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Network Pharmacology and Molecular Docking Identifies the Mechanisms of Xiaoying Daotan Decoction in the Treatment of Hashimoto's Thyroiditis

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Abstract

Background: Hashimoto's thyroiditis (HT) is considered as one of the most common clinical expressions of autoimmune thyroid disease (AITD). Xiaoying Daotan Decoction (XYDTD) is the main traditional Chinese medicine (TCM) formula used for the treatment of HT, but its mechanism has not been fully elucidated. To predict the potential mechanism of XYDTD for HT, this study conducted network pharmacology analysis and also verified it by molecular docking.

Methods: The potential therapeutic targets were got by taking the intersection between the action targets of XYDTD from TCMSP database and the disease targets of HT from GeneCards database. Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted via RStudio's ggplot2 software package, while herb-ingredient-therapeutic target network and protein-protein interactions (PPI) network were contrasted via Cytoscape 3.8. Molecular docking experiments were carried out by using AutoDock Vina 1.1.2.

Results: The study identified 72 potential therapeutic targets after analysis. It showed that XYDTD might exert its effect on HT mainly by inhibiting immune inflammation through AGE-RAGE signaling pathway, TNF signaling pathway and IL-16 signaling pathway, and pro-inflammation cytokines like IL-6, IL-1β, TNF-α and AKT1 are the key genes, which were verified by the result of molecular docking.

Conclusion: The network pharmacology analysis executed in this study provides convincing evidence and helps to promote the understanding of the underlying mechanisms of XYDTD for HT.

Keywords: Hashimoto's thyroiditis; Xiaoying Daotan Decoction; network pharmacology

Introduction

Hashimoto's thyroiditis (HT) is a chronic inflammation of the thyroid gland and now considered the most common autoimmune thyroid disease[1]. It results from a dysregulation of the immune system which leads to an immune attack on the thyroid, of which the mechanisms still remain unclear[2]. In early phase of the disease, the most common manifestation is goiter with or without hypothyroidism, which is the clinical hallmark of HT in the later stages. The mean respectively 3.5-5/1000 in women and 0.6-1/1000 incidence and the prevalence of spontaneous hypothyroidism were in men [3]. The current treatment of HT is mainly symptomatic treatment and based on the administration of synthetic thyroid hormones to correct the hypothyroidism as needed [4]. However, there are still certain unresolved problems of HT with current western symptomatic treatment. Traditional Chinese medicine (TCM) in the treatment of HT has promising efficacy with evidence to support. Successful clinical applications of Chinese herbal have been reported for the treatment and remission of HT. It has shown that a certain combination of compounds can shrink and soften the enlarged thyroid gland [5]. Unsatisfactorily, the potential active compounds, underlying pharmacology mechanisms and definite targets of

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TCM are still not fully revealed, thus hindering the modernization of TCM. Network pharmacology, a novel approach to discover TCM on the molecular level, provides a promising research paradigm for the transformation of TCM from experience-based medicine to evidence-based medicine[6]. In this work, network pharmacology was used to uncover the mystery and complicated mechanisms of TCM.

Xiaoying Daotan decoction (XYDTD), a traditional Chinese medicine prescription for the treatment of HT, takes Xiakucao, Tubeimu and Chaihu as Jun (monarch); Muli, Chuanshanlong, Lujiaoshuang, Yujin, Zhixiangfu as Chen (minister); Hangshao, Chuanxiong as Zuo (adjuvant) and Gancao as Shi (messenger). The whole prescription has the effect of soothing the liver and regulating qi, resolving phlegm and dispersing knots. And it is proved from the network pharmacology on molecular level that the four essential components of this prescription are Tubeimu (Bolbostemma paniculatum), Xiakucao (Prunella vulgaris), Chaihu (Radix Bupleuri) and Yujin (Curcumae Radix). These four essential components make a major contribution to the whole therapeutic effect respectively: Tubeimu has been proved to possess significant anti-inflammatory effects[7]; Xiakucao has been shown to have therapeutic value for HT such as alleviating thyroid goiter [8]; Chaihu helps to soothe the liver and promote yang and qi [9]; Yujin helps to soothe the liver to regulate qi by promoting blood circulation and removing blood stasis [10]. It could be seen that this work will promote our understanding of the underlying mechanisms of XYDTD and provide more convincing evidence for clinical application of XYDTD in HT (Figure 1 depicts a flowchart of the entire research procedure).

