Original Article

Identification of key genes, biological functions, and pathways of empagliflozin by network pharmacology and its significance in the treatment of type 2 diabetes mellitus

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Background: To explore the key genes, biological functions, and pathways of empagliflozin in the treatment of type 2 diabetes mellitus (T2DM) through network pharmacology.

Methods: The TCMSP (a traditional Chinese medicine system pharmacology database and analysis platform) was used to screen empagliflozin's active components and targets. The target genes of T2DM were screened according to the GeneCards and OMIM databases, and a Venn diagram was constructed to obtain the target for T2DM treatment. Cytoscape 3.7.2 software was adopted to construct the drug-component-target-disease network. Functional annotation of Gene Ontology (GO) and enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were performed using R software.

Results: Target genes with a probability >0 were selected, among which Compound 012, Compound 060, Compound 093, Compound 111, and Compound 119 Swiss Target Prediction suggested that no similar active substances or predictable target genes were found. A "compound-target gene-disease" network was constructed, in which *SLC5A2*, *SLC5A1*, *SLC5A4*, *SLC5A11*, *ADK*, and *ADORA2A* were the core genes of T2DM. The key factors of the GO summary map included chemical reaction, membrane organelle, protein binding, and so on. The KEGG pathway summary map included the *AMPK* pathway, insulin resistance, the *MAPK* pathway, longevity-related pathway regulation, and so on. The top 10 pathways were endocrine resistance, the *NF-κB* signaling pathway, the *HIF-1* signaling pathway, apoptosis, cell senescence, the Ras signaling pathway, the *MAPK* signaling pathway, the *FoxO* signaling pathway, the *P13K-Akt* signaling pathway, and the p53 signaling pathway. The binding of active compounds to key proteins was verified based on the Swiss Dock database, and the molecular docking of 193 bioactive compounds was finally verified. Among them, *SLC5A2*, *SLC5A1*, *LDHA*, *KLK1*, *KLF5*, and *GSTP1* had better binding to the protein molecules.

Conclusions: Empagliflozin may regulate the targets of *SLC5A2*, *SLC5A1*, *LDHA*, *KLK1*, *KLF5*, and *GSTP1*. There are numerous ways of treating T2DM with empagliflozin, including by regulating apoptosis, cell aging, as well as the *NF-κB*, *HIF-1HIF-1*, *Ras*, *MAPK*, *FoxO*, *P13K-Akt*, and *p53* pathways.

Keywords: Network pharmacology; empagliflozin; type 2 diabetes mellitus (T2DM); key genes; pathway analysis

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Introduction

Type 2 diabetes mellitus (T2DM) is a complex endocrine disease, which is mainly characterized by chronic hyperglycemia and insulin resistance causing pathological changes in several organs (brain, pancreas, liver, skeletal muscle, fat, etc.) (1-3). Long-term hyperglycemia and accompanying glucose and lipid metabolism disorders are likely to induce a series of complications, such as obesity, cardiovascular and cerebrovascular diseases, nervous system diseases, urinary system diseases, digestive system diseases, endocrine and metabolic system diseases, and optic nervous system diseases (4), which significantly harms the health of patients. Therefore, the effective control of blood glucose is the key to treating T2DM (5,6).

According to the International Diabetes Federation, it is estimated that 151 million people worldwide suffer from T2DM; this number was 194 million in 2003, 246 million in 2006, 285 million in 2009, 366 million in 2011, 382 million in 2013, and 415 million in 2015 (7). In recent decades, the prevalence of T2DM in China has risen sharply, from 5.5% in 2001 to 10.9% in 2013 (8), with about 110 million people suffering from T2DM, illustrating that China has the largest number of diabetes patients in the world (9). As a result, preventative and therapeutic measures for T2DM have become important issues in our nation's health policy. At present, hypoglycemic drugs such as insulin, biguanides, thiazolidinediones, dipeptidyl peptidase IV inhibitors, sulfonylureas, α-glucosidase inhibitors, and glucagon-like-1 agonists are widely used (10).

Highlight box

Key findings

- Empagliflozin may regulate the targets of *SLC5A2*, *SLC5A1*, *LDHA*, *KLK1*, *KLF5*, and *GSTP1*.

