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Study on the molecular mechanism of Huang jing in the treatment of type 2 diabetes mellitus based on network pharmacology

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Abstract: **Objective** To explore the possible mechanism of Huang jing in the treatment of T2DM by using network pharmacology. **Methods** Using TCMSP database to obtain the active components and targets of Xanthine, combined with OMIM, GeneCards, DrugBank, DisGeNET and other databases to screen T2DM-related gene targets, extracting drug-disease target intersection with R software, and constructing drug-component-disease-target network diagram. The intersection target PPI network is constructed through the String database and Cytoscape software. The GO function and KEGG pathway enrichment analysis of the target of T2DM are performed using R software and Metascape database. **Results** 12 active ingredients and 76 targets are selected from Huang jing. Key proteins involved AKT1, VEGFA, PPARG, CASP3, FOS, MAPK14, MMP9, etc. 818 items are obtained by GO functional enrichment analysis, KEGG pathway enrichment screening obtained 80 signaling pathways is mainly concerned with the signaling pathways including whereas resistance, age-rage signaling pathway in the diabetic outcome, MAPK signaling pathway, and Relaxin signaling pathway. **Conclusion** The possible mechanism of action of xanthine in the treatment of T2DM is predicted by using network pharmacology method. The useful components of Xanthine mainly coordinated the intervention of T2DM by coordinating inflammatory signaling pathway, insulin-related pathway and antioxidation-related signaling pathway, providing new ideas and clues for further clinical application research.

1. Introduction

Since the 1990s, the incidence of diabetes has risen sharply in China and even globally, with type 2 diabetes (T2DM) accounting for 93.7% [1]. At present, Western medicine treatment of T2DM mainly focuses on sulfonylureas, biguanides, glinides and other drugs, combined with diet, exercise and other drugs to control blood sugar [2]. However, the treatment is still difficult. In the history of medicine globally, the earliest understanding of this disease in Chinese medicine is "quenching thirst". The discussion is very detailed, and the name of quenching thirst is first seen in "Su Wen Strange Diseases". From the perspective of traditional Chinese medicine, the aetiology of this disease includes: insufficient endowment, improper diet, emotional disorder, excessive desire to

work, etc., the disease involves multiple viscera such as lung, spleen, stomach, kidney, etc., the primary pathogenesis of this disease is Yin deficiency, Jin deficiency, and excessive heat [3]. The main clinical manifestations are "three more and one less": polydipsia, polyphagia, polyuria, and weight loss, but its pathogenesis needs to be further clarified.

Sound clinical effects have been achieved by analyzing the pathogenesis and implementing syndrome differentiation in treating diabetes with traditional Chinese medicine [4]. Huang jing for medicine edible Chinese herbal medicine, 2015 edition of "Chinese pharmacopoeia" their sexuality umami, spleen, lung, kidney meridian, has the effect of invigorating spleen, kidney, lung, Qi and Yin. Modern pharmacological studies have shown that Huang jing has many pharmacological effects, such as lowering blood sugar, anti-ageing and anti-depression [5], its hypoglycemic effect may be achieved through anti-inflammatory action [6]. In addition, Huang jing traditional Chinese medicine preparation in the treatment of T2DM is not as inadequate as western medicine alone [7]. It also has an excellent lipid-lowering effect and has the potential to be used in the treatment of complications of T2DM [8]. However, the molecular mechanism of its resistance to T2DM has not been fully elucidated. The multi-component and multi-target characteristics of Huang jing can hardly be explained by a single experimental method. The emergence of network pharmacology provides new research ideas and methods for the study of traditional Chinese medicine.

2. Materials and methods

2.1 Screening of effective components of Huang jing

All the chemical components of Huang jing are retrieved from TCMSP (https://tcmspw.com/tcmsp.php), the traditional Chinese medicine system's pharmacology database and analysis platform.

2.2 Screening of active components and target proteins

According to pharmacokinetic parameters, two ADME attribute values with oral bioavailability (OB) greater than 30% and drug-likeness (DL) greater than 0.18 are set for preliminary screening of active ingredients, so as to obtain active compounds and protein targets of their action.