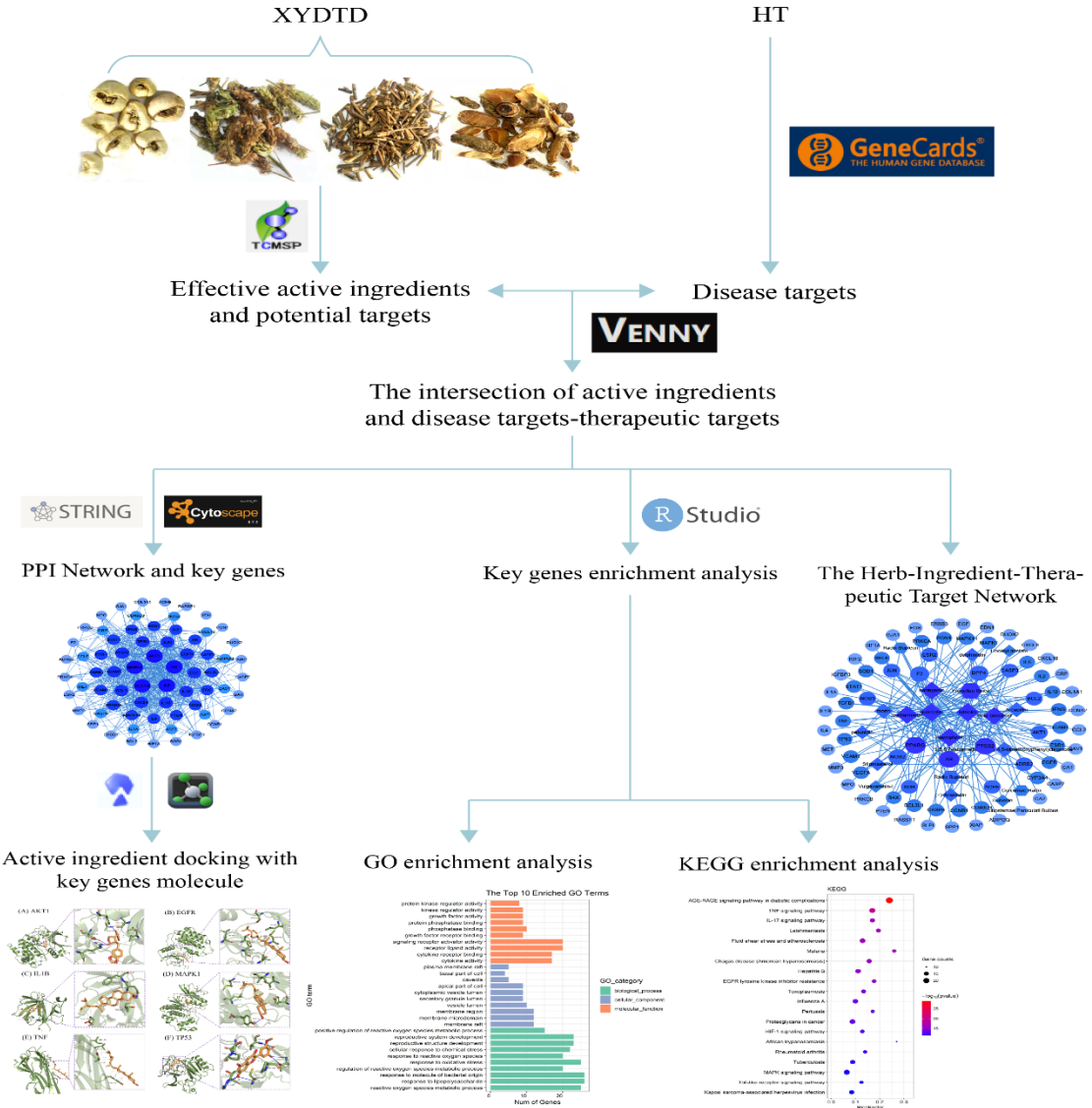

Figure 1. **A schematic diagram of the network pharmacology-based strategies for determining the pharmacological mechanisms of the herbal formula XYDTD on HT**

1. Methods

1.1. Screening of active ingredients and target prediction of XYDTD from TCMSP

According to the standards of OB≥30% and DL≥0.18, the active ingredients of "Bolbostemae Paniculati Bulbus", "Prunellae Spica", "Radix Bupleuri" and "Curcumae Radix" were retrieved in the TCMSP database (https://tcmspw.com/tcmsp.php). The targets in the above active ingredients were collected and collated in the TCMSP database, and verified with the help of Uniprot database (https://www.uniprot.org). Finally, the active ingredient-target database was established.

1.2. Screening of disease targets

Using the Genecard database(https://www.genecards.org) to search and screen the disease genes, with the retrieval word as “Hashimoto's thyroiditis”. Establishing the disease targets database.

1.3. Collating common herb-disease targets as therapeutic targets

The target data of the above two databases was imported to the Venny website (https://bioinfogp.cnb.csic.es/tools/venny/), and the intersection of the two databases was taken. The intersecting targets were the therapeutic targets.

1.4. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway functional Enrichment Analysis

GO analysis and KEGG Pathway Analysis were performed using the RStudio’s clusterProfiler package with the therapeutic targets. According to the results of Gene Ontology analysis, the top ten enrichment maps of BP (Biological Process), MF (Molecular Function) and CC (Cellular Component) were made by the RStudio’s ggplot2 software package; the top twenty pathways enrichment terms was also made by ggplot2 software package.

1.5. Herb-ingredient-therapeutic target Network Construction

Searching the corresponding key ingredients and herbs by the therapeutic targets in the ingredient-target database, and using Cytoscape 3.8 to construct and visualize the herb-ingredient-therapeutic target network. After calculating the node topology parameter, take the mean value of closeness and betweenness and twice of the average degree as the standard to get the key genes which are playing an important role in the network of herb-ingredient-therapeutic target, and may be the main action protein of formula.

1.6. PPI Network Construction and Screening of key genes

The therapeutic targets were imported to the String database (https://string-db.org) for Protein-Protein Interactions, with a setting of which the high confidence is equal to 0.700. Using Cytoscape 3.8 to construct PPI network and calculate the node topology parameter. Take the mean value of closeness and betweenness and twice of the average degree as the standard to get the key genes which are important in biological networks.

1.7. Molecular docking

Molecular docking experiments were carried out on the key targets (targets corresponding to key genes that are important in biological networks) of XYDTD and their key active ingredients using AutoDock Vina 1.1.2[11] to verify their interaction activity. Autodock Vina adopts semi-flexible molecular docking, that is to say, in the docking process, the effective molecules are flexible while the proteins remain rigid. The results were evaluated by semi empirical free energy function. The specific steps are as follows:

1) Download the structure file of active ingredients on TCMSP database ("mol2" format). Add hydrogen atom and select mmff94 force field to charge and minimize energy for the molecular structure using Open Babel. Finally, AutoDock tools was used to convert the ingredients into “pdbqt” format files.
2) Download the crystal structure of the key target protein on the PDB database (http://www.rcsb.org/). The selection of key target protein meets the following three conditions: ①Human protein; ②the protein structure contains eutectic ligands, and the eutectic ligands have biological activity; ③choose the crystal structure with smaller resolution value. For proteins that do not have eutectic ligand structure in PDB database and cannot obtain specific active sites by searching literatures, molecular docking experiments are not carried out.
3) Autodock tools is used to separate the target protein and its ligand, add hydrogen atom, calculate the charge, and then export it to pdbqt format file. Then Autodock tools is used to determine the size and center of docking box.
4) At last, the active ingredients and the target protein were butted by Vina in turn, and the

affinity was extracted and the results were analyzed and plotted by PyMOL 2.4.0.

