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Study on the Mechanism of Liuwei Dihuang Pill in Treating Hypothyroidism Based on Network Pharmacology

Ruyu Teng[1,a], Yuanshan Tang[2,b,*], Lu Cheng[1,c], Xiaojing Zhu[1,d]

[1]Shaanxi University of Chinese Medicine, Xianyang, 712046, China
[2]Xi'an Hospital of Traditional Chinese Medicine, Xi'an, Shaanxi, 710021, China
[a]2239574563@qq.com, [b]1553077709@qq.com, [c]1453129704@qq.com, [d]1192095498@qq.com
[*]Corresponding author

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Abstract: Objective: To study the mechanism of Liuwei Dihuang Pill (LWDP) in the treatment of hypothyroidism from the perspective of network pharmacology. Methods: The active ingredients of Liuwei Dihuang pill were screened by TCMSP platform database and the target prediction was carried out. GeneCards database was used to screen genes associated with hypothyroidism.The target of Liuwei Dihuang pill in treating hypothyroidism was obtained by Venny platform. The protein-protein interaction (PPI) network, gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of these targets were performed to reveal the possible mechanism of Liuwei Dihuang pill in treating hypothyroidism. Results: A total of 28 active components and 189 targets were identified from Liuwei Dihuang Pills, and 4502 targets were identified from GeneCard database. The PPI network contains 124 nodes and 323 edges. 662 biological processes (BP), 72 cell components (CC) and 127 molecular functions (MF) were obtained by GO enrichment. A total of 149 signaling pathways were obtained by KEGG enrichment analysis, and the top 20 signaling pathways were visualized and analyzed.The signal pathways related to hypothyroidism include AGE-RAGE signal pathway, lipid and atherosclerosis signal pathway, HIF-1 signal pathway, PI3K-Akt signal pathway and MAPK signal pathway. Conclusion: The main components of Liuwei Dihuang pill in treating hypothyroidism are quercetin, kaempferol, β-sitosterol, diosgenin, kadsurenone, hederagenin, sitosterol and stigmasterol. The main targets of Liuwei Dihuang pill are MAPK1, TP53, TNF, AKT1 and EGFR, and they play a therapeutic role through AGE-RAGE, lipid and atherosclerosis, HIF-1, PI 3K-Akt and MAPK signal pathways.

1. Introduction

Hypothyroidism is a systemic hypometabolic syndrome caused by variety of causes, such as Hashimoto 's thyroiditis, which is also the main cause of hypothyroidism [1].Studies have shown that hypothyroidism can lead to heart failure [2], osteoporosis [3] and other diseases.At present, oral

levothyroxine (L-T4) is mainly used in modern medicine. Although it can return the content of TSH and thyroid hormone to the normal range, it is generally taken for life [4].

Qu Zhuqiu [5] thinks that the treatment of this disease should be based on kidney, and Liu Wei Di Huang Pill is selected to treat hypothyroidism of deficiency of both yin and yang.Chen Xiabo [6] believes that due to deficiency of kidney essence and spleen qi, long-term illness, deficiency of yin and yang and imbalance of qi and blood, Erxian Decoction can be selected, which is mainly composed of Liuwei Dihuang Pill, which has the function of nourishing yin and filling essence and tonifying qi and blood.Based on the method of network pharmacology, this study explored the mechanism of hypothyroidism through network construction and network topology analysis, in order to provide theoretical support for clinical treatment of hypothyroidism.

2. Materials and Methods

2.1. Screening of active ingredients and targets of Liuwei Dihuang Pill

The active ingredients and targets of Liuwei Dihuang Pill were searched and collected on TCMSP (https://old.tcmsp-e.com/) platform. Oral bioavailability (OB) ≥30%, class drug (DL)≥0.18. The target gene corresponding to the target protein was searched by UniProt (https://www.uniprot.org/).

2.2. Prediction of the target of Liuwei Dihuang pill on hypothyroidism

Using GeneCard (https://www.genecards.org/) database, using the term "hypothyroidism" as the search term, to obtain the disease target of hypothyroidism. Liuwei Dihuang pill and the predicted targets of hypothyroidism were input into Venny2.1 to obtain the intersection targets of Liuwei Dihuang pill on hypothyroidism, and the active component-target network was constructed by Cytocape3.8.0 software.