2.3 Standardized target gene names

For the standardization of protein targets information, unified Uniprot compound action of protein targets can be converted to target protein database (https://www.uniprot.org/), enter the UniprotKB, set species, choose the authenticated as a result, download data files, after processing the active ingredients and drug target matching and get all the protein targets of correction for Uniprot ids, and get the corresponding gene name, delete duplicate values. If it cannot be matched, the target protein will be discarded by returning UniprotKB for manual matching one by one. If the match is still not possible, the target protein is discarded.

2.4 Screening of disease genes

Potential T2DM targets are searched in OMIM (https://omim.org), GeneCard (https://www.genecards.org/), DrugBank (https://www.drugbank.ca), Disgenet (http://disgenet.org/) and other databases with the keywords "Type 2 Diabetes Mellitus". If there are too many targets, the target with Score greater than the median is set as the disease's potential target. After merging the results of multiple disease databases, the disease target is obtained by removing duplicate genes.

2.5 Intersection of active ingredient-disease target

The software R (Rguix64 4.0.2) is used to take the intersection of Huang jing's active component targets and disease targets to obtain the potential targets of Huang jing to treat T2DM, and the corresponding Venn diagram is drawn.

2.6 Construction of the active component-target network

The Network diagram of "TCM-component-disease-target" is drawn by Cytoscape3.8.0 software, and the Network topology is analyzed by Network Analyzer plug-in to screen the main active components.

2.7 PPI network construction

Intersection targets are submitted to String11.0 online database (https:// string-db.org/cgi/ input.pl) for Protein-Protein Interaction (PPI) analysis. Set the species as "Homo sapiens", the minimum interaction threshold as "highest confidence" > 0.9, hide the free nodes, and the rest as default values to get the PPI network graph. PPI data are imported into Cytoscape software, and the Network Analyzer plug-in is used for further topology analysis to screen the core targets.

2.8 GO function and KEGG pathway enrichment analysis

Using Metascape Online Database (http://www.webgestalt.org/) and Bioconductor (http://www.bioconductor.org/) in the "dosed", "pathview", "clusterprofile" and "org. Hs.Eg.Db" biomedical data packets, such as combining with R software to GO overlap between gene function and KEGG pathway enrichment analysis of enrichment process with P 0.05 or less for filter conditions, significant output 20, before making a histogram and bubble chart. With microscopic letter platform (http://www.bioinformatics.com.cn/) Biological Process (in the Process, BP), cells (Cellular Component, CC) and Molecular functions (Molecular Function, MF) three sets of representative data for triad view.

3. Results and analysis

3.1 Screening of active components and target proteins

Table 1 Basic information of some active components of Huang jing

Mol ID	Component name	The molecular weight	OB(%)	DL
MOL001792	DFV	256.27	32.76	0.18
MOL002714	baicalein	270.25	33.52	0.21
MOL002959	3'-Methoxydaidzein	284.28	48.57	0.24
MOL000358	beta-sitosterol	414.79	36.91	0.75
MOL000359	sitosterol	414.79	36.91	0.75
MOL003889	methylprotodioscin_qt	446.74	35.12	0.86
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	256.27	71.12	0.18
MOL000546	diosgenin	414.69	80.88	0.81
MOL006331	4',5-Dihydroxyflavone	254.25	48.55	0.19
MOL009760	sibiricoside A_qt	432.71	35.26	0.86
MOL009763	(+)-Syringaresinol-O-beta-D-glucoside	580.64	43.35	0.77

A total of 38 active ingredients are obtained by using TCMSP database. According to OB≥30% and DL≥0.18, the ingredients lacking target prediction data are eliminated, and 12 active ingredients are screened out. The specific information is shown in Table 1.

3.2 Intersection of the active ingredient and disease target

A total of 149 potential targets are screened by TCMSP database, and 76 standardized targets are obtained after matching. A total of 842 potential disease targets are obtained through the retrieval of keywords in T2DM by OMIM, Genecards, Ddrugbank, Disgenet and other databases, and 33 components-disease target intersections are obtained by R software, as shown in Figure 1.