2. Result

2.1. Active ingredients and targets in formula

After eliminating ingredients that not meet the OB, DL requirements and without targets, 25 eligible active ingredients were screened. Four of them belong to Bolbostemae Paniculati Bulbus, eleven belong to Prunellae Spica, thirteen belong to Radix Bupleuri, three belong to Curcumae Radix. The specific active ingredients are shown in the table.1 below. Also, in the TCMSP database, we found 240 targets that related to the active ingredients.

Table 1. **Active Ingredients of XYDTD**

ID	Ingredient	OB/%	DL	Herb
MOL010316	Δ7,16,25,26-stigmastatrienol	46.21	0.76	Bolbostemae Paniculati Bulbus
MOL010318	Δ7,22,25-triene-3-ol	46.67	0.76	Bolbostemae Paniculati Bulbus
MOL000358	beta-sitosterol	36.91	0.75	Bolbostemae Paniculati Bulbus Prunellae Spica Curcumae Radix
MOL000359	sitosterol	36.91	0.75	Bolbostemae Paniculati Bulbus Curcumae Radix
MOL000422	kaempferol	41.88	0.24	Prunellae Spica Radix Bupleuri
MOL004355	Spinasterol	42.98	0.76	Prunellae Spica
MOL000449	Stigmasterol	43.83	0.76	Prunellae Spica Radix Bupleuri
MOL004798	delphinidin	40.63	0.28	Prunellae Spica
MOL000006	luteolin	36.16	0.25	Prunellae Spica
MOL006767	Vulgaxanthin-I	56.14	0.26	Prunellae Spica
MOL006772	poriferasterol monoglucoside_qt	43.83	0.76	Prunellae Spica
MOL006774	stigmast-7-enol	37.42	0.75	Prunellae Spica
MOL000737	morin	46.23	0.27	Prunellae Spica
MOL000098	quercetin	46.43	0.28	Prunellae Spica Radix Bupleuri
MOL001645	Linoleyl acetate	42.1	0.2	Radix Bupleuri
MOL002776	Baicalin	40.12	0.75	Radix Bupleuri
MOL000354	isorhamnetin	49.6	0.31	Radix Bupleuri
MOL004598	3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl) chromone	31.97	0.59	Radix Bupleuri
MOL004609	Areapillin	48.96	0.41	Radix Bupleuri
MOL013187	Cubebin	57.13	0.64	Radix Bupleuri
MOL004624	Longikaurin A	47.72	0.53	Radix Bupleuri
MOL004653	(+)-Anomalin	46.06	0.66	Radix Bupleuri
MOL004718	α-spinasterol	42.98	0.76	Radix Bupleuri
MOL000490	petunidin	30.05	0.31	Radix Bupleuri
MOL004328	naringenin	59.29	0.21	Curcumae Radix

2.2. Screening of disease targets and collating common herb-disease targets as therapeutic targets

After searching in the Genecards database and correcting the retrieval results into corresponding targets by Uniprot database, we got 692 disease targets. The target data of disease and formula was imported to the Venny website, and 72 common herb-disease targets (therapeutic targets) were found. The therapeutic targets are shown in the

table.2 below.

Table 2. **Therapeutic Targets of XYDTD for HT**

Number	Gene name	Uniprot ID	Number	Gene name	Uniprot ID	Number	Gene name	Uniprot ID
1	PTGS2	P35354	25	VCAM1	P19320	49	CAV1	Q03135
2	ADRB2	P07550	26	SLPI	P03973	50	GJA1	P17302
3	BCL2	P10415	27	CA2	P00918	51	IL1B	P01584
4	BAX	Q07812	28	EGFR	P00533	52	CCL2	P13500
5	JUN	P05412	29	VEGFA	P15692	53	CXCL8	P10145
6	CASP3	P42574	30	BCL2L1	Q07817	54	PRKCB	P05771
7	CASP8	Q14790	31	MAPK1	P28482	55	DUOX2	Q9NRD8
8	PRKCA	P17252	32	IL10	P22301	56	COL1A1	P02452
9	TGFB1	P01137	33	IL6	P05231	57	PTEN	P60484
10	PON1	P27169	34	TP53	P04637	58	IL1A	P01583
11	NOS2	P35228	35	CASP7	P55210	59	MPO	P05164
12	AR	P10275	36	IL2	P60568	60	CRP	P02741
13	PPARG	P37231	37	CCNB1	P14635	61	CXCL10	P02778
14	DPP4	P27487	38	IFNG	P01579	62	SPP1	P10451
15	F2	P00734	39	IL4	P05112	63	RASSF1	Q9NS23
16	NOS3	P29474	40	XIAP	P98170	64	IGFBP3	P17936
17	ACHE	P22303	41	CD40LG	P29965	65	IGF2	P01344
18	AKT1	P31749	42	MET	P08581	66	ERBB3	P21860
19	TNF	P01375	43	EDN1	P05305	67	ESR1	P03372
20	XDH	P47989	44	MMP3	P08254	68	ESR2	Q92731
21	STAT1	P42224	45	FOS	P01100	69	MAPK14	Q16539
22	CYP3A4	P08684	46	EGF	P01133	70	CCNA2	P20248
23	ICAM1	P05362	47	SOD1	P00441	71	CAT	P04040
24	SELE	P16581	48	HIF1A	Q16665	72	ADIPOQ	Q15848

2.3. Gene ontology and pathway functional enrichment analysis of therapeutic Targets

By clusterProfiler package, there were 1741 terms in BP, 23 in CC, 68 in MF, and 156 pathways. We chose the top 10 enriched GO terms including BP, CC, MF (Figure 2) and top 20 pathways for enrichment terms (Figure 3) below. It can be seen from the figure that AGE-RAGE signaling pathway and TNF signaling pathway are enriched with more genes and have lower *P* values, indicating that AGE-RAGE signaling pathway and TNF signaling pathway may be the main action pathways of XYDTD in treating HT. Detailed enrichment maps of these two pathways are shown in figure 4. The red nodes are the genes enriched to these two important pathways.