2.3. Construction of PPI network of common target of Liuwei Dihuang pill and hypothyroidism

Use STRING11.0 database (https://cn.string-db.org/) to establish protein interaction (PPI) network model, set biological species as "Homo sapiens," hide unconnected nodes, select maximum confidence value>0.900 to obtain PPI network, download TSV file to CytoScape 3.8.0 for drawing.Calculate the Degree value of the whole PPI network through Tools-Analyze Networks in CytoScape.

2.4. Enrichment analysis of active ingredients of Liuwei Dihuang Pill-functional pathway of hypothyroidism target

The target of Liuwei Dihuang Pill for treating hypothyroidism was entered into DAVID (https://david.ncifcrf.gov/) platform to analyze GO function and KEGG pathway.Image visualization was performed using the Microbiology Online Platform (http://www.bioinformatics.com.cn).

3. Results

3.1. The active components and targets of Liuwei Dihuang Pill

The active components of Shu Di, Shan Yao, Shan Zhuyu, Fu Ling,Ze Xie,Mu Danpi were 2, 16,

20, 15, 10, 11.After removing the repeated values and the components without target, it was found that Liuwei Dihuang Pill contained 28 active ingredients, and there were 189 drug target genes corresponding to the active ingredients after removing the repeated values.

3.2. Target of Liuwei Dihuang Pill on Hypothyroidism

In the GeneCard database, a total of 4502 targets related to hypothyroidism were obtained, and compared with the pharmacodynamic targets of Liuwei Dihuang Pill, a total of 124 targets were obtained (see Figure 1).Study of drug-active ingredient-target using Cytoscape visualization (see Figure 2).The results showed that the main constituents were quercetin, kaempferol, β-sitosterol, diosgenin, kadsurenone, hederasaponin, sitosterol and stigmasterol.

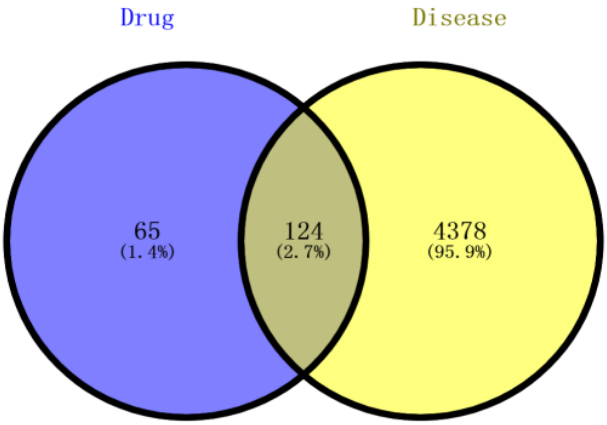

Figure 1: Drug-disease intersection target plot

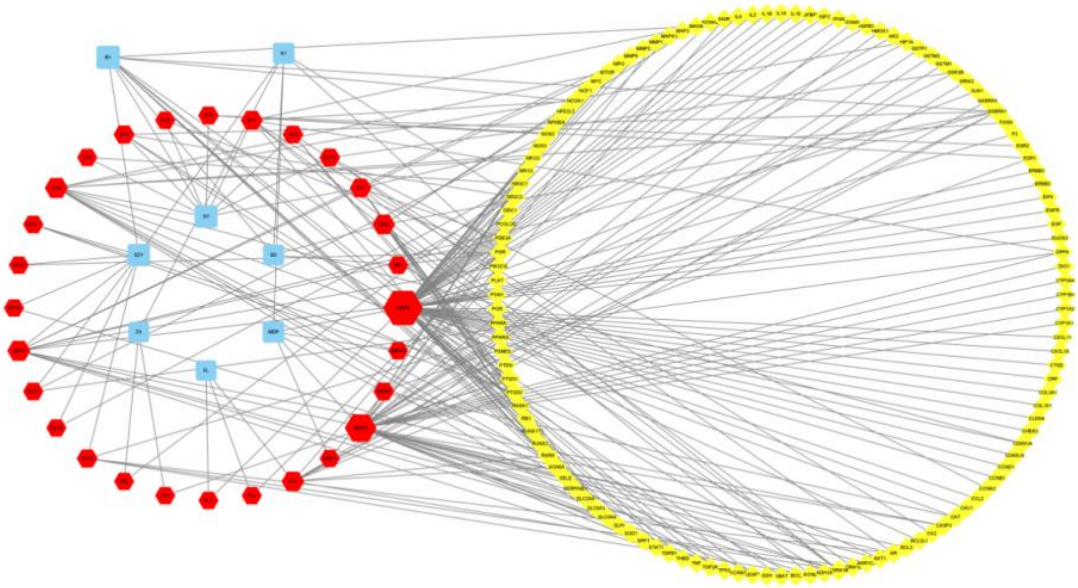

Figure 2: Drug-component-target network diagram of Liuwei Dihuang pill on hypothyroidism