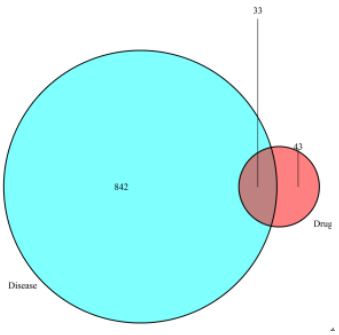


Figure. 1 Intersection target of Huang jing -T2DM

3.3 Network diagram of traditional Chinese medicine-chemical composition-disease-target

Import the prepared Network file and Type file into Cytoscape software, and get the network as shown in Figure 2. Among them, yellow is the traditional Chinese medicine Huang Jing, blue is the disease T2DM, purple is the main chemical component, light green is the potential target of Huang Jing, pink is the common target of Huang Jing and T2DM.

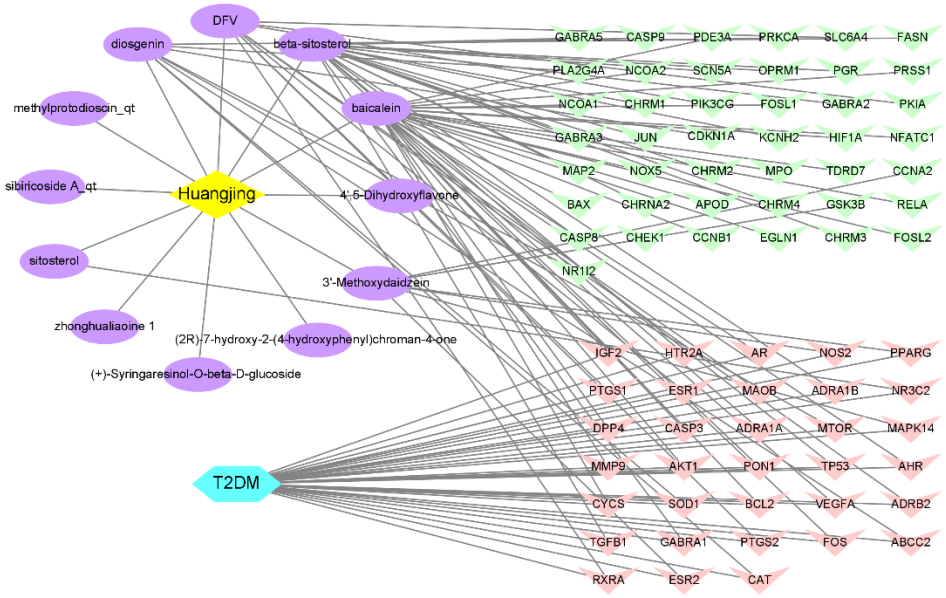
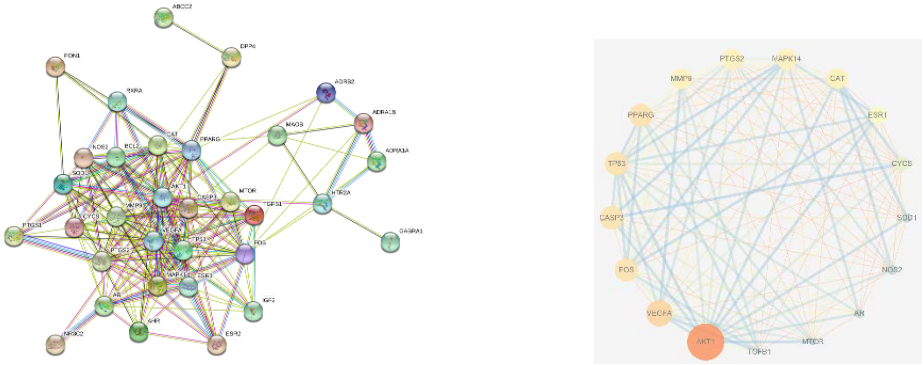