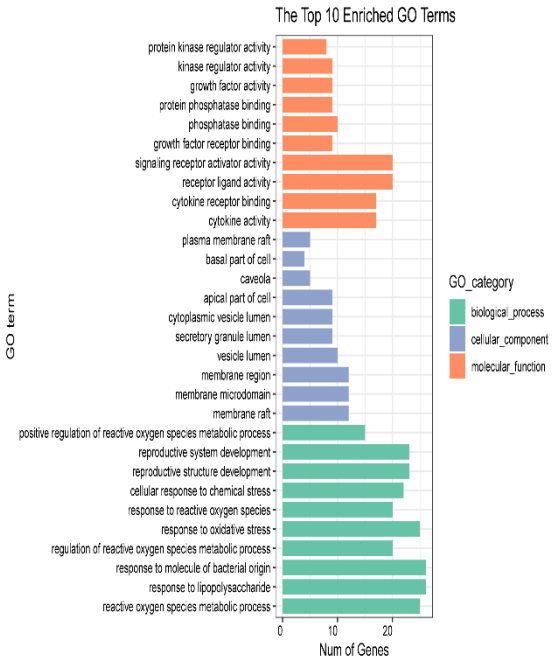

Figure 2. **The Top 10 Enriched GO Terms**

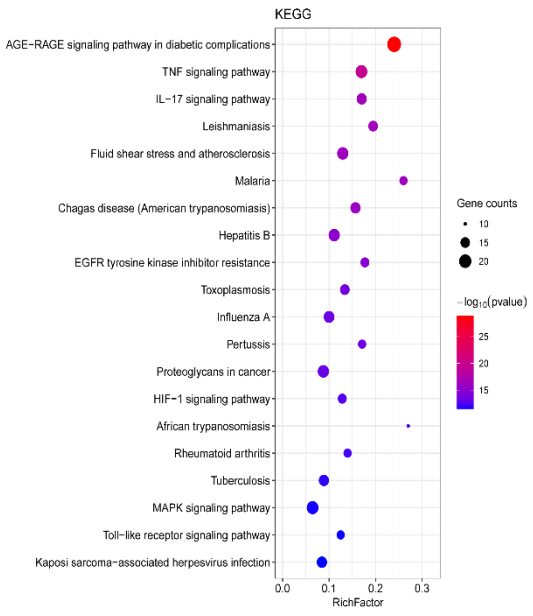

Figure 3. **The Top 20 Enriched Pathway Terms**

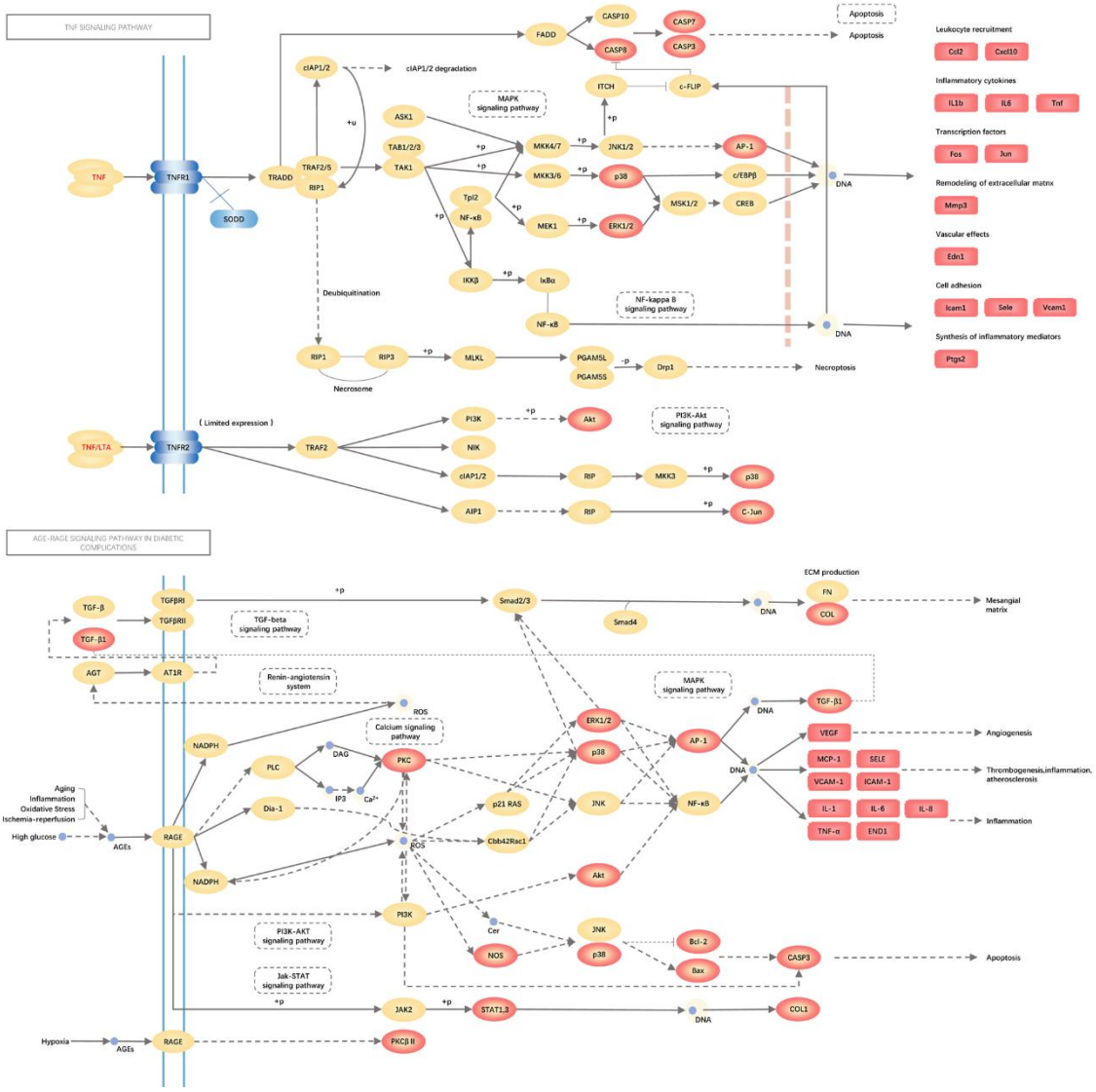

Figure 4. **The Enrichment Map of AGE-RAGE Signaling Pathway and TNF Signaling Pathway**

2.4. Constructing the herb-ingredient-therapeutic target network and screening of key genes

Importing the data of therapeutic targets and their corresponding ingredients and herbs into Cytoscape, constructing the herb-ingredient-therapeutic target network (Figure

5). The hexagon represents herbs, the diamond represents ingredient, and the circle represents therapeutic targets in the figure. The color from light to dark indicates that the degree of the node increases gradually.

Take the mean value of closeness centrality and betweenness centrality and twice of the average degree as the standard to get the key genes, and get 15 genes. These genes may be playing an important role in the network of herb-ingredient-therapeutic target, and may be the main action proteins of the formula (Table.3).

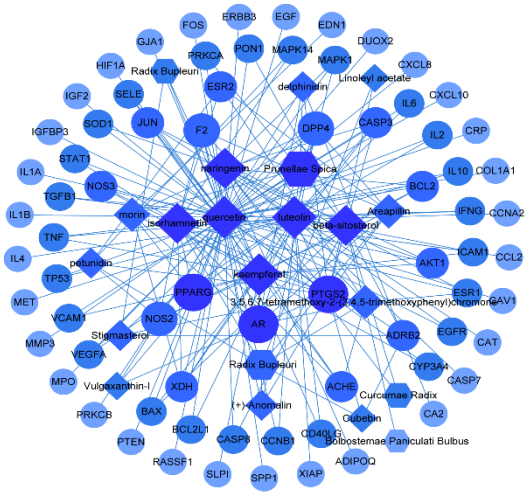

Figure 5. **The Herb-Ingredient-Therapeutic Target Network**

Table 3. **Key Genes of the Herb-Ingredient-Therapeutic Target Network**

Gene name	Betweenness Centrality	Closeness Centrality	Degree
PTGS2	0.13600567	0.54819277	16
AR	0.03751592	0.47894737	8
PPARG	0.02675848	0.47894737	8
DPP4	0.02536354	0.46907216	7
CASP3	0.01775394	0.45959596	5
F2	0.01620465	0.44174757	6
XDH	0.01564755	0.45959596	5
AKT1	0.01203646	0.45049505	4
BCL2	0.01132555	0.4375	4
ADRB2	0.00937248	0.41743119	4
ACHE	0.00934228	0.43333333	4
MAPK1	0.00893264	0.4375	3
JUN	0.00837014	0.44174757	4
NOS2	0.00719595	0.32269504	6
NOS3	0.00575326	0.42924528	4

