accounting for 5.0% of all head and neck cancers [1]. Over the last decade, the incidence has increased by approximately 2% per year [2]. More than 95% of thyroid carcinomas are of follicular cell origin whereas the rest 3 to 5% are medullary thyroid carcinomas arising from C cells. The former type can be further divided into Papillary Thyroid Carcinoma (PTC), Follicular Thyroid Carcinoma (FTC), Hurthle Cell Carcinoma (HCC), Poorly Differentiated Thyroid Carcinoma (PDTC), and Anaplastic Thyroid Carcinoma (ATC) [3]. THCA treatment includes surgical resection, radioactive iodine therapy (usually following surgery), inhibition of Thyroid-Stimulating Hormone (TSH) [4] and inhibition of kinase-based target therapies [5]. The most common side effects of treatment include fatigue, anemia, nausea/weight change/dietary issues etc., [5]. Currently, patients with TC are pursuing safer therapies. Natural herbs are considered to be safer than chemical drugs [6]. Therefore, alternative or complementary treatments are needed, together with active prevention of TC. Currently, the roots of Ranunculus Ternatus (RT) are mainly used to cure lymphatic tuberculosis and cancer [7] in China with positive curative effects. However, only a few anticancer research result available [8,9], the detailed molecular mechanism of its effect remains unclear.

Network pharmacology is a new discipline based on systems biology theory, biological system network analysis, and multi target drug molecule design-specific signal node selection [10]. It has been widely used to study the molecular mechanisms underlying Chinese Medicine. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allows us to characterize the behavior of small molecules in the binding site of target proteins and elucidate fundamental biochemical processes [11].

Therefore, we combined network pharmacology and molecular docking to explore the mechanism of action of RT in TC treatment.

Materials and Methods

Collection of the potential active compounds of RT

Potential pharmacologic active compounds of RT were collected from the TCMSP database [12], this study used OB≥30% and DL≥0.18 as the screening conditions. Nine active compounds were selected and used in the follow-up network pharmacological analysis.

Putative target prediction of RT

Putative targets of the active compounds were obtained from TCMSP, PubChem, and Swiss Target Prediction databases. Cytoscape 3.9.1 software was used to visualize network diagrams of the active compounds and targets. Cytoscape is a software environment that is used for integrated models of biomolecular interaction networks.

Identification of THCA related targets

"Thyroid carcinoma" and "Thyroid cancer" and thyroid cancer were used as keywords to identify relevant RT targets from the database. The following databases were used: DrugBank, TTD, UniProtKB,GeneCards, CanProVar, ICGC, UCSC Xena, and TCGA. Total of 30711 thyroid cancer genes were identified.

Protein-Protein Interaction (PPI) analysis

The overlapping targets in the two databases of drug and disease targets were used as candidates for the mechanism of action of RT on THCA. These related RT targets were then entered into the STRING tools software [13]. The data analysis mode was set to "Multiple proteins," and the species was limited to "Homo sapiens." After screening the data, we set the confidence level to ≥0.90, hidden the isolated proteins, and exported a TSV file. The "cytoHubba" section was used to obtain the core protein based on the degree value, the "network analyzer" tool was used to analyze the network topology; Cytoscape 3.9.1 software was used to draw the PPI diagram. We also use "CytoNCA2.1.6" to get core protein for the next step molecular docking use. We set degree ≥ 22, Betweenness Centrality (BC)≥146.2701, CC(Closeness Centrality)≥0.380342, and Neighborhood Connectivity (NC)≥9.31345.

Gene Ontology (GO) and KEGG pathway enrichment analysis

Overlapping targets were imported into the Functional Annotation tool of the Database for Annotation, Visualization, and Integrated Discovery (DAVID) 6.8 [14]. The enriched P-values of the functional annotations were corrected using the Bonferroni ($P<0.05$) and Benjamini ($P<0.05$) methods [15]. The enrichment results were plotted using bioinformatics, an online platform for data analysis and visualization.

Molecular docking

Macro molecular protein preparation: To obtain the PDB ID, overlapping RT and THCA proteins were uploaded to the STRING tool and used to obtain the protein structure from the RCSB Protein Data Bank. PyMol2.5.0, modified the downloaded protein structure to remove original ligands and water molecules. The AutoDock Tools1.5.6 was used to add hydrogen and set the docking parameters. The "Grid box" was set to perform the blind docking.