Figure. 2 Huang jing-component-T2DM-target network

3.4 PPI network

The drug-disease common targets are imported into the String analysis platform's online database, and the PPI network graph is obtained, as shown in Figure 3a, including 33 nodes and 197 edges. The node represents each gene protein, and the line between the nodes represents the interaction relationship between the two proteins connected by the straight line. The thicker the line is, the stronger the interaction relationship is. The obtained string_interactions.tsv file is imported into Cytoscape, and the Network Analyzer plug-in is used for further topology analysis. Core targets are screened according to the median of Degree value card (Fig. 3b). The larger the Degree value, the larger the circle area and the redder the color are. The top 10 targets are AKT1, VEGFA, PPARG, CASP3, TP53, FOS, CAT, MAPK14, MMP9 and PTGS2, which indicated that it is very possible for Huang jing to prevent and control T2DM by acting on these 10 targets.



a. The PPI network is the common target of Huang jing -T2DM

b. PPI topology core targets

Figure. 3 a. The PPI network is the common target of Huang jing -T2DM; b. PPI topology core targets

3.5 GO functional enrichment analysis

A total of 818 biological processes, including female pregnancy, response to steroid hormone and blood circulation are obtained by GO enrichment analysis of 33 common targets screened using Metascape online database. There are 38 cells including membrane raft, RNA polymerase II transcription factor complex, secretory granule lumen and so on. It has 45 molecular functions such as nuclear receptor activity, protein homodimerization activity and protein phosphatase binding. The item with the best P value is selected from the representative item cluster for visual analysis (Fig. 4), and the histogram of difference and bubble diagram of the first 20 GO biological process analysis is made by R language (Fig. 5).