The rhombus is that intersection target of the medicine and the disease, the hexagon represent the composition of the traditional Chinese medicine, and the square represents the name of the traditional Chinese medicine SD: Shu Di;SY: Shan Yao;SZY: Shan Zhuyu;ZX:Ze Xie;FL:Fu Ling;MDP: Mu Danpi), A1 (MOL000359) is common component of Shu Di, Ze Xie, Shan Zhuyu,

and Mu Danpi, and B1 (MOL000449) is common component of Shu Dia, Shan Zhuyu, and Shan Yao.The size of the graph represents the strength of the action.

3.3. PPI network construction

Importing the intersection target points into the STRING database, hiding the stray nodes, and setting the maximum confidence value>0.900, a PPI network diagram containing 124 nodes and 323 frames can be obtained.Download the TSV file and import it into CytoScape 3.8.0 software for plotting (see Figure 3).The results showed that MAPK1, TP53, TNF, AKT1, EGFR, etc. had higher Degree values.

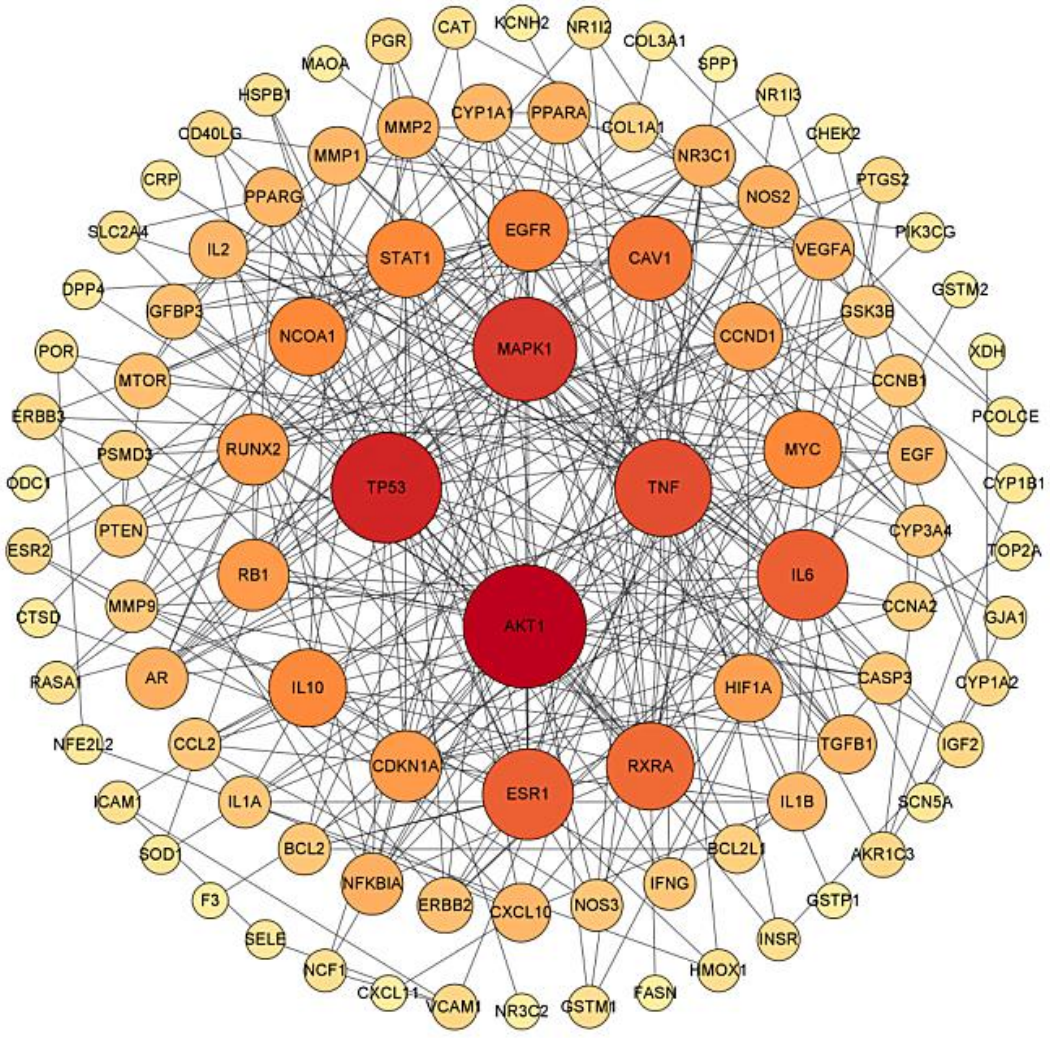

Figure 3: PPI Network Diagram of Liuwei Dihuang Pill in Treating Hypothyroidism

3.4. GO function and KEGG pathway enrichment analysis of targets

The common target information of Liuwei Dihuang pill active ingredients and hypothyroidism was imported into DAVID system for GO analysis. The species was set as human, and at most 20 gene items were selected from BP, MF and CC respectively according to P<0.05.GO analysis chart (see Figure 4)was made by using microbiology information (http: // www. bioinformatics. com. cn/);Take the top 20 pathways with the number of genes and draw bubble diagram for KEGG

pathway analysis.(See Figure 5)