What is known and what is new?

- Empagliflozin can reduce glomerular pressure and increase distal tubular sodium transport, and it has been considered to inhibit lipid deposition in the kidney.
- There are many ways to treat T2DM with empagliflozin, including by regulating apoptosis, cell aging, as well as the *NF-κB*, *HIF-1HIF-1*, *Ras*, *MAPK*, *FoxO*, *P13K-Akt*, and *p53* pathways.

What is the implication, and what should change now?

- Empagliflozin may regulate the *NF-κB*, *HIF-1HIF-1*, apoptosis, cell senescence, *Ras*, *MAPK*, *FoxO*, *P13K/Akt*, and *p53* signaling pathways (among others) by regulating the targets of *SLC5A2*, *SLC5A1*, *LDHA*, *KLK1*, *KLF5*, and *GSTP1*.

However, most of the existing hypoglycemic drugs often lead to some side effects, such as weight gain and hypoglycemia, which limit their use to a certain extent and make it difficult to effectively regulate metabolism (11). As a result, the research and development of new hypoglycemic drugs, such as empagliflozin, has continued. Empagliflozin, an orally-active inhibitor of the sodium-glucose co-transporter 2 (SGLT2) (12), was approved by the Food and Drug Administration (FDA) of the United States on August 1, 2014, and entered the Chinese mainland via the China Food and Drug Administration (CFDA) on September 20, 2017. Unlike traditional hypoglycemic drugs, it has a unique hypoglycemic mechanism, primarily targeting *SGLT-2* in the proximal tubule of the kidney (13). This protein belongs to the SGLT family, and together with other molecules from the same family, SGLT-1 mediates glucose reabsorption in the glomerular filtrate. The *SGLT-2* protein also plays a leading role in this process, accounting for about 90% (14). Empagliflozin can inhibit the physiological function of these proteins and reduce reabsorption, and excess glucose is excreted through the urine to improve the blood glucose levels of patients (15). Since its mechanism of action is not dependent on insulin, a considerable degree of side effects can be avoided. At the same time, recent clinical studies have shown that diabetes type 2 patients can benefit from empagliflozin by increasing high-density lipoprotein cholesterol (HDL-C) levels in peripheral blood, relieving hypertension, and reducing the risk of cardiovascular disease (16,17). It also plays a positive role in reducing the development of diabetic nephropathy. Moreover, empagliflozin can reduce glomerular pressure and increase distal tubular sodium transport, and it has also been considered to inhibit lipid deposition in the kidney (18,19). Although existing studies (17-19) have confirmed that empagliflozin has a certain regulatory effect on glucose and lipid metabolism in patients with type 2 diabetes, little is currently known about its mechanism. Recently, research shows that engegliptin can significantly reduce the renal tubular biomarker KIM-1, thus playing a protective role in patients with heart failure and reducing cardiac remodeling. These cardiovascular benefits may not be explained by the anti-inflammatory effects of engegliptin (19,20).

Network pharmacology is based on systems biology, bioinformatics, molecular pharmacology, multi-directional pharmacology, computational biology, and other emerging cross-disciplines. It utilizes high-throughput screening, network construction, and molecular exchange verification from the systematic and overall perspectives to study the

interaction between drugs and body networks, so as to reveal the mechanism of drug action (20). Cyber pharmacology is a new discipline that employs a comprehensive, relevant, and diverse approach that uses the target as the research strategy, which is contrary to the previous "single ingredient-single target-single disease" concept of drug development. Network pharmacology is an effective method of revealing the relationship between the action targets of drugs and diseases' targets through network construction at different levels and observing the intervention and effects of complex drugs on multi-target diseases at the micro level. In this study, a network pharmacology approach was applied to screen empagliflozin's active components and targets, the related targets of known T2DM cases were searched, and the potential pathway of empagliflozin's intervention on type 2 diabetes was explored via the Database for Annotation, Visualization, and Integrated Discovery (DAVID) database and Clue Go analysis to provide new ideas for the clinical treatment of T2DM. We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6406/rc).

