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Using Network Pharmacology to Explore the Mechanism of Capsicum in Treating Type 2 Diabetes Mellitus

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Abstract [**Objectives**] The paper was to explore the mechanism of capsicum (*Capsicum annuum* L.) in treating type 2 diabetes mellitus (T2DM) and search for new targets. [**Methods**] The active ingredients of capsicum were queried from TCMSP database to obtain the corresponding target proteins. The related targets of T2DM were screened from GeneCards database, and the target intersection of active ingredients of capsicum and diabetes mellitus was obtained via Venny software. The protein-protein interaction (PPI) network of the compounds was constructed using STRING database, and the GO bio-function and KEGG pathway enrichment were further analyzed using Metascape database. [**Results**] Through TCMSP database query and conditional screening, 14 candidate active molecules, 93 potential targets and 225 related pathways were obtained. [**Conclusions**] The results of GO and KEGG enrichment analysis show that the main active ingredients of capsicum play a role in the treatment of T2DM by regulating cancer pathways, chemical carcinogenesis—receptor activation, proteoglycans in cancer, and prostate cancer pathways, which will provide an important theoretical basis for subsequent research.

Key words Network pharmacology, Capsicum, Type 2 diabetes mellitus, Mechanism of action

1 Introduction

Diabetes mellitus (DM) is a chronic metabolic syndrome caused by the decrease of insulin level in the body, among which type 2 diabetes mellitus (T2DM) accounts for about 95% of DM cases. At present, the pathogenesis of T2DM is still unclear. The records of DM in TCM literature indicate that it belongs to the category of "symptom-complex of excessive eating". The disease is due to congenital deficiency, improper diet, emotional disorder, fatigue and internal injury, *etc.* The modern clinical manifestations are "drinking, eating and urinating too much". The most common clinical manifestations are fatigue, emaciation or high urine sugar, and patients of long duration of the disease are often accompanied by various complications[1]. According to the ancient medical books, the pathogenesis of symptom-complex of excessive eating is mainly yin deficiency and dryness heat. After years of exploration, TCM has accumulated a lot of effective scientific experience in the theory and practice of treating DM[2]. In recent years, ethnic medicine has also made certain achievements in the treatment of DM and its complications[3-4].

Capsicum (*Capsicum annuum* L.) belongs to *Capsicum*, Solanaceae. The fruits and roots of capsicum are often used medically, and it is one of the annual or limited perennial herbs for both medicinal and edible purposes[5]. Capsicum, native to tropical South America, is generally harvested from July to October each year when the fruits are mature. Nowadays, capsicum is widely cultivated in many provinces and cities in China, such as Inner Mongolia, Shandong, Xinjiang and Yunnan[6]. Historical records show that China was one of the first countries to use capsicum as a medicine. Capsicum is acrid in taste, hot in nature, bitter, with the meridian tropism of heart and spleen. There are discussions on the effects of capsicum like "warming heat and dispelling cold, dispelling wind and heat, dispelling cold and heat, expelling phlegm and dispelling dampness" in a herbal medicine literature *Yaoxingkao*. Therefore, capsicum is used in TCM prescriptions to treat various diseases such as stomach cold wind and rheumatic pain[7]. There are many kinds of chemical substances in capsicum, mainly alkaloids, flavonoids and steroids, as well as terpenoids, phenolic compounds and unsaturated fatty acids[7]. According to pharmacological studies, capsicum has a variety of biological activities, such as analgesic effect, anti-cancer effect, antibacterial effect, effect on cardiovascular system, effect on digestive system, and hypoglycemic and lipid-lowering effect, so it can be used to treat diseases such as DM and obesity[8]. At present, capsicum has been approved as an OTC drug by FDA. With the further research on the chemical composition of capsicum, the pharmacological activity of capsicum has also become a hot spot in the development and research of TCM[7-8].

Network pharmacology is a novel bioinformatics technology for drug target prediction, which explains the mechanism of TCM treatment from multiple aspects by constructing a "drug-ingredient-target" interaction network with overall and systematic characteristics. This method is in line with the characteristics of "multiple formulations, approaches and targets" of traditional Chinese medicine, and can be used to study and clarify the material basis of TCM efficacy[9].