2.5. PPI Network Construction and Screening of key genes

After the data of therapeutic genes have been processed with the database, we used Cytoscape to construct PPI network (Figure 6. The larger the node is, the darker the color is, the greater its degree value is, indicating that node genes play a more important role in biological networks.) and calculate the node topology parameter for key genes that may be important in biological networks, with the standard mentioned earlier. The key genes are shown in the table.4 below.

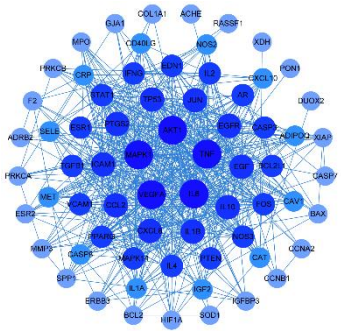

Figure 6. **The PPI Network**

Table 4. **Key Genes of the PPI Network**

Gene	Betweenness Centrality	Closeness Centrality	Degree
AKT1	0.120314496	0.744444444	44
IL6	0.113327505	0.744444444	44
TNF	0.035704988	0.683673469	39
VEGFA	0.051002467	0.670000000	37
MAPK1	0.057855355	0.683673469	36
EGFR	0.050147544	0.656862745	35
TP53	0.057596376	0.650485437	34
JUN	0.026005055	0.650485437	34
IL1B	0.028298137	0.644230769	34

2.6. Molecular docking

Autodock Vina's evaluation of the binding of small molecules to proteins is mainly expressed by the binding energy (affinity), that is, the value of ΔG obtained by fitting after calculation. If the binding energy is less than 0, it means that the ligand can spontaneously bind to the receptor, and the smaller the value is, the higher the binding energy is, and the easier the active ingredient is to bind to the receptor. Topological analysis of protein interaction network was carried out, with the results of twice of the average degree, the average value of betweenness centrality and closeness centrality, that 9 core genes were obtained under the condition, and 6 proteins suitable for molecular docking were obtained after screening.