Ligand preparation: The ligand 2D structures were downloaded from the PubChem database in SDF format. Avaodro2-1.97.0, and Open Babel version 3.1.1, were used to mechanically convert and optimize the 3D ligand and save it in the PDBQT format.

Molecular docking: Autodock Tools1.5.6 was used to verify the ligand-protein binding affinity and the results of the pharmacological network. The PyMol2.5.0 and Discovery Studio4.5 software were used to visualize the binding results.

Results

RT active compounds and target network results

In the TCMSP database, subject word "Mao zhua Cao" as the search term and OB≥30% and DL≥0.18 as the screening conditions to obtain nine active compounds. These identified compounds accounted for the RT components with known active effects (Figure 1).

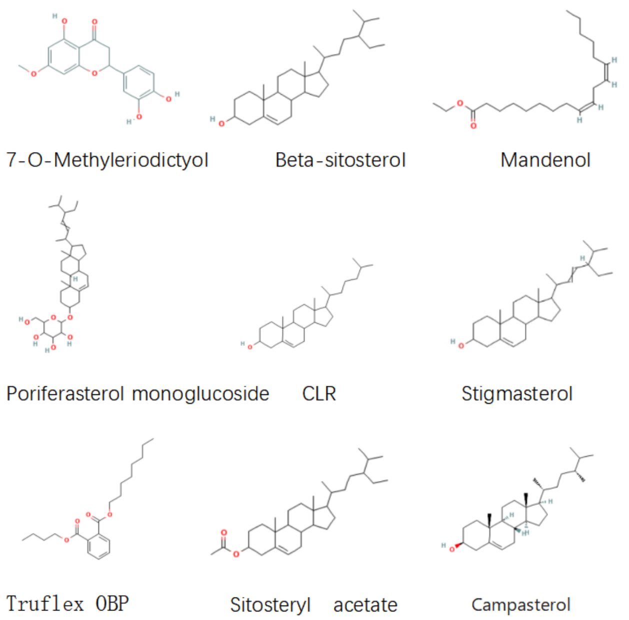


Figure 1: Structure information for the active compounds of RT.

Two hundred seventy-nine hypothetical targets were identified to be associated with these nine compounds. To further investigate the relationship between these compounds and their related targets at the system level, a compound-target network was mapped (Figure 2). Considering the information offered and the degree value from the compound-target network, the top six compounds were chosen for further analysis: Truflex OBP, beta-sitosterol, Mandenol, CLR, Stigmasterol, and 7-O-methyleriodictyol.

THCA target network results

A total of 30711 genes were recorded as THCA drug-disease related targets.

Compound-THCA targets PPI network

Based on the targets screened above, bioinformatics SRplot online tools were used to map 279 drug-related targets to 30711 THCA-related targets, resulting in 271 overlapping targets (Figure 3). The PPI protein interaction analysis was performed on 271 targets, the "cytoNCA" section was adopted to screen. The top 170 targets are illustrated (Figure 4) according to their degree values. The colors of the nodes are illustrated from red to yellow in descending order of degree values. The top 9 targets were chosen to do molecular docking, these were as followings: SRC (degree=144), EGFR (epidermal growth factor receptor) (degree=142), PPARG (degree=134), PTGS2 (The prostaglandin-endoperoxide synthase-2) (degree=128), HIF1A (degree=128), ESR1 (degree=128), MAPK1 (degree=108), MMP9 (degree=106), ERBB2 (degree=106).

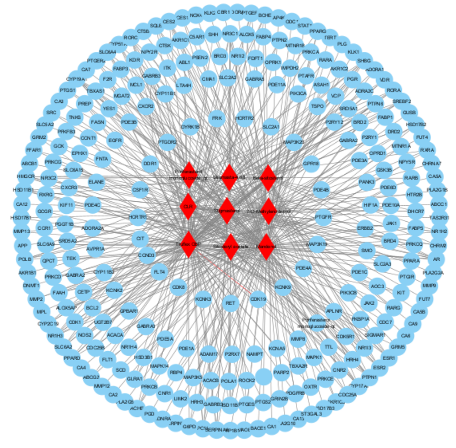


Figure 2: Compound-target network. Red diamond nodes represent active compounds of RT and blue circular nodes represent corresponding targets.