Therefore, on the basis of previous work, this study intends to construct a "capsicum-capsicum active ingredient-target" net-

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work from the two aspects of disease targets and active ingredient targets of capsicum to explore the mechanism of action of capsicum against T2DM. The results will lay a foundation for the discovery of new drug targets and the verification of pharmacodynamic substances of TCM and the in-depth research on pharmacodynamic material basis.

2 Materials and methods

2.1 Screening of active ingredients and targets In Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (http://tcmspw.com/tcmsp.php), the retrieval was carried out with the keyword "capsicum", and eligible active ingredients of capsicum could be obtained from the screening results with drug likeness (DL) ≥ 0.18 and oral bioavailability (OB) ≥ 30% as the screening conditions. The active ingredients were input into SwissTargetPrediction database (http://www.swisstargetprediction.ch/index.php) to retrieve the corresponding target proteins. The names of human target genes and target proteins were screened through UniProt database (https://www.uniprot.org/).

2.2 Screening of disease candidate targets The related genes of T2DM were obtained by inputting the key word "type 2 diabetes mellitus" in GeneCards database (http://www.genecards.org/). A relevance score > 30 was set to screen eligible genes as disease candidate targets.

2.3 Construction of PPI network Venny software was used to obtain the genes at the intersection of the active ingredient target of capsicum and the gene target of T2DM, that is, the potential target, and Cytoscape software was used to construct the network diagram of "capsicum-capsicum active ingredient-target". The potential targets were imported into STRING database (https://string-db.org/), with species "human" as the screening condition via Multiple proteins tool, and the confidence was set as 0.7. The network display was set to hide disconnected nodes in the network, and the screened protein-protein interaction (PPI) network was downloaded in TSV format. Cytoscape software was utilized to visually display the documents in TSV format, thus obtaining the PPI network diagram and key core target proteins.

2.4 Analysis of GO bio-function and KEGG pathway enrichment Metascape database was used for user-defined analysis, and "Homo sapiens" was selected as the species parameter. The cellular component (CC), molecular function (MF) and biological process (BP) were analyzed through Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. On this basis, the key proteins related to the target protein were screened, and the relevant signaling pathway was analyzed to further study the mechanism of action of the target protein in capsicum treatment against T2DM.

3 Results and analysis

3.1 Acquisition of active ingredients According to the conditions in Section **2.1**, 215 candidate target genes of active ingredients of capsicum were screened from TCMSP database, and 14 active ingredients of capsicum were obtained, such as beta-carotene, Diop, sitosterol, *etc.* (Table 1).

Table 1 Active ingredients of capsicum after screening

No.	TCM name	Chemical ingredient and name	OB//%	DL
MOL002773	Capsicum	Beta-carotene	37.18	0.58
MOL002879	Capsicum	Diop	43.59	0.39
MOL000359	Capsicum	Sitosterol	36.91	0.75
MOL000433	Capsicum	FA	68.96	0.71
MOL012362	Capsicum	Capsanthin	47.52	0.51
MOL008688	Capsicum	Capsicastrine_qt	31.22	0.83
MOL008690	Capsicum	(2E,4Z,6Z,8E,10E,12E,14Z,16Z,18E)-1,20-bis[(1R,4S)-4-hydroxy-1,2,2-trimethylcyclopentyl]-4,8,13,17-tetramethylicosa-2,4,6,8,10,12,14,16,18-nonaene-1,20-dione	33.00	0.49
MOL008691	Capsicum	Alpha-carotene/beta, epsilon-carotene	34.51	0.58
MOL008693	Capsicum	Cryptocapsin	42.47	0.54
MOL010234	Capsicum	Delta-carotene	31.80	0.55
MOL008698	Capsicum	Dihydrocapsaicin	47.07	0.19
MOL008723	Capsicum	Mutatoxanthin	60.65	0.56
MOL008745	Capsicum	3-Methoxy-4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxybenzoic acid	33.20	0.25
MOL000953	Capsicum	CLR	37.87	0.68

3.2 Construction of target network A total of 14 329 genes related to T2DM were retrieved through GeneCards database, and 1 318 target genes were obtained with relevance scores > 30 as the restriction condition. The above results were taken as the disease candidate genes in this study, which was then intersected with candidate genes of capsicum, and 93 potential targets were obtained (Fig. 1). The obtained effective active ingredients and potential target genes of capsicum were displayed visually using CytoScape software. The edge, endpoint and other properties of the network are shown in Fig. 2. The network analysis intuitively exhibited that the network contained 229 nodes and 386 edges; the pattern in green represented active ingredient, and those in red and blue represented capsicum and target, respectively. It can be seen that capsicum has the characteristics of multi-ingredient and multi-target action in preventing T2DM.