Methods

The data sources and analysis platforms used in this study included the traditional Chinese medicine system pharmacology database and analysis platform (TCMSP) as well as the GeneCards, OMIM, UniProt, and String databases. TCMSP includes chemicals, targets and drug-target networks, and associated drug-target-disease networks, as well as pharmacokinetic properties for natural compounds involving oral bioavailability, drug-likeness, intestinal epithelial permeability, blood-brain-barrier, aqueous solubility, etc. This breakthrough has sparked a new interest in the search of candidate drugs in various types of traditional Chinese herbs. We also utilized Cytoscape (version 3.7.2, Institute for Systems Biology, Washington, USA) and R (version 3.8.6, R Core Team, Vienna, Austria). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Research method

The potential target acquisition of compound Swiss Target Prediction was based on the similarity to the two- and three-dimensional structures of known compounds to predict the target genes of compounds, which could be respectively predicted in human, rat, and mouse species. The International Chemical Identifier (InChI) of all compounds was converted into Simplified Molecular-Input Line-Entry System (SMLIES), the target of the compound was predicted by Swiss Target Prediction, and the human target protein was queried using the UniProt database. The target protein was then converted into the corresponding target gene, and finally, the abbreviated name of the target gene was obtained.

The disease potential target was obtained using "type 2 diabetes mellitus" as the key phrase, and the T2DM-related disease target gene information was screened using the GeneCards and OMIM databases. The repetitive gene was deleted from Excel (Microsoft Corporation, USA) and combined to obtain the target gene of T2DM. Next, the T2DM target gene information was matched with the target gene of empagliflozin to obtain the common target gene, which was the potential target gene for treating T2DM.

A compound-target gene network map was constructed using the relationship between the compound corresponding genes and the disease differential genes predicted by Swiss Target Prediction and the Super Pred database. The compound-target gene-disease relationship table was obtained, the relationship was visually displayed by Cytoscape 3.7.2, and the "compound-target gene-disease" network map was constructed.

A protein interaction network was constructed, the compound target and T2DM target were intersected, and the intersection target was uploaded to the STRING database (https://cn.string-db.org/) to build the protein-protein interaction (PPI) network. The species was set to "Homo sapiens", the PPI network was obtained, and the PPI network TSV file was imported into Cytoscape 3.9.0 software for visualization.

Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

GO enrichment analysis (http://www.geneontology.org) is a comprehensive database established by the Union of Gene Ontology, which classifies and summarizes all gene-related research results worldwide. In this database, genes and their products are standardized based on the biological terms of genes and proteins, which can be applied to a variety of species and describe the functions of genes and proteins in a standardized and meaningful manner. GO is the internationally accepted classification system for describing gene functions. By intersecting the disease genes and drug target genes to obtain the intersection target genes, an

analysis of GO gene distribution in the experiment would clarify the embodiment of sample differences in the gene functions.

Statistical analysis

According to the GO functional enrichment analysis, the GO items of the intersecting target gene are noticeably enriched compared to the genomic background, which facilitates the determination of the biological functions of the target genes that are significantly related. In this analysis, all the intersection target genes were first mapped to each GO term (http://www.geneontology.org/). Moreover, each term was counted for the number of genes, and then the hypergeometric test or other statistical methods were employed to discover the GO items that were significantly enriched in the intersecting target genes compared with the whole genome background. The formula for calculating the hypergeometric distribution score was as follows (21):

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}} \quad [1]$$

In this equation, N represents the total number of genes with GO annotation, n denotes the number of intersection target genes in N, M is the general number of genes annotated with that specific GO term, and m is the number of intersection target genes annotated as a particular GO term.

GO annotation of intersection target genes

The enriched GO data were screened, and $P<0.05$ was set as the standard.

KEGG enrichment analysis in organisms

Different genes coordinate with each other to exercise their biological functions, and the most important biochemical metabolic pathway and signal transduction pathway involved in differentially expressed genes can be determined by analyzing the significantly enriched pathway. The KEGG (http://www.genome.jp/kegg/) database is a large knowledge base for the systematic analysis of gene function, contact genome information, and functional information. Genome information on the KEGG database is mainly obtained from databases, such as the National Center for Biotechnology Information (NCBI), including complete and partially sequenced genome sequences, which are stored in the KEGGGENES database. A more advanced form of functional information involves graphical representations of cellular processes such as metabolism, membrane transport, signal transduction, cell cycle, etc., as well as homologous conserved sub-pathways, which are stored in the KEGGPATHWAY database.