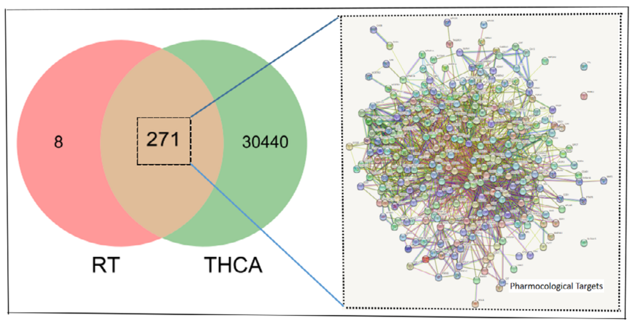


Figure 3: Venn diagram of intersecting targets of RT and THCA. The PPI network map of protein interaction was derived from STRING.

Enrichment analysis of GO and KEGG pathway

To further clarify the mechanism of RT treatment in THCA, we conducted enrichment analysis of 271 target genes using DAVID. Based on the gene counts and P- values, the top 10 KEGG pathways (Figure 5) and GO enrichments (Figure 6) were selected. For KEGG pathway enrichment analysis, the targets were primarily enriched in the following pathways:(1) extracellular signal-regulated kinase 1/2 (ERK1/2) cascade, which is a central signaling pathway that regulates a wide variety of stimulated cellular processes, including proliferation, differentiation, and survival, as well as apoptosis and stress response. (2) Chemicals Carcinogen-receptor activation, which induces and/or enhances carcinogenic processes. These receptors include cell surface receptors and some intracellular receptors, which result in biological responses, including gene transcription. The latter

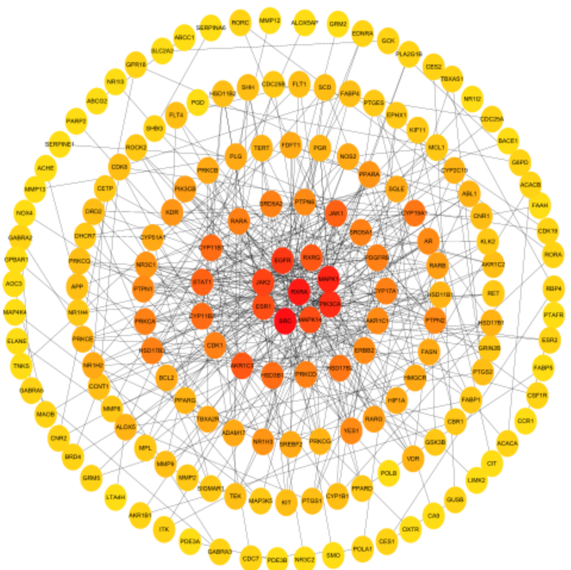


Figure 4: THCA targets PPI network. The colors of the nodes are illustrated from red to yellow in descending order of degree values.

translocates into the nucleus and acts as a transcription factor that regulates gene expression. (3) Pathways in cancer: These kernel regulatory factors contribute to the initiation and progression of cancer. (4) EGFR tyrosine kinase inhibitor Resistance to EGFR-TKIs regulates cell cycle progression and proliferation. (5) Metabolic pathways refers to nucleotide metabolism. In addition, other regulatory processes are also involved (e.g., inflammatory responses). These results demonstrate the primary mechanism of RT treatment for THCA.

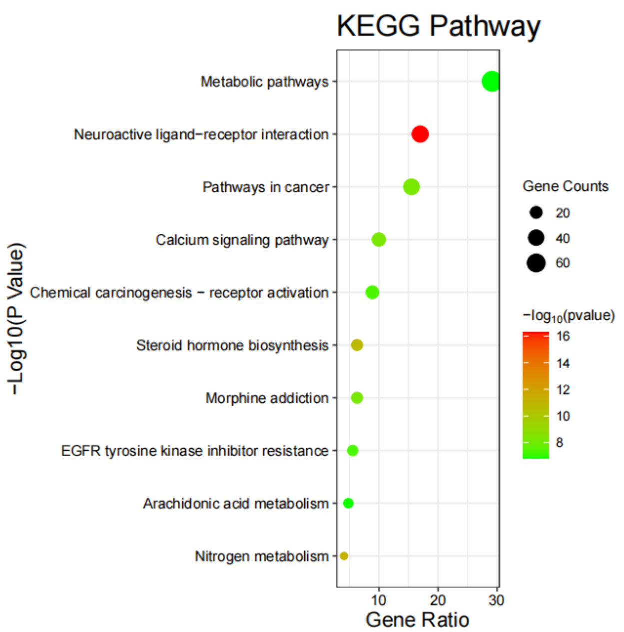


Figure 5: KEGG pathway analysis. The sizes of the bubbles are illustrated from big to small in descending order of the number of the potential targets involved in the pathways.