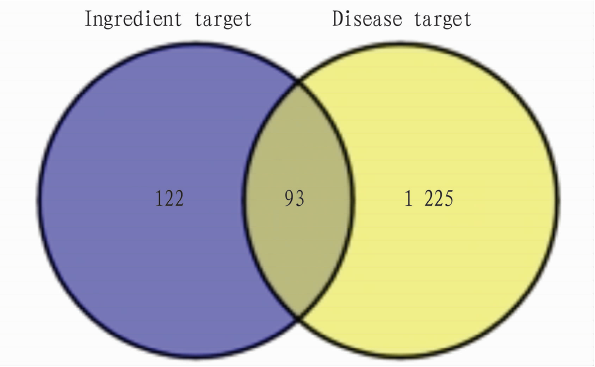

Fig. 1 Venn diagram of capsicum ingredient target- T2DM disease target

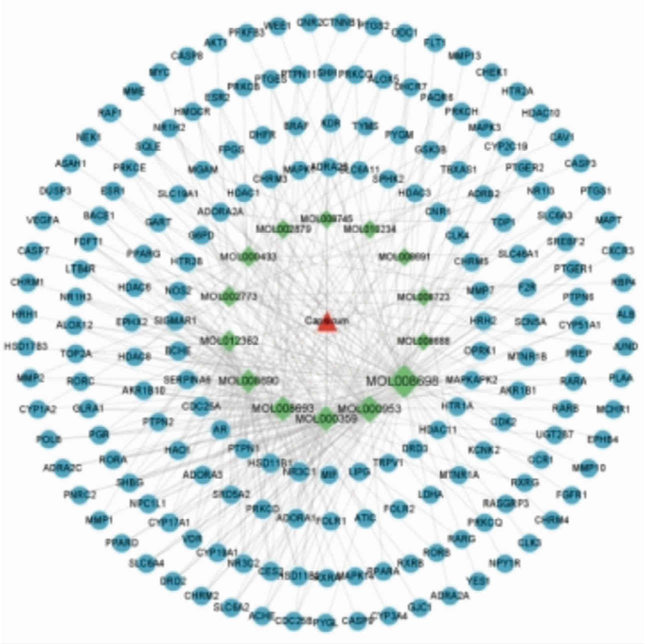

Fig. 2 Network diagram of capsicum-capsicum active ingredient-target

3.3 Construction of PPI network

PPI network analysis can obtain the target protein genes of drugs more effectively and intuitively from a systematic perspective, as shown in Fig. 3. The PPI network diagram constructed using Cytoscape drawing software consisted of 85 nodes. The 93 targets obtained in Section **3.2** were sorted out using Excel table data and then imported into STRING database. The results were saved in TSV format, and PPI network diagram was constructed by importing Cytoscape drawing software. When the degree of the target protein increased, the size of the nodes also increased, and the larger the node was, the darker the color was (Fig. 3). The screening criteria for the 13 core targets in Table 2 were degree > triple median values.

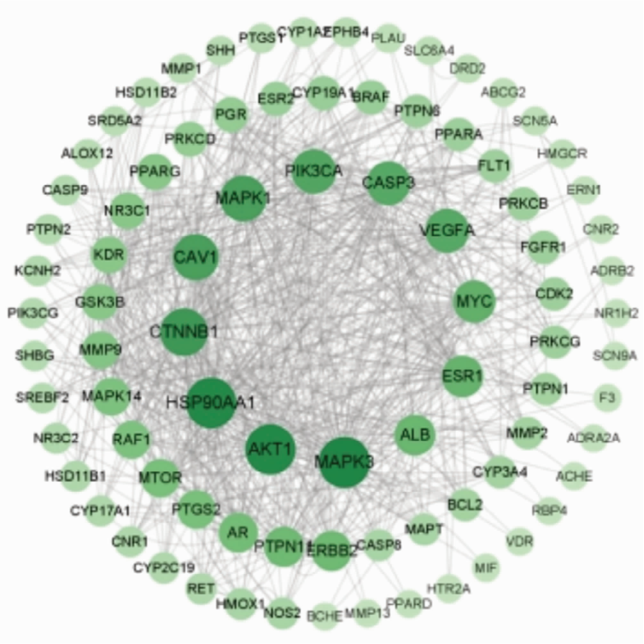

Fig. 3 PPI network of potential targets of capsicum

3.4 GO bio-function and KEGG pathway enrichment analysis

Using Metascape database, the species parameter "Homo sapiens" was selected for user-defined analysis, and 5 543 BP results