Therefore, all 16 key ingredients were butted with the protein crystals of the above 6 genes one by one, and then they were sorted from small to large after the docking score was extracted. The top

five ingredients with strong binding force to each protein are shown in Table 5. Select the ingredient with the lowest binding energy corresponding to each target protein, analyze its key residues and interactions, and make a binding ideograph, as shown in Figure 7 (the blue solid line in the diagram indicates hydrogen bond, and the gray dotted line indicates hydrophobic effect). It can be seen from the figure that the ingredient enters the active site of the target protein, and the analysis of the binding mode shows that the active ingredient mainly includes hydrogen bond and hydrophobic Interactions. MOL013187 and AKT1's (Figure 7(A)) active site residues form three hydrogen bonds, MOL000358 and MAPK1 (Figure 7(D)) form two hydrogen bonds, MOL000490 and TP53 (Figure 7(F)) form six hydrogen bonds. In addition, the above ingredients form hydrophobic interactions with the target protein active site residues. However, there is no hydrogen bond between MOL000358 and EGFR (Figure 7(B)), MOL000449 and IL1B (Figure 7(C)), MOLl000358 and TNF (Figure 7(E)), which is mainly combined with hydrophobic action. (MOL013187: cubebin; MOL000358: beta-sitosterol; MOL000490: petunidin; MOL000449: Stigmasterol)

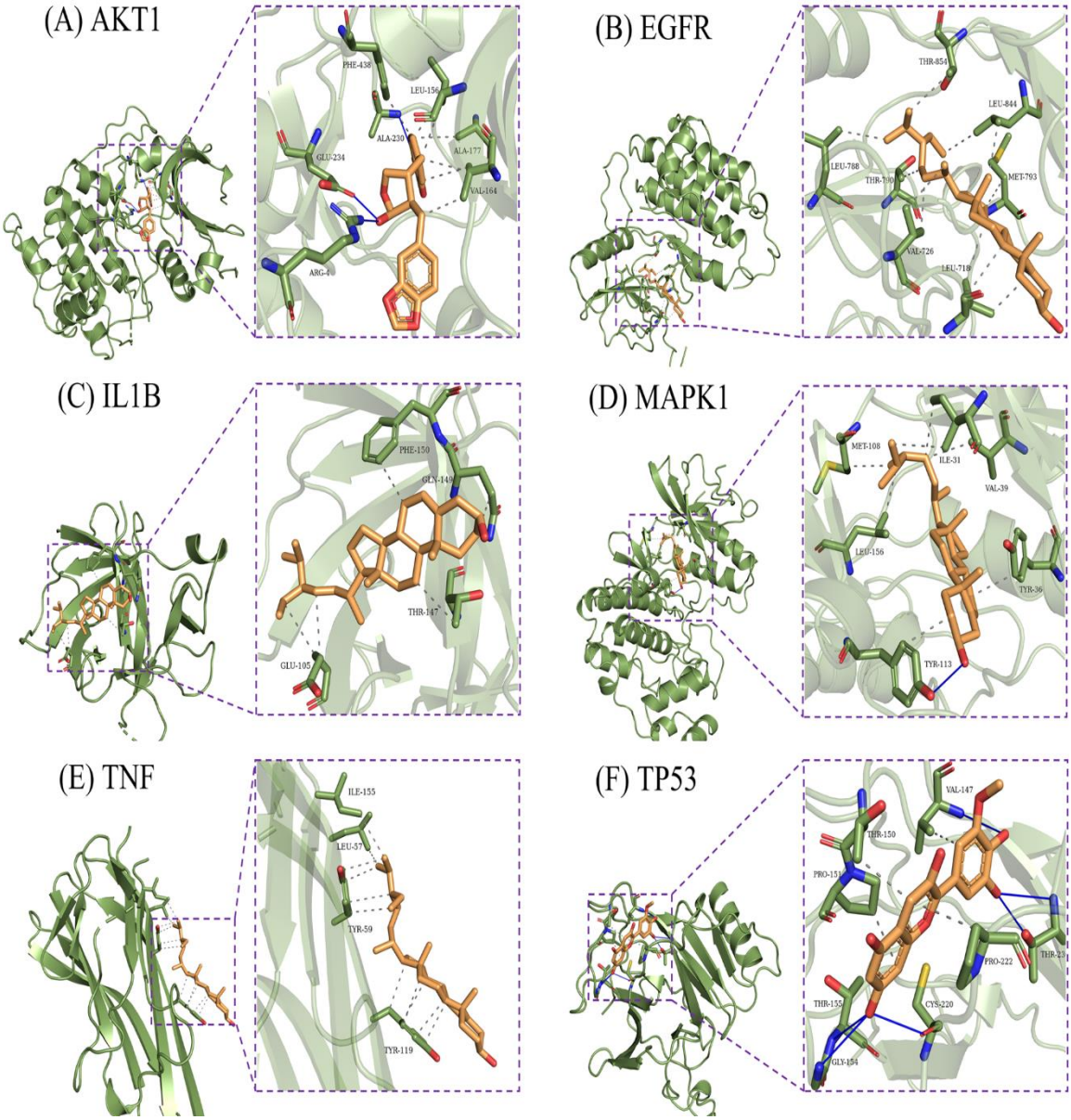

Figure. 7 **Docking result of the active ingredients on Key genes((A) The docking results of MOL013187 and AKT1, (D) The docking results of MOL000358 and MAPK1, (F) The docking results of MOL000490 and TP53, (B) The docking results of MOL000358 and EGFR, (C) The docking results of MOL000449 and IL1B, (E) The docking results of MOLl000358 and TNF)**

Table 5. **Docking Result**

Gene	PDB ID	ingredient	Affinity(kcal/mol)
AKT1	3MV5	MOL013187	-8.4
		MOL000737	-7.6
		MOL000354	-7.4
		MOL000490	-7.4
		MOL000006	-7.3
		XFE※	-6.9
EGFR	2ITY	MOL000358	-8.9
		MOL000449	-8.9
		MOL004653	-8.2
		MOL004798	-7.9
		MOL000490	-7.8
		IRE※	-8.7
IL1B	6Y8M	MOL000449	-5.9
		MOL000358	-5.5
		MOL000006	-5.3
		MOL000737	-5.3
		MOL000098	-5.2
		SX2※	-4
MAPK1	5NHV	MOL000358	-9.1
		MOL004653	-8.9
		MOL013187	-8.9
		MOL000006	-8.4
		MOL000098	-8.2
		8QB※	-7.9
TNF	2AZ5	MOL000358	-6.4
		MOL000449	-6.4
		MOL004653	-6.2
		MOL000006	-5.8
		MOL013187	-5.7
		307※	-6.8
TP53	6GGC	MOL000490	-7.8
		MOL000098	-7.7
		MOL000006	-7.6
		MOL000422	-7.6
		MOL000737	-7.6
		EXN※	-8.7