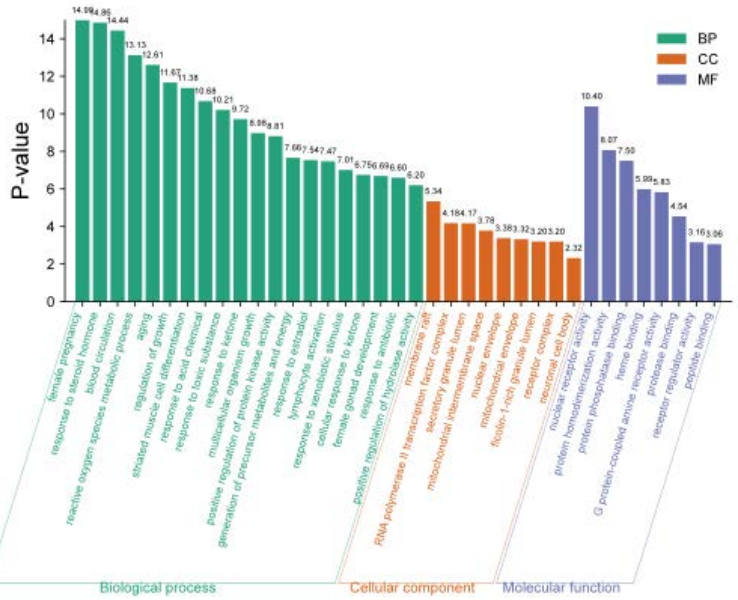


Figure. 4 Three-in-one view of common target GO analysis

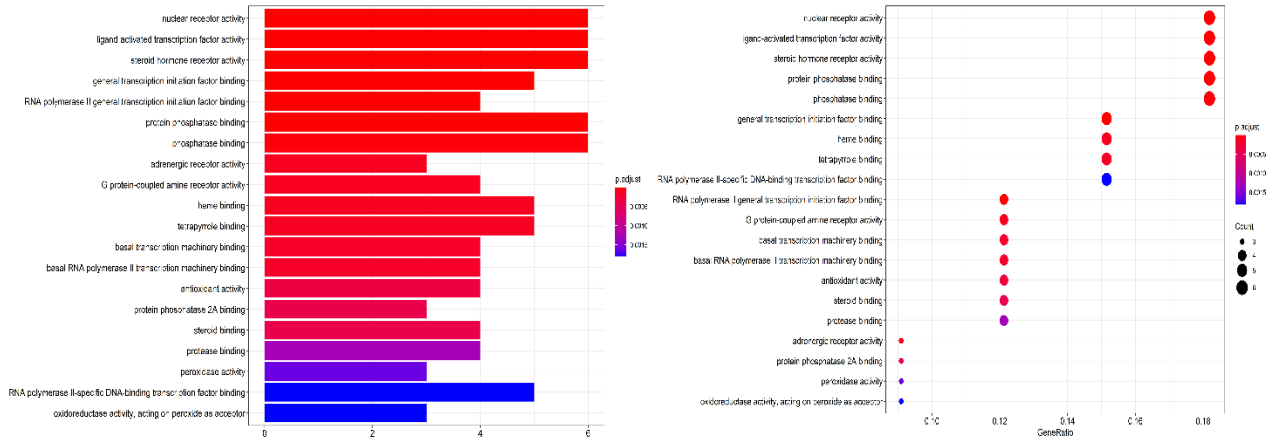


Figure. 5 Bar chart and bubble chart of the top 20 GO biological processes

3.6 KEGG pathway enrichment analysis

The software R is used to carry out KEGG enrichment analysis on 33 key targets, and 80 enrichment pathways are obtained. Visualization analysis is performed on the first 20 pathways to obtain the histogram, bubble diagram (Figure 6) and pathway diagram of KEGG enrichment analysis, showing the pathway diagram with the highest enrichment degree and P value (Figure 7). The analysis showed that the key targets are mainly concentrated in Endocrine resistance, age-range signaling pathway in diabetic complications, MAPK signaling pathway, Relaxin signaling pathway and other related signaling pathways.