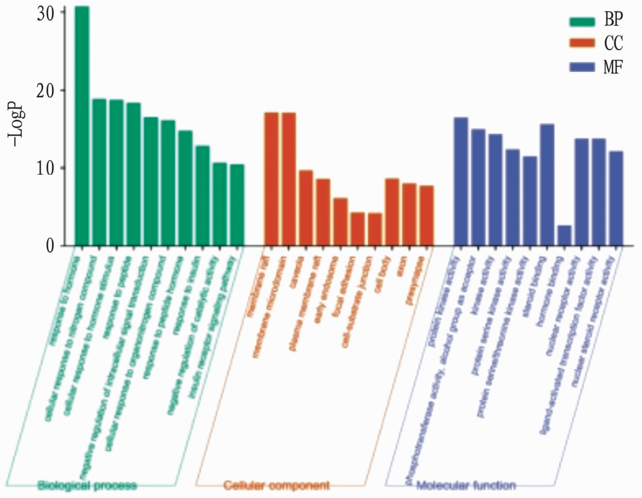

Fig. 4 GO functional enrichment analysis

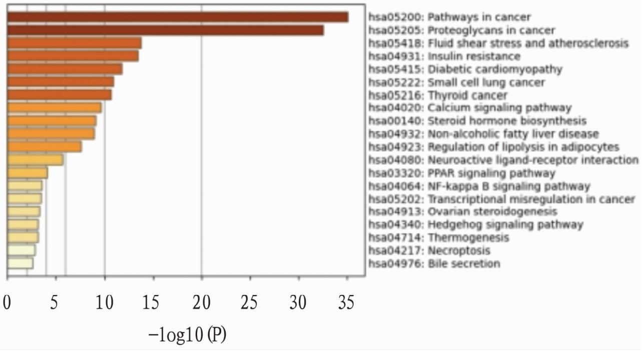

Fig. 5 KEGG pathway enrichment analysis

Table 2 Degree of core targets

Abbreviation	English name	Degree
MAPK3	MAP kinase-activated protein kinase 3	64
HSP90AA1	Heat shock protein HSP 90-alpha	62
AKT1	Potassium channel AKT1	62
CTNNB1	Catenin beta-1	54
CAV1	Caveolin-1	50
MAPK1	Mitogen-activated protein kinase 1	50
PIK3CA	PI3-kinase subunit alpha	46
VEGFA	Vascular endothelial growth factor A	44
CASP3	Casparian strip membrane protein 3	44
MYC	Myc proto-oncogene protein	40
ESR1	Ethylene-responsive transcription factor ESR1	38

were obtained. The GO functional enrichment analysis obtained was composed of CC, MF and BP results, and the top 10 enrichment results were visualized (Fig. 4). The results showed that BP mainly regulated response to hormone, cellular response to nitrogen compound, cellular response to hormone stimulus, *etc.* in T2DM treatment with capsicum; CC mainly included membrane raft, membrane microdomain, caveola, *etc.*; MF mainly included protein kinase activity, phosphotransferase activity, phosphotransferase activity (alcohol group as acceptor), kinase activity, *etc.*

KEGG pathway enrichment was analyzed and explored through Metascape database, and a total of 225 signal pathways were obtained. The results of pathway enrichment were visualized using Metascape online tool. These pathways mainly involved cancer pathways, chemical carcinogenesis—receptor activation, proteoglycans in cancer, prostate cancer and other pathways, and it could be inferred that these pathways are the main regulatory pathways of the active ingredients of capsicum in the treatment of T2DM (Fig. 5).