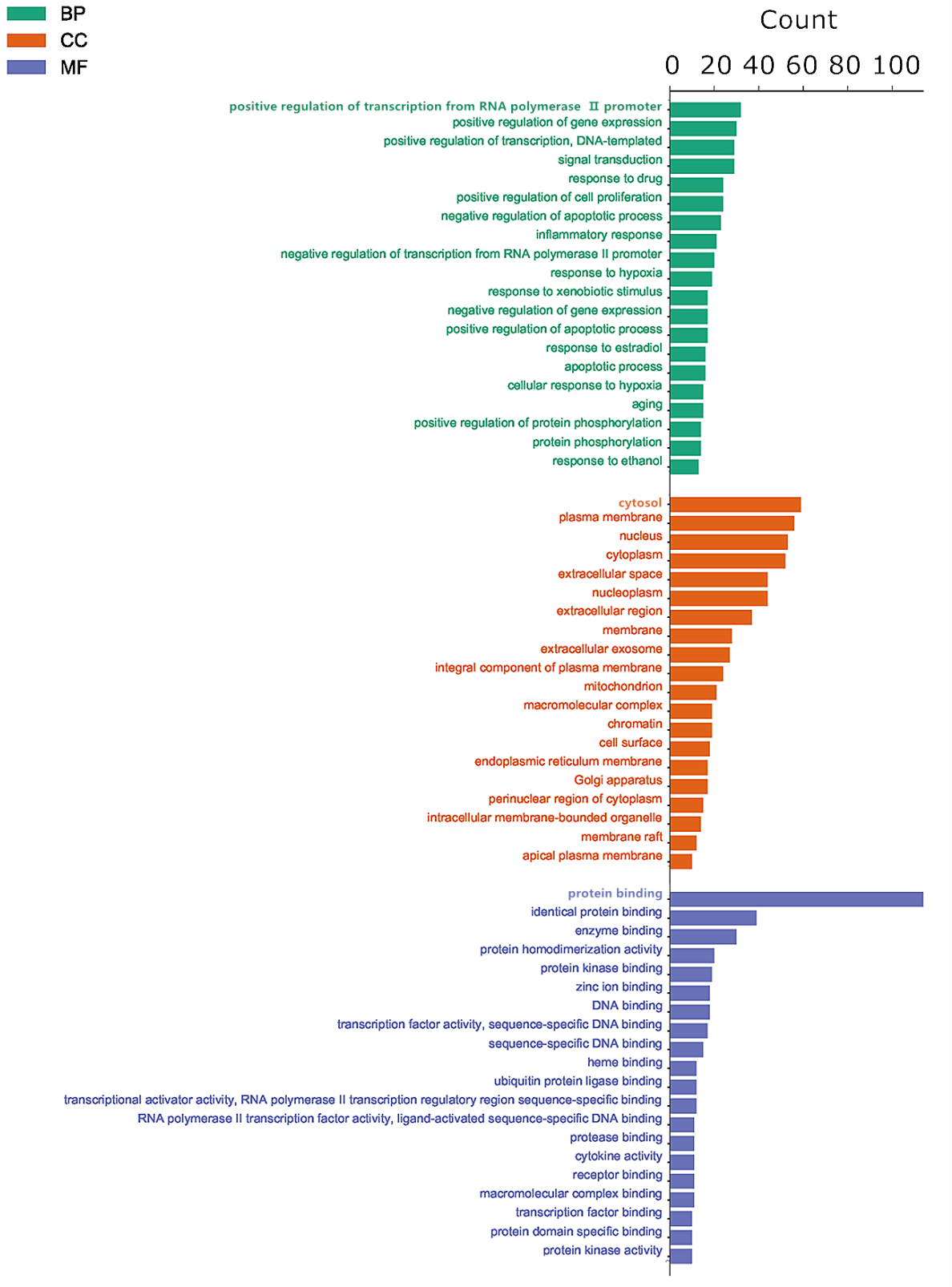

Figure 4: GO enrichment analysis

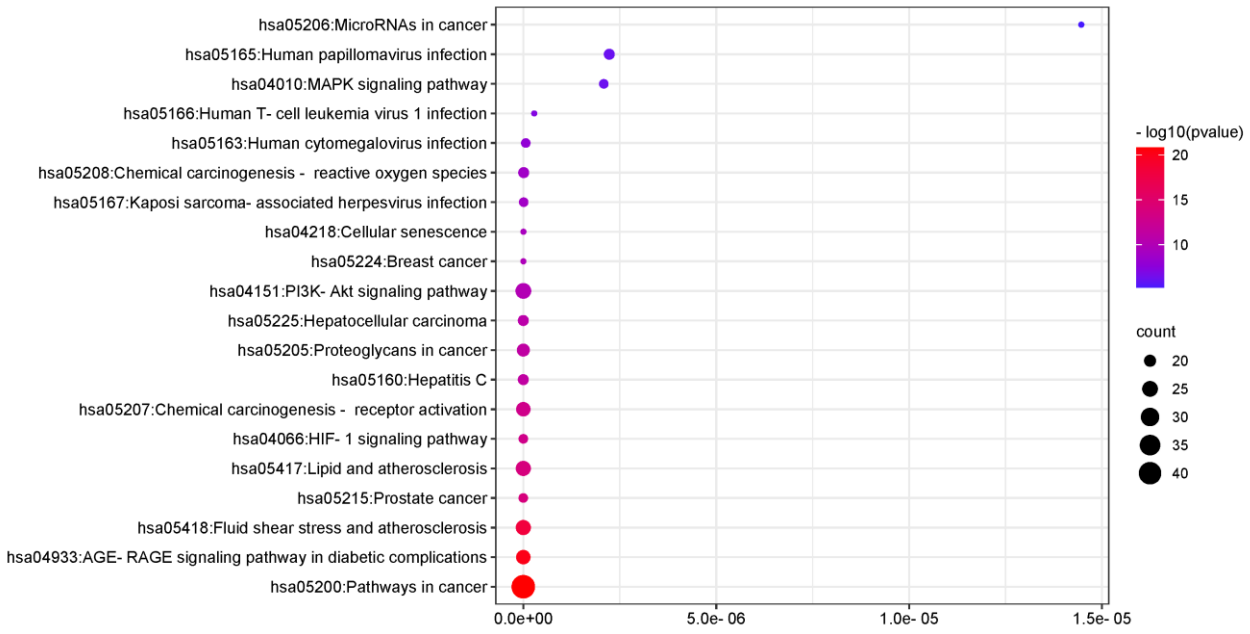

Figure 5: KEGG pathway analysis

From the enrichment analysis of GO in Figure 4, it can be seen that:positive regulation of gene expression, drug response, hypoxia response, estradiol response, positive regulation of transcription, positive regulation of cell proliferation, inflammatory response, negative regulation of apoptotic processes, transcription regulation of RNA polymerase II promoter, positive regulation of apoptotic processes, positive regulation of protein phosphorylation and senescence.

The KEGG pathway analysis results (See Figure 5) showed that the signal pathways of Liuwei Dihuang Pill on hypothyroidism included cancer pathway, AGE-RAGE signal pathway, lipid and atherosclerosis, HIF-1 signal pathway, PI3K-Akt signal pathway, MAPK signal pathway, etc.

4. Discussion

Hypothyroidism is one of the most prevalent disorders of the endocrine system [7], and is more common in women and the elderly [8].The clinical manifestations are somnolence, weight gain [9], memory impairment, unresponsiveness [10], intolerance of cold, skin pruritus and constipation [11].At present, the pathogenesis of Hashimoto's thyroiditis with hypothyroidism mainly includes apoptosis, oxidative stress and cytokines [12].Studies have shown that autoimmune thyroiditis is associated with apoptosis signaling pathways [13-14].Animal experiments have demonstrated that hypothyroidism in rats is associated with oxidative stress processes [15].Cytokines play a key role in the regulation of the immune system and are associated with the pathogenesis of hypothyroidism [16].