※Eutectic ligands of the protein structure

3. Discussion

In the theory of traditional Chinese medicine, HT usually refers to a disease called "Ying", which majorly caused by Qi stagnation and phlegm coagulation, while “Xiaoying Daotan” means to removing phlegm and dissipating “Ying”. Although XYDTD has been frequently used for the treatment of HT in China for a long time, the mechanism of it still remains unclear due to its multiple components and multiple targets. Using network pharmacology, we hope to break the limitation of traditional single pharmacology study and elucidate the potential mechanism of XYDTD against HT.

Through the top 10 enriched GO terms we got via GO enrichment, it is revealed that the therapeutic targets of XYDTD are mainly related to the response to lipopolysaccharide, the response to oxidative stress, the regulation of reactive oxygen species metabolic process and the response to molecule of bacterial origin, indicating that XYDTD may have an inhibitory effect on immune inflammation and bacterial infections. This is consistent with previous research on the pathogenesis of HT[12].

The result of KEGG signal pathway enrichment suggested that XYDTD has an effect on multiple related signal pathways, among which AGE-RAGE signaling pathway, TNF signaling pathway, IL-17 signaling pathway, MAPK signaling pathway, Toll−like receptor signaling pathway seem to play an important role. Studies have shown that AGE-RAGE signaling pathway could cause oxidative stress and activate nuclear factor-κB (NF-κB), a key mediator of inflammation. After being activated, NF-κB enters the nucleus and promotes specific sequences binding with several genes, which could cause the expression and release of a large number of adhesion molecules, growth factors, and proinflammatory cytokines (i.e., IL-6, IL-8, TNF-α), and eventually cause tissue damage[13-15]. It could be predicted that XYDYD might be able to block AGE-RAGE pathway, inhibit the activation of NF-κB and the release of inflammatory mediators it caused, thereby avoiding cell damage and dysfunction. TNF signaling pathway is also closely related to inflammatory immune response. It can initiate NF-κB signaling pathway and MAPK pathway, resulting to the release of proinflammatory cytokines[16]. It could be found that the pathways can play a synergistic role in the process of XYDTD for HT, mainly working on the inflammation immune response.

By constructing the herb-ingredient-therapeutic target network, it’s identified that XYDTD is composed of 16 compounds and 72 genes regulating the main signaling pathways of HT. We were able to get 15 key genes and they may be the main action proteins of XYDTD, namely prostaglandin-endoperoxide-synthase-2 (PTGS2), peroxisome proliferator-activated receptor gamma (PPARG), DPP4, BCL2, etc. It’s known that PTGS2 is

an inflammatory factor, which mainly causes inflammation and oxidative stress injury as an agonist of T lymphocytes activation. By inhibiting the expression of PTGS2, it works to diminish pro-inflammatory factors (i.e., IL-1β, IL-6, and TNF-α), thereby regulating macrophage recruitment for improving the immune microenvironment and playing an anti-apoptosis effect[17-19]. Similarly,

PPARG participates in inflammation. Once activated by a ligand, it binds to PPAR response elements (PPRE) and modulates the transcription of its target genes, so thus participating in various physiological and pathological processes, including adipocyte differentiation, inflammatory response, apoptosis, obesity, arteries Atherosclerosis and cancer[20,21]. It could act as a critical regulator by suppressing NF-κB-mediated proinflammatory responses.

The PPI network analysis based on the targets of XYDTD on HT illustrated the interaction relationship between targets. The key genes of them are AKT1, IL6, TNF, VEGFA, MAPK1, EGFR, TP53, JUN and IL1B, indicating an important position in the biological network. Genes like AKT1, VEGFA, MAPK1, EGFR and TP53 are cell cycle regulatory proteins, participating in the regulation of cell growth, proliferation, differentiation and apoptosis, while genes like IL6, TNF, IL1B are pro-inflammatory cytokines. IL-1β and TNF-α are known to affect thyroid function by stimulating IL-6 secretion and modifying epithelium integrity through alteration of junction proteins expression[22-24]. Moreover, it's demonstrated in vitro studies that IL-1β can directly modulate junction protein, thereby mediating thyroid follicular cells destruction in HT[25]. IL-6 and TNF-α are believed to promote the production of IL-22. It's also reported that after stimulating by IL-6 in vitro, the peripheral T lymphocytes from the patients of HT show enhanced differentiation into Th22 cells, a subpopulation of T helper cells that produce IL-22. Studies showed that IL-22 plays a specific part in HT, as it has stronger expression in the thyroid gland of HT patients and its levels correlate with TPO antibody levels in patients with HT[26,27]. Moreover, studies showed both IL-6 and TNF-α can regulate type 2 iodothyronine 50-deiodinase of anterior pituitary and affect TSH releasing, causing nonthyroidal illness syndrome[28,29]. These evidences all indicate the critical role for these cytokines in antibody production and the process of HT. It also could be found that the result of PPI network analysis can correspond to the contents of enriched GO terms.

To verify the interaction between the compounds and proteins, we conducted the molecular docking experiment between the 16 core compounds from the herb-ingredient-therapeutic target network and the 6 core genes from PPI network. The results showed they have relatively strong binding ability, indicating the credibility of the above analysis.