4 Discussion

Many chemicals with anti-diabetic effect can be extracted from TCM, such as alkaloids, flavonoids, peptides, polysaccharides and amino acids[10]. Capsicum contains a lot of alkaloids and flavonoids and other chemical ingredients. A large number of literatures have shown that the bioactive molecules in capsicum have significant hypoglycemic effects and can regulate the physiological metabolism of glycolipids in the body. Alkaloids are regarded as preventive and therapeutic drugs for endocrine diseases, especially DM, and play a certain role in promoting the discovery of new anti-diabetic drugs[11]. Capsaicin is an important alkaloid in capsicum, and is an important compound that causes the spicy taste of capsicum[8]. Capsaicin is an alkaloid composed of capsaicin, dihydrocapsaicin and other homologues. Of all the capsicum, only nonivamide is a strong tasting and stimulating substance that can be either extracted from capsicum or synthesized chemically[12]. At present, many research groups at home and abroad have conducted researches on the alkaloid components of capsicum. For example, capsaicin can stimulate the intestinal peristalsis of rats to some extent, affect the absorption of small intestine, and has a certain inhibitory effect on carbohydrate digestive enzymes[13]. Capsaicin can increase glucagon level by inhibiting the absorption of glucose in the intestine, thereby improving glucose metabolism and achieving hypoglycemic effect[14]. Studies have shown that capsaicin can also promote the secretion of insulin, thereby reducing the glucose level in the human body[15]. Natural capsaicin (including capsaicin and dihydrocapsaicin) extracted from capsicum, or capsaicin with a purity greater than 95%, can significantly reduce blood glucose levels in rats with DM induced by strep-tozocin (STZ), and can also regulate the glucose metabolism disorder and intestinal glucose absorption, thereby promoting insulin secretion[16]. It has significant effects on glucose metabolism disorder and insulin resistance, and can activate the PI3K/Akt related signaling pathway, playing a positive effect on the improvement of T2DM[17]. Capsicum also contains a variety of flavonoids, such as quercetin (flavonols) and luteolin (flavonoids)[18]. Studies have shown that quercetin can not only reduce glucose in the blood, but also improve insulin secretion. Quercetin is recommended as an anti-diabetic lead compound and has therapeutic effects on T2DM[19-20].

Previous studies reveal that there are 14 potential active ingredients and 93 potential targets in capsicum, indicating that there may be a synergistic effect of multi-components and multi-targets in capsicum. Based on PPI network, effective ingredients against T2DM were screened, such as beta-carotene, Diop, *etc.* According to the degree calculated by the software, 12 core targets were selected, and these nodes played a key role in the PPI network. In order to explore the core targets of capsicum in therapy against T2DM, significant targets were obtained, including AKT1, MAPK3, HSP90AA1, CTNNB1, CAV1, *etc.* AKT1 has the largest node in PPI, which may be an important target for capsicum treatment of T2DM. AKT1, protein kinase-1, is involved in regulating many processes, including metabolism, cell survival, cell proliferation and growth, and angiogenesis[15]. In this study, GO pathway enrichment of the target of capsicum treatment against T2DM showed that BP mainly regulated response to hormone, cellular response to nitrogen compound, cellular response to hormone stimulus, *etc.* in T2DM treatment with capsicum; CC mainly included membrane raft, membrane microdomain, caveola, *etc.*; MF mainly included protein kinase activity, phosphotransferase activity, phosphotransferase activity (alcohol group as acceptor), kinase activity, *etc.* KEGG signal pathway enrichment analysis showed that these pathways mainly involved cancer pathways, chemical carcinogenesis—receptor activation, proteoglycans in cancer, prostate cancer and other pathways, and it could be inferred that these pathways are the main regulatory pathways of the active ingredients of capsicum in the treatment of T2DM. The predicted data in this paper provide some theoretical supports for experimental verification in the later stage, and the research results will be used as a basis for further research on pharmacodynamic evaluation and serum pharmacochemistry.

Capsicum has a variety of pharmacological activities. This paper conducted a study based on the method of network pharmacology, and used relevant databases and software to screen its effective ingredients and protein targets for the treatment of T2DM through network analysis. The results show that capsicum contains active

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ingredients for the treatment of T2DM, and its specific targets need further research and experimental verification in the later stage, in order to ensure the accuracy of network pharmacological predictions. The discussion of capsicum network pharmacology can further promote the development and utilization of capsicum.

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