According to the drug-active ingredient-target network analysis, quercetin, kaempferol, beta-sitosterol, diosgenin, kadsurenone, hederagenin, sitosterol and stigmasterol may play a main role in the treatment of hypothyroidism.Modern pharmacological studies have shown that [17] quercetin has anti-inflammatory, antioxidant, immunomodulatory and anti-tumor effects.Studies have shown [18] that quercetin can activate TPO of rats, make them produce well-differentiated metabolite 3,5-L-diiodotyrosine, and promote the expression of TPO and the production of L-thyroxine.Quercetin also has certain regulatory effect on inflammatory release and apoptosis of

epithelial cells [19] ;Stigmasterol has the effects of anti-inflammation, lowering cholesterol and improving memory [20-22] ;β-sitosterol can inhibit apoptosis of granulosa cells, which is related to regulation of endocrine hormone, neuroprotection and anti-inflammation [21-24] ;Kaempferol has antioxidant, anti-inflammatory, heart protection, nerve protection, anti-diabetes, anti-osteoporosis, anti-anxiety effects [25].Diosgenin can improve cardiac function and reduce inflammatory reaction, which is related to cell apoptosis and endocrine hormone regulation process [26-27].Kadsurenone inhibits inflammatory mediators[28].Hederagenin is neuroprotective, anti-inflammatory, anti-apoptotic, and lipid-lowering [29-30].Conclusion: These components may be the core components of Liuwei Dihuang pill in the treatment of hypothyroidism, and have certain clinical research value.

The PPI network of Liuwei Dihuang pill showed that mitogen-activated protein kinase 1 (MAPK1), tumor suppressor factor p53(TP53), tumor necrosis factor (TNF) and serine/threonine protein kinase 1 (AKT1) were the key targets.MAPK1 plays an important role in transcriptional regulation, cell proliferation, cell differentiation and apoptosis [31].TP53 can control cellular stress response by coordinating the expression of many target genes, leading cells to cell cycle arrest or apoptosis after DNA damage or other disturbances [12].It was found [16] that the cytokine concentration in the serum of patients with hypothyroidism after thyroidectomy showed a large amount of TNF-α, and their functions included proliferation and differentiation, cell death, immunomodulatory activity, lipid metabolism and endothelial function.Thus, the increased TNF-α concentrations suggest possible changes in these parameters, which may be related to hypothyroidism.AKT1 can regulate cell apoptosis, cell proliferation and inflammatory response [32].

GO enrichment is mainly involved in the regulation of inflammation, cell proliferation and apoptosis.KEGG pathway analysis shows that some studies [33] show that hypothyroidism can be treated by regulating PI3K-Akt signaling pathway.Lipid and atherosclerotic signaling pathways regulate hypothyroidism.Lipidomic analytical methods are powerful in providing complementary insights into the pathophysiology of hypothyroidism [34].HIF-1 signaling protects cells from apoptosis [35].Wang Shiqi et al. [36] found that the levels of HIF-1α and key glycolytic enzymes hexokinase 2, phosphofructokinase and pyruvate kinase in autoimmune thyroiditis mice were significantly higher than those in control group, indicating the role of HIF-1 signaling pathway in Hashimoto's thyroiditis.AGE-RAGE signal transduction pathway is related to the release of inflammatory factors, and its mechanism of action is to activate inflammatory factors to cause tissue damage [37]. After the activation of AGE-RAGE pathway, it can induce the differentiation of CD4+ T lymphocytes into Th1 cells, thus causing damage to thyroid follicular cells [38].MAPK signaling pathways mediate gene expression, motility, metabolism, differentiation, proliferation, and programmed cell death [39].Most of these signaling pathways are related to inflammation, cell proliferation, apoptosis and lipid signaling pathways.

In conclusion, Liuwei Dihuang Pill may achieve the effects of anti-inflammation, anti-apoptosis, anti-oxidation and immunoregulation through multiple targets and multiple pathways, so as to achieve the effect of treating hypothyroidism.This provides further theoretical support for the clinical and basic research of Liuwei Dihuang pill in the treatment of hypothyroidism.

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