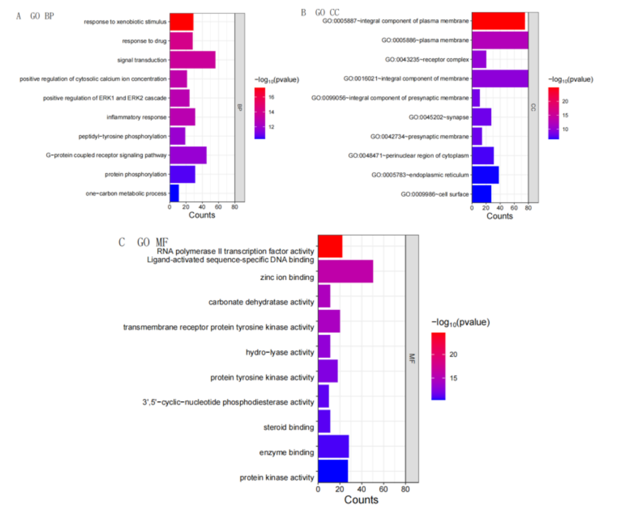


Figure 6: GO enrichment analysis. The top 10 terms of each part are shown. (A) BP: biological processes (B) CC: cell component (C) MF: molecular function.

Molecular docking analysis

Nine target genes (receptors) and their corresponding compounds (ligands) were selected for the molecular docking analysis. During the docking process, binding free energy is released due to bond formation or interaction with the protein ligand. The Binding free energy at the active site can be used to estimate binding affinity. The lower the free energy, the tighter is the binding and affinity. It is generally believed that a binding energy of <-4.25 kcal/mol indicates a certain binding activity between a small ligand and receptor proteins. The binding energy <-5.0 kcal/mol indicated that there was a good binding activity between the two. Binding energy <-7.0 kcal/mol indicates strong binding activity between the ligand and the receptor [16]. In addition to the binding energy, another evaluation index must be introduced. Some researchers believe that the number of hydrogen bonds should also be included as an evaluation index, requiring each small molecule to form two hydrogen bonds with Hinge when binding with a protein [17]. A total of 17 docking activity results were screened (Table 1). Figure 7 displays the docking patterns of the 17 complexes, including Truflex-OBP--EGFR (-7.4 kcal/mol), Truflex-OBP--ERBB2 (-8.0 kcal/mol), Truflex-OBP--PTGS2 (-9.5 kcal/mol), Truflex-OBP--ESR1 (-8.1 kcal/mol), Truflex-OBP--HIF1A (-6.6 kcal/mol), 7-O-Methyleriodictyol--ESR1 (-7.1 kcal/mol), 7-O-Methyleriodictyol--MMP9 (-7.3 kcal/mol), 7-O-Methyleriodictyol--PPARG (-5.6 kcal/mol), 7-O-Methyleriodictyol--SRC (-7.3 kcal/mol), Mandenol--MAPK1(-8.5 kcal/mol), Mandenol--SRC (-8.5 kcal/mol), Mandenol--PPARG (-7.4 kcal/mol), Mandenol--PTGS2 (-10.5 kcal/mol), CLR--ESR1 (-8.5 kcal/mol), CLR--PPARG (-7.7 kcal/mol), Stigmasterol--ESR1 (-8.5 kcal/mol), beta-sitosterol--ESR1 (-8.0 kcal/mol). In Figure 7, using Truflex-OBP--EGFR as an example, the small-molecule ligand Truflex-OBP fits into the interfaced pocket formed by EGFR in the protein (Figure 7 (b)). The results showed that five hydrogen bond formations were involved in Met769 (distance of 2.84 Å and 2.94Å)、Thr830(distance of 2.91Å)、Glu738(distance of 3.45Å、2.82Å), six alkyl hydrophobic formations were involved in Ala719、Leu820、Lys721、Val702、Gly695、Leu694.

Therefore, Truflex-OBP binds to EGFR through various interactions including hydrogen bonding and alkyl hydrophobicity. Furthermore, from table 2, all the binding energies of the 17 receptor-ligand complex <-5.0 kcal/mol indicated good binding activity between them. Except Truflex-OBP--HIF1A (-6.6 kcal/mol) and 7-O-Methyleriodictyol--PPARG (-5.6 kcal/mol), all the others <-7.0 kcal/mol indicates strong binding activity between ligand and receptor.