In addition, information about chemical substances, enzyme molecules, enzymatic reactions, and other related information is stored in the KEGG database. In organisms, different genes were analyzed for their specific biological functions through orderly coordination. Therefore, by analyzing the abundant pathway information in the KEGG database, we can better understand the biological functions of genes, such as metabolic pathways, genetic information transmission, cellular processes, and other complex biological functions, which significantly improve the value of the database in terms of practical production and application.

KEGG pathway annotation of the intersecting target genes screened the enriched KEGG pathway data with $P<0.05$.

Molecular docking selected empagliflozin as the ligand and predicted the target gene (intersection gene) acting on the disease as the receptor for molecular docking verification. The program database (PDB) file of the 3D structure of the key target protein was downloaded from the PDB database, and the PDB file of the key target protein 3D structure was dehydrated and hydrogenated using PyMOL (Version 2.0, Schrödinger L & DeLano W, Oregon, USA). The compound ligand utilized the RD Kit to convert its corresponding SMILES into a 3D structure PDB file. Finally, the PDB file of receptor and ligand was converted into Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) format using MGLTOOLS (version 1.5.7, The Scripps Research Institute, La Jolla, CA, USA). Then, using the protein as a receptor and the small molecule as a ligand, the active pocket with the highest score of drug Score was selected as the active site of molecular docking according to the prediction results of Proteins Plus, and the binding ability was predicted by Auto Dock Vina software (version 4.2, The Scripps Research Institute, La Jolla, CA, USA). Low binding energies were associated with more stable conformations.

Results

Active components and target screening selected target genes with a probability >0, in which Compound 012, Compound 060, Compound 093, Compound 111, and Compound 119 Swiss Target Predictions suggested that

Table 1 The activated targets of the compounds

Drug	Gene
Empagliflozin	*SLC5A2*
Empagliflozin	*SLC5A1*
Empagliflozin	*SLC5A4*
Empagliflozin	*SLC5A11*
Empagliflozin	*ADK*
Empagliflozin	*ADORA2A*

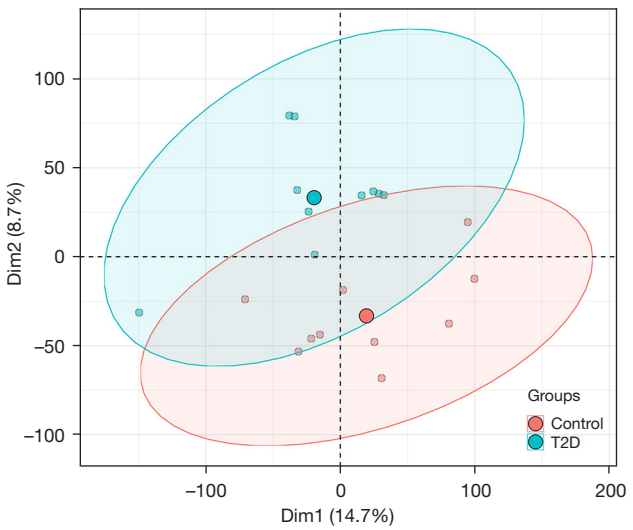

Figure 1 PCA analysis of T2D and control groups. PCA, principal component analysis. T2D, type 2 diabetes.

no similar active substances or predictable target genes were found. The Super Pred database was constructed by extracting the compound-target interaction data from Super Target, chemical database of the European Molecular Biology Laboratory (ChEMBL), and Binding Database (Binding DB). Super Pred was used for Extended-Connectivity Fingerprints (*ECFP*) molecular fingerprints to calculate structural similarity and remove some weakly bound compound-protein interactions [such as inhibitory constant (Ki), inhibitory concentration 50% (IC_{50}) >10 μM]. The database contained about 341,000 compounds, 1,800 targets, and 665,000 compound-target interactions. The SMLIES of all compounds was predicted using Super Pred, and the action targets of the compounds were obtained. After combining the results, all of the target genes were obtained: *SLC5A2*, *SLC5A1*, *SLC5A4*, *SLC5A11*, *ADK*, and *ADORA2A* (*Table 1*).