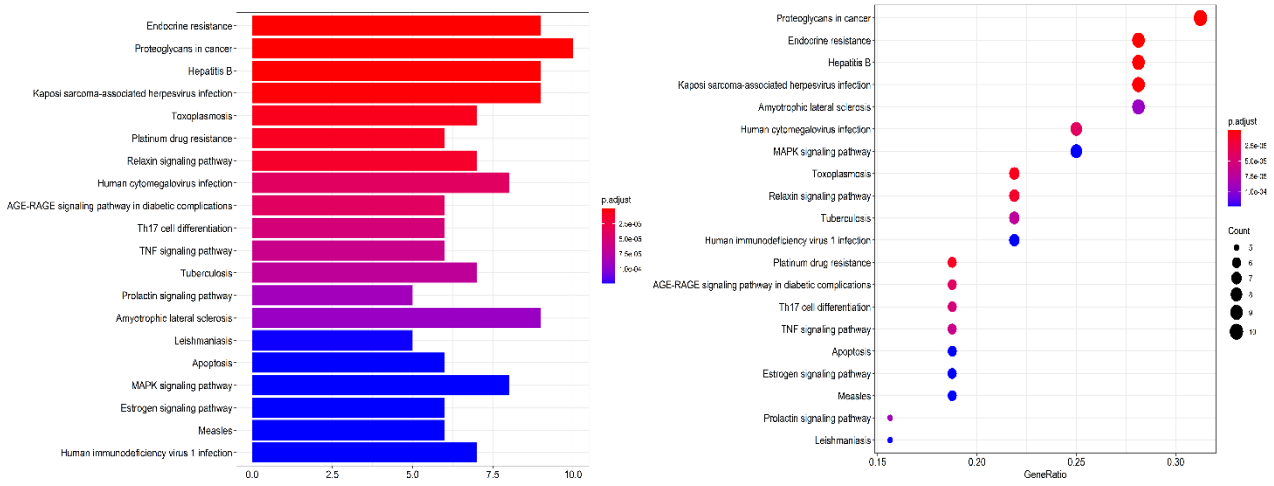


Figure. 6 Histogram and bubble diagram of enrichment analysis of KEGG pathway

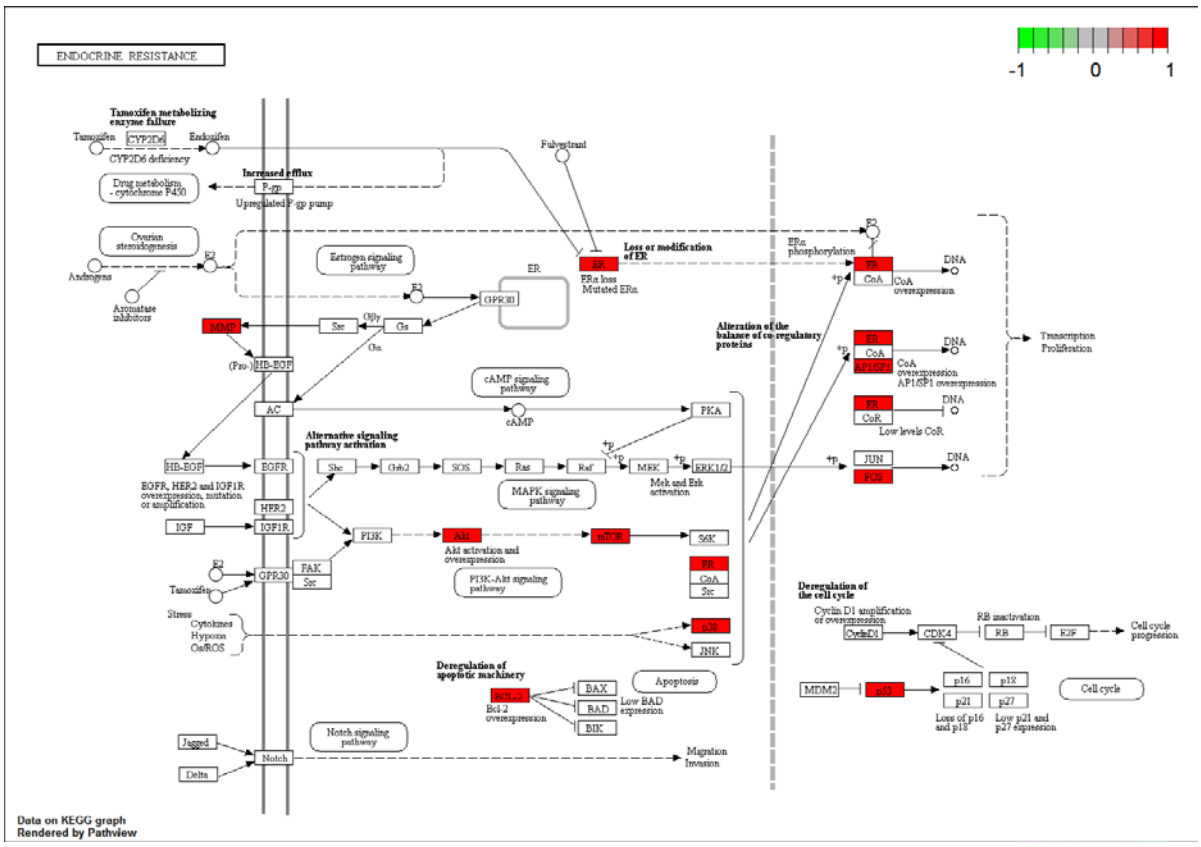


Figure. 7 The signaling pathways of the medically defined syndrome

4. Discussion

T2DM is a metabolic disease caused by the interaction of genetic and environmental factors. Two crucial links in the pathogenesis of T2DM are islet β-cell dysfunction and insulin resistance. However, no new treatments have been found for diabetes, which remains a lifelong disease. The

treatment goal is to normalize blood sugar, correct metabolic disorders, and prevent or reduce complications.