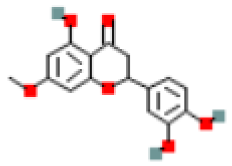
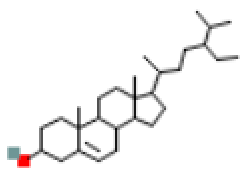
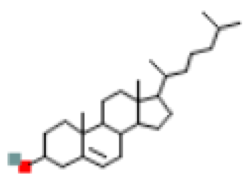
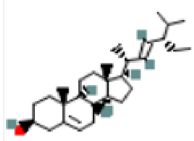
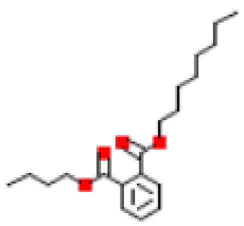
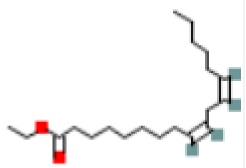
Number	Compound	Molecular Formula	Structure
1	7-O-Methyleriodictyol	C16H14O6	
2	Beta-sitosterol	C29H50O	
3	CLR	C27H46O	
4	Stigmasterol	C29H48O	
5	Truflex-OBP	C20H30O4	
6	Mandenol	C20H36O2	

Table 1: Chemical information for the active compounds of RT.

No.	Hub Jenes	PDB ID	Compound	Docking Affinity(kcal/mol)	Hydrogen bond position and length	Hydrophobic action position
1	EGFR	2gs2	Truflex-OBP	-7.4	Met769(2.84Å, 2.94Å) Thr830(2.91Å) Glu738(3.45Å, 2.82Å)	Ala719 Leu820 Lys721 Val702 Gly695 Leu694
2	ERBB2	1n8z	Truflex-OBP	-8	Ser441(2.90Å) Asn280(3.31Å) Tyr279(2.86Å)	Thr5 Thr1 Asn466 His468
3	ESR	6sbo	O-Methyleriodictyol	-7.1	NO	Leu391 Leu428 Phe425 Met421 Met343 Ile424 Leu525 Gly521 Leu384 Leu346 Ala350 Leu387 Leu349 Phe404
4	ESR	6sbo	beta-sitosterol	-8	His516(3.12Å)	His513、Ile510、Thr431、Ala430、Arg434、Leu509、Ser512 、
5	ESR	6sbo	CLR	-8.5	NO	Leu508、His513、His516、Ser512、Arg515、Leu511、Ile451、
6	ESR	6sbo	Stigmasterol	-8.5	NO	Leu508 Ser512 Arg515 Leu511 Asn455 Ile451 Leu479
7	ESR	6sbo	Truflex-OBP	-8.1	NO	Trp383 Ala350 Leu525 Leu387 Leu349 Phe404 Leu391 Leu384 Met343 Thr347

8	HIF1A	4h6j	Truflex-OBP	-6.6	Thr288(2.75Å)	Ser284 Tyr276 His291 Phe295
9	MAPK1	2y9q	Mandenol	-8.5	Thr159(3.38Å)	Thr110 Lys114 Tyr113 Thr118 Lys117 Leu115
10	MMP9	6esm	O-Methyleriodictyol	-7.3	Ala189(3.33Å) Gln227(3.08Å, 2.98Å) His236(2.96Å) His226(2.80Å)	Val223 Leu222 Leu188 Tyr248 Leu187 Tyr179 His190
11	PPARG	7awc	7-O-Methylerіodictyol	-5.6	Ser342(2.81Å)	Ile341 Val339 Leu330 Arg288 Met348 Met364 GLy284 Arg280 Gly284 Ile281 Leu255 Glu259 Ile262
12	PPARG	7awc	CLR	-7.7	NO	Arg288 Gly284 Ile281 Leu255 Gln273 Arg280 Phe287 Ser341
13	PPARG	7awc	Mandenol	-7.4	NO	Val339 Arg288 Gly284 Glu259 Gln272 Gln273 Arg280 Cys285 Ile341 Leu330
14	PTGS2	5f19	Mandenol	-10.5	NO	Arg120 Leu359 Ser353 Leu352 Ala527 Val523 Gly526 Val116 Phe381 Tyr385 Phe209 GLy533 Leu534 Val344 Tyr348 Val349 Leu531 Tyr355
15	PTGS2	5f19	Truflex-OBP	-9.5	Met522(3.20Å) Leu534(3.28Å) Gly533(3.21Å) Phe529(3.01Å)	Ser353 Ala527 Val523 Val349 Gly526 Phe381 Tyr385 Phe205
16	SRC	2src	7-O-Methyleriodictyol	-7.3	NO	Met314 Leu325 Phe405 Thr338 Val323 Leu407 Leu393 Tyr340 Ala293 Leu273 Gly274 Val281 Ala403 Ile336
17	SRC	2src	Mandenol	-8.5	NO	Gly344 Ser345 Ala403 Val281 Lys295 Val323 Thr338 Ile336 Ala293 Leu393 Asp404 Leu273 Tyr340