4. Conclusion

In this study, network pharmacology was applied to reveal the potential mechanism of XYDTD in the treatment of HT. The result indicated that XYDTD exerts its pharmacological effect against HT mainly by 16 core compounds (i.e., beta-sitosterol, kaempferol, stigmasterol, delphinidin, luteolin) and 9 key target genes (i.e., IL-6, TNF, IL-1β, AKT1, EGFR, TP53, MAPK1), which might be through pathways like AGE-RAGE pathway, TNF pathway, IL-16 pathway. These comprehensive analyses can provide a theoretical basis for further study. It's worth noting that the ingredients of herbs may change during the process of boiling in practice, and the study lacks of experimental verification, all requiring further study.

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References

[1] R L, R G, C T, PER PPJPer. Hashimoto's Thyroiditis. 2003:205-11; discussion 11.
[2] A A, SM F, A C, A DD, reviews FPJA. Autoimmune thyroid disorders. 2015; 14:174-80.
[3] F R, P F, G E, et al. Hashimotos' thyroiditis: Epidemiology, pathogenesis, clinic and therapy. 2019; 33:101367.
[4] P C, A DR, reviews RNJA. Hashimoto thyroiditis: clinical and diagnostic criteria. 2014; 13:391-7.
[5] BJ F, LH S, XQ Z, medicine QYJZxyjhxbJoCi. [Effects of Ruanjian Xiaoying Decoction on chronic lymphocytic thyroiditis]. 2006; 4:355-7.
[6] S L, medicines ZBJCjon. Traditional Chinese medicine network pharmacology: theory, methodology and application. 2013; 11:110-20.
[7] Y B, H L, QY L, et al. Therapeutic effects of Smilax glabra and Bolbostemma paniculatum on rheumatoid arthritis using a rat paw edema model. 2018; 108:309-15.
[8] H Q, J Z, Q G, et al. Prunella vulgaris L. attenuates experimental autoimmune thyroiditis by inducing indoleamine 2,3-dioxygenase 1 expression and regulatory T cell expansion. 2020; 128:110288.
[9] F Y, X D, X Y, W W, L Y, international NJJBr. Radix Bupleuri: A Review of Traditional Uses, Botany, Phytochemistry, Pharmacology, and Toxicology. 2017; 2017:7597596.
[10] Z C, L H, Y L, et al. Different Processed Products of Curcumae Radix Regulate Pain-Related

Substances in a Rat Model of Qi Stagnation and Blood Stasis. 2020; 11:242.

[11] O T, chemistry OAJJoc. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. 2010; 31:455-61.

[12] RA A, Hormone WAJ, metabolisme mrH-uSHe. The Pathogenesis of Hashimoto's Thyroiditis: Further Developments in our Understanding. 2015; 47:702-10.

[13] F L, Y F, C W, et al. The expression of GPR109A, NF-kB and IL-1β in peripheral blood leukocytes from patients with type 2 diabetes. 2014; 44:443-8.

[14] Y S, J Q, Q Z, et al. Advanced glycation end products increased placental vascular permeability of human BeWo cells via RAGE/NF-kB signaling pathway. 2020; 250:93-100.

[15] W C, J C, Y C, et al. Advanced glycation end products induced immune maturation of dendritic cells controls heart failure through NF-κB signaling pathway. 2015; 580:112-20.

[16] J M, X C, G X, Fish LXJ, immunology s. Chronic exposure to the ionic liquid [Cmim]Br induces inflammation in silver carp spleen: Involvement of oxidative stress-mediated p38MAPK/NF-κB signalling and microRNAs. 2019; 84:627-38.

[17] Z Z, C L, S M, et al. Silencing of PTGS2 exerts promoting effects on angiogenesis endothelial progenitor cells in mice with ischemic stroke via repression of the NF-κB signaling pathway. 2019; 234:23448-60.

[18] R B, R V, laboratory FNJTrtjo, medicine c. Prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2), a complex target for colorectal cancer prevention and therapy. 2018; 196:42-61.

[19] S L, M J, L W, Biomedicine YSJ, Biomedecine p, pharmacotherapie. Combined chemotherapy with cyclooxygenase-2 (COX-2) inhibitors in treating human cancers: Recent advancement. 2020; 129:110389.

[20] SH P, HJ C, H Y, et al. Endoplasmic reticulum stress-activated C/EBP homologous protein enhances nuclear factor-kappaB signals via repression of peroxisome proliferator-activated receptor gamma. 2010; 285:35330-9.

[21] S H, B Z, IS C, et al. PPAR-γ in Macrophages Limits Pulmonary Inflammation and Promotes Host Recovery following Respiratory Viral Infection. 2019;93.

[22] P E, S A, C A, et al. Disruption of tight junction structure in salivary glands from Sjögren's syndrome patients is linked to proinflammatory cytokine exposure. 2010; 62:1280-9.

[23] SK S, BN P, YC L, et al. Reduced expression of junctional adhesion molecule and platelet/endothelial cell adhesion molecule-1 (CD31) at human vascular endothelial junctions by cytokines tumor necrosis factor-alpha plus interferon-gamma Does not reduce leukocyte transmigration under flow. 2001; 159:2281-91.

[24] C G, P T, S J, Cytokine NMJ. Co-culture of human monocytes and thyrocytes in bicameral chamber: monocyte-derived IL-1alpha impairs the thyroid epithelial barrier. 2000; 12:32-40.

[25] SA R, M K-K, I C, H A, N B-E, Immunobiology P-RSJ. IL-1β and TSH disturb thyroid epithelium integrity in autoimmune thyroid diseases. 2013; 218:285-91.

[26] N F-V, M A-P, I B, et al. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. 2010; 95:953-62.

[27] X B, J S, W W, et al. Increased differentiation of Th22 cells in Hashimoto's thyroiditis. 2014; 61:1181-90.

[28] Davis PJ %J Current opinion in endocrinology d, obesity. Cytokines and growth factors and thyroid hormone. 2008; 15:428.

[29] A B, K B, H J, endocrinology KJJTJo. Effects of proinflammatory cytokines on anterior pituitary 5'-deiodinase type I and type II. 2000; 167:505-15.