This study used network pharmacology and bioinformatics methods to explore the therapeutic effect of Huang Jing on diabetes. The active ingredient targets showed that baicalein (baicalein), beta-sitosterol (β-sitosterol) and diosgenin (diosgenin) had the main therapeutic effects. Studies have shown that baicalein can delay the occurrence of diabetic peripheral neuropathy through antioxidant stress, inhibition of PKC, p38MAPK and activation of the polyol pathway [9]. The mechanism may be to enhance the pancreas' antioxidant defense, reduce oxidative stress, and ultimately inhibit the expression of CASP3, thereby reducing the apoptosis of β cells [10]. β-sitosterol is a kind of natural pharmaceutical component that has the effects of lowering blood sugar, lowering blood lipid, anti-inflammatory, anti-oxidation, anti-arteriosclerosis, etc.[11]. Ponnulakshmi R etc. [12] studies have shown that β-sitosterol can improve blood glucose level through IR and GLUT4 in adipose tissue of T2DM rats. In the network diagram, diosgenin is linked to apoptosis targets AKT1, oxidative stress targets SOD1, CAT, etc., which is consistent with experimental literature reports [13]. Wu Leitao [14] studies have shown that diosgenin regulates AKT phosphorylation through ERK/Akt signaling pathway, thereby promoting cell cycle transition and inhibiting cell apoptosis, and finally leading to cell proliferation.

In this study, PPI network analysis shows that the key targets of Caspar on T2DM are mainly AKT1, VEGFA, PPARG, CASP3, TP53, FOS, CAT, MAPK14, MMP9, PTGS2, etc. AKT1 is a serine/threonine-protein kinase essential for intracellular reactions that initiate phosphorylation of insulin receptor substrate -1 (IRS-1) and ultimately regulate sugar and lipid metabolism [15]. Yu Hong etc. [16] experimental results showed that AKT1 is overexpressed in renal tissue of diabetic rats, and Danqi mixture could reduce the expression level of AKT1, block renal fibrosis, and improve diabetic nephropathy. PPARG is a crucial regulator of adipocyte differentiation, lipid and carbohydrate metabolism, and can increase target tissues' sensitivity to insulin [17]. The expression of PPARG in the liver and skeletal muscle of diabetic rats is significantly increased. High levels of liver PPARG can improve insulin sensitivity and moderate insulin resistance [18]. Liu Yanmin [19] observed that the pathogenesis of diabetic rats might be related to the over-expression of C-fos protein at the cellular and molecular level. MAPK14 is a member of the mitogen-activated protein kinase signaling pathway and plays an essential role in proinflammatory factor-induced cellular cascades. MAPK14 leads to the production of pro-inflammatory cytokines such as IL-1β and TNF-α and inflammation-related enzyme activators [20].

This study showed that the KEGG pathway is mainly enriched in endocrine resistance, AGE-RAGE signaling pathway, MAPK signaling pathway, and relaxin signaling pathway in diabetes complications. Currently, multiple endocrine resistance mechanisms have been found, Huang Li et al. [21] studies have shown that neuroendocrine-related hormones in patients with T2DM can affect insulin resistance, and the disorder of endocrine hormones directly leads to the progression of the disease. An AGE-RAGE signaling pathway is one of the classical theories of T2DM. Combined with RAGE, AGE-RAGE activates multiple signal transduction pathways in cells and produces various pathological cell responses, promoting the formation and development of T2DM and its complications [22]. MAPK signaling pathway plays a significant role in inflammation. Xiang Cui et al. [23] studies have shown that Scutellaria baicalensis and Coptidis coptidis can play a better anti-inflammatory effect by blocking the MAPK pathway, and further improve the glucose and lipid metabolism of T2DM rats. Relaxin signaling pathway is involved in slowing down and delaying diabetic complications. Studies have found that exogenous relaxin can improve insulin sensitivity in diabetic patients [24]. It fully shows that traditional Chinese medicine can treat diseases through multiple components, multiple targets and multiple ways.

In summary, the key targets of Huang Jing in the treatment of T2DM are screened and predicted

preliminarily, and the potential molecular mechanisms are discussed. It can be seen that the active component of Chrysostom interferes with T2DM mainly through coordinating inflammatory signaling pathways, insulin-related pathways, and antioxidant related signaling pathways, which has guiding significance for efficacy verification experiments. At the same time, this study only analyzed and studied the signaling pathways with a strong correlation with T2DM, and did not consider the subtle relationships between signaling pathways and pathways or between proteins. In addition, the combined application and the changes of the chemical composition after transfusion are not considered. Therefore, the research results need further verification and exploration.

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