Table 2: Results of 9 hub genes and compounds of RT molecular docking.

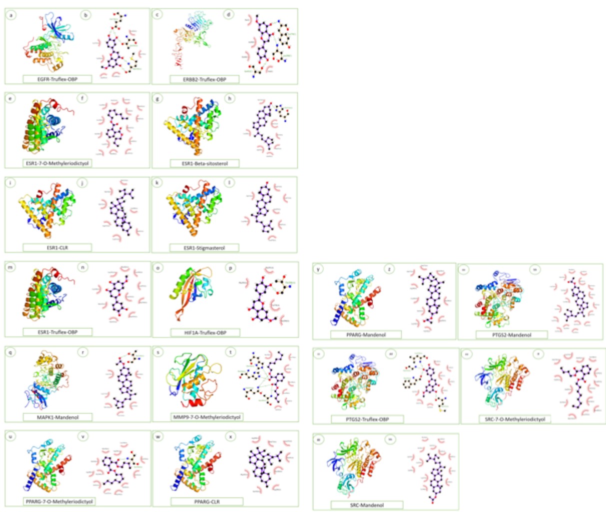


Figure 7: Molecular docking models of active compounds binding to potential targets. The top 17 pairs of molecular docking simulation are shown. Schematics (3D) represent that molecular model of the compound is in the binding pocket of the protein. The compounds are shown as stick model with yellow colored. The amino acid residues surrounding are represented by surface. Schematics (2D) show the interactions between compounds and surrounding residues. The green dashed lines represent hydrogen bonds and the interaction distances are indicated beside to the bonds. The red eyelash arc represent hydrophobic action residues.

Compound-target -pathway network

The degree value was used as the screening condition to perform enrichment analysis of the screening results of the compound-target-pathway. Ultimately, nine essential genes, SRC, EGFR, PPARG, PTGS2, HIF1A, ESR1, MAPK1, MMP9, and ERBB2, involving six compounds and five pathways, were screened (Figure 8). This indicates that RT could treat THCA by regulating the expression of the nine target genes.

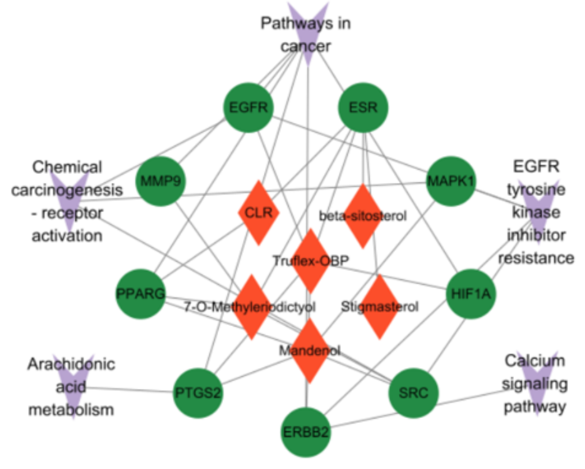


Figure 8: Compound-target-pathway network. Diamond nodes represent compounds, circle nodes represent hub genes, and 9 key targets are distinguished using green color. Arrow nodes represent pathways.

Discussion

Traditional is an effective and complementary therapy that can be used to treat THCA. RT exerts therapeutic effects on THCA; however, its underlying mechanisms remain unclear. In the present study, the network pharmacology method was adopted to elucidate the relationships between the active compounds, key targets, and signaling pathways, thereby revealing the potential therapeutic mechanisms of RT.

In this study, six active compounds, nine key targets, and five pathways were predicted for the THCA treatment. The diversity of compounds, multiple key targets, and pathways embodies the principles of comprehensive treatment [18]. RT is a Chinese herbal medicine with multi target therapeutic effects. The association between these active compounds and THCA requires further investigation. This study identified SRC, EGFR, PPARG, PTGS2, HIF1A, ESR1, MAPK1, MMP9, and ERBB2 as the nine hub protein targets related to THCA.

Both 7-O-Methyleriodictyol and Mandenol have good binding abilities for the 1st core target, SRC. The protein encoded by SRC belongs to the SRC Family of Kinases (SFKs), and SRC is a non-receptor tyrosine kinase. SRC kinase can activate the corresponding signaling pathways, including MAPK (The mitogen-activated protein kinase), PI3K/AKT, and EGFR. SRC is one of the best-studied oncoproteins and has been shown to regulate cancer hallmarks that ultimately control the behavior of transformed cells and contribute to tumor progression and metastasis [19]. Studies have shown that SRC is differentially expressed in PTC [20,21]. Targeting SRC blocked THCA tumor growth *in vivo* [22-24] and induced apoptosis by mediating the PI3K/AKT pathway [25].

Truflex-OBP has good binding ability to the 2nd core target EGFR. EGFR is a receptor tyrosine kinase that regulates a series of important events, including proliferation, migration, differentiation, apoptosis, and intercellular communication during development [26]. The primary downstream signaling pathways of EGFR are the MAPK, PI3K/Akt/PTEN/mTOR, and RAS/RAF/MEK/ERK (Extracellular signal–related kinase) pathway [27]. EGFR is overexpressed in thyroid cancer [28,29]. The EGFR family is among the most investigated receptor protein-tyrosine kinase groups, owing to its general role in signal transduction and oncogenesis, and several dozen FDA-approved small-molecule protein kinase inhibitors, such as afatinib, osimertinib, dacomitinib, avitinib, olmutinib, pelitinib, and neratinib [30-32].

CLR, Mandenol and 7-O-Methyleriodictyol all have a good binding ability to the 3rd core target PPARG, which is a protein-coding gene. It has been shown to inhibit cell proliferation, induce cell cycle termination and apoptosis in multiple cancer cells, promote intercellular adhesion, and cripple the inflamed state of the tumor microenvironment [33]. PPARG gene fusion results in the production of the PPARG fusion protein, denoted PPFP, and is found in approximately 30 - 35% of follicular thyroid carcinomas, as well as in a subset of follicular variants of papillary thyroid carcinomas [34]. A large body of evidence suggests that PPARg functions as a tumor suppressor, as activation of the PPARg/RXRa signaling pathway in different types of cancer, including bladder cancer [35-37] and thyroid cancers [38], inhibits cell growth, decreases tumor invasiveness, and reduces the production of pro-inflammatory cytokines [39].

Both Truflex-OBP and Mandenol have good binding ability to the 4th core target, PTGS2, which is one of the two isozymes of prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase, the key enzyme in prostaglandin biosynthesis, and acts both as a dioxygenase and as a peroxidase. COX-2 is a product of prostaglandin-endoperoxide synthase 2 (PTGS2) gene expression. High PTGS2 expression is associated with extra-thyroidal extension, lymph node metastasis, and higher tumor stage and is also an independent predictor of poor disease-free survival (DFS) [40-42]. COX-2 inhibitors have been suggested to inhibit the immunosuppression of PGE2 and may enhance or reverse the response to Immune Checkpoint Inhibitors (ICIs) [43]. PGE2 may be related to macrophages in the microenvironment, and COX-2 inhibition may counteract thyroid tumor cell growth [44].

Truflex-OBP has good binding ability to the 5th core target, HIF1A. HIF1A is considered to be the master transcriptional regulator of cellular and developmental response to hypoxia [45]. It has been recognized as an important cancer drug target. Studies have provided convincing evidence of a strong correlation between elevated HIF-1 levels and tumor metastasis, angiogenesis, poor patient prognosis, and tumor resistance therapy [46]. HIF1A expression is associated with desmoplastic stromal reactions and lymph node metastasis [47,48]. Moreover, HIF-1 is strongly associated with invasion, metastasis, and chemo-/radioresistance of cancer cells [49-51]. Hif-1α Inhibitors Could successfully inhibited the progression of differentiated thyroid Cancer *in vitro* [52].

Truflex-OBP, Stigmasterol, beta-sitosterol, CLR, and O-methyleriodiyol had very good binding abilities to the 6th core targets ESR1. ESR1 is a major ligand-activated transcription factor and member of the family nuclear of receptors [53]. Genes regulated by ESR1 / SP1 play a role in cell cycle regulation and proliferation, purine/pyrimidine biosynthesis and metabolism, immune responses, and regulation of lipid metabolism. Estrogen receptor-mediated pathways in thyroid cancer include the (a) PI3K/AKT/mTOR, (b) Ras/Raf/MEK/extracellular signal-regulated kinase (ERK), and (c) reactive oxygen species (ROS)-related pathways [54]. Higher ESR1 expression and ESR ratios were associated with aggressive prognostic factors and worse overall survival in female PTC patients [55]. Targeting Estrogen receptor inhibits PTC tumor growth [56].

Mandenol has a good binding ability to the 7th core target MAPK1. MAPK1 is also known as ERK, p38, p40, p41, ERK2, ERT1, NS13, ERK-2, MAPK2, PRKM1, PRKM2, P42MAPK, p41mapk, and p42-MAPK and encodes a member of the MAP kinase family. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act as integration points for multiple biochemical signals and are involved in a wide variety of cellular processes, such as cancer cell proliferation, migration, and invasion [57]. Hyperactivation of the mitogen-activated protein kinase (MAPK) pathway (RAS-RAF-MEK-ERK) occurs in approximately 60% of papillary cancers and 45% of anaplastic cancers [58]. Studies suggest that single-agent selective mitogen-activated protein kinase (MAPK) pathway inhibitors can restore the expression of the sodiumiodide symporter, rendering radioactive iodine-refractory differentiated thyroid cancer patients amenable to RAI therapy [59]. The combination of dabrafenib and trametinib was approved by the FDA for BRAF-mutated ATC, targeting different parts of the MAPK pathway [60].

7-O-Methyleriodictyol has a good binding ability to the 8th core target MMP9. MMP9 plays a key role in tumorigenesis by regulating migration, epithelial-to-mesenchymal transition, survival of cancer cells, induction of the immune response, angiogenesis, and formation of the tumor microenvironment [61]. Enhanced activation of matrix metalloproteinase-9 correlates with the degree of papillary thyroid carcinoma infiltration [62]. MMP9 is involved in cell migration and invasion ability [63]. MMP-9 knockdown inhibits cell invasion and metastasis [64]. Decreases the levels of MMP-9 blocked lymph node metastasis in PTC and angiogenesis in ATC [65].

Truflex-OBP has good binding ability to the 9th core target ERBB2. ERBB2 is commonly referred to as HER2 (Human epidermal growth factor receptor 2), and aliases include NEU, NGL, HER2, TKR1, CD340, HER-2, MLN 19, and HER-2/neu. It encodes a member of the EGF receptor family of receptor tyrosine kinases, and overexpression of this gene has been reported in numerous adenocarcinomas [66-69] as well as in advanced or metastatic PTC with poor prognosis [70]. ERBB2 inhibitors are widely used for the treatment of many malignancies [71]. Trastuzumab was the first targeted therapy approved by the FDA in September 1998 for HER2-positive breast cancer, whereas others include lapatinib, Margenza, Perjeta.

From the point of view of the ability of the ingredients, Truflex-OBP showed good receptor binding to the following five targets: EGFR, PTGS2, HIF1A, ESR1, and ERBB2, while 7-O-Methyleriodictyolto the following four targets: ESR1, EMMP9, PPARG and SRC; and Mandenol to the following four targets: SRC, PPARG, PTGS2, and MAPK1. Therefore, Truflex-OBP, 7-O-Methyleriodictyol and Mandenol may be the main ingredients of RT for anti-THCA.

Conclusion

In summary, based on network pharmacology and molecular docking, we predicted nine critical targets from complex network analysis and provided a comprehensive explanation of the therapeutic mechanism of RT for THCA, which may be related to proliferation, apoptosis, and immunity. In addition, the SRC, EGFR, PPARG, PTGS2, HIF1A, ESR1, MAPK1, MMP9, and ERBB2 signaling pathways may be critical for THCA treatment. Truflex-OBP, 7-O-Methyleriodictyol and Mandenol may be the main active ingredients of RT. Our study provides new insights into the treatment of THCA. Further in vivo and in vitro experimental verifications should be conducted in the future.

Disclosure

The author reports no conflicts of interest in this work.

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