Disease target screening was searched with "type 2 diabetes mellitus" as the keyword in the Gene Expression Omnibus (GEO) database. The disease-related gene set GSE20966 was selected, and the gene expression matrix was obtained. Subsequently, we used principal component analysis (PCA) to reduce the dimension of clustering (*Figure 1*). Differentially expressed genes were analyzed using Limma, and the differentially presented genes were screened for |log2 (FC)| >0.5 Magi Rdegdegp (*Table 2*). Ggplot2 was applied to draw the volcano map of the difference analysis results, in which red represented the up-regulated differential genes, blue represented the down-regulated differential genes, and gray denoted genes that did

Table 2 The lists of DEGs

Gene	logFC	AveExpr	*t*	P value	adj.P.val	B
MDFIC	−1.4982752	6.212814	−8.398945	0.0e+00	0.0007706	8.126831
EFHD2	−0.9313277	5.303572	−7.703826	1.0e-07	0.0015536	6.999102
FXYD3	−1.0418585	7.136242	−6.921565	7.0e-07	0.0053737	5.632009
PCOLCE2	−3.1995486	6.824960	−6.727917	1.1e-06	0.0061347	5.277615
IGFBP3	1.1066074	5.593753	6.437339	2.2e-06	0.0093031	4.734203
SLC2A2	1.8595008	7.277278	6.321798	2.8e-06	0.0100271	4.51432

Only the top six items are shown in the table above. DEGs, differentially expressed genes; logFC, fold of difference after taking $\log_2$; AveExpr, mean expression after taking log2; adj.P.val, corrected P value; B, pair of probabilities of differential gene expression.

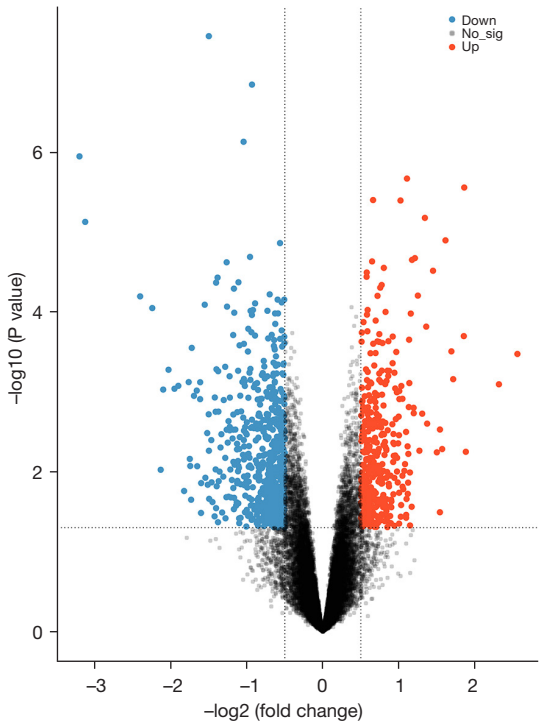

Figure 2 The heap map of DEGs. DEGs, differentially expressed genes.

not meet the differential gene screening criteria (*Figure 2*). The first 30 genes were selected in ascending order of their P values, and a heat map was drawn using the heatmap package (*Figure 3*). In total, 1,038 potential disease targets were identified.

The prediction of potential drug targets on the disease was analyzed using python (version 2.7, Scotts Valley, CA, USA), and 170 drug targets and 1,038 disease targets were utilized to draw a Wayne diagram. After intersecting the two, ten drug-disease common targets were identified, including 160 drug-specific target genes and 10 overlapping target genes (see *Figure 4*).

Compound-target gene network diagram

The relationship between target genes and disease differential genes predicted using the Swiss Target Prediction and the Super Pred database was used to obtain *Table 3*, and the relationship was visually displayed by Cytoscape 3.7.2 to construct a "compound-target gene-disease" network map (*Figure 5*). Among them, *SLC5A2*, *SLC5A1*, *SLC5A4*, *SLC5A11*, *ADK*, and *ADORA2A* were identified as the core genes of T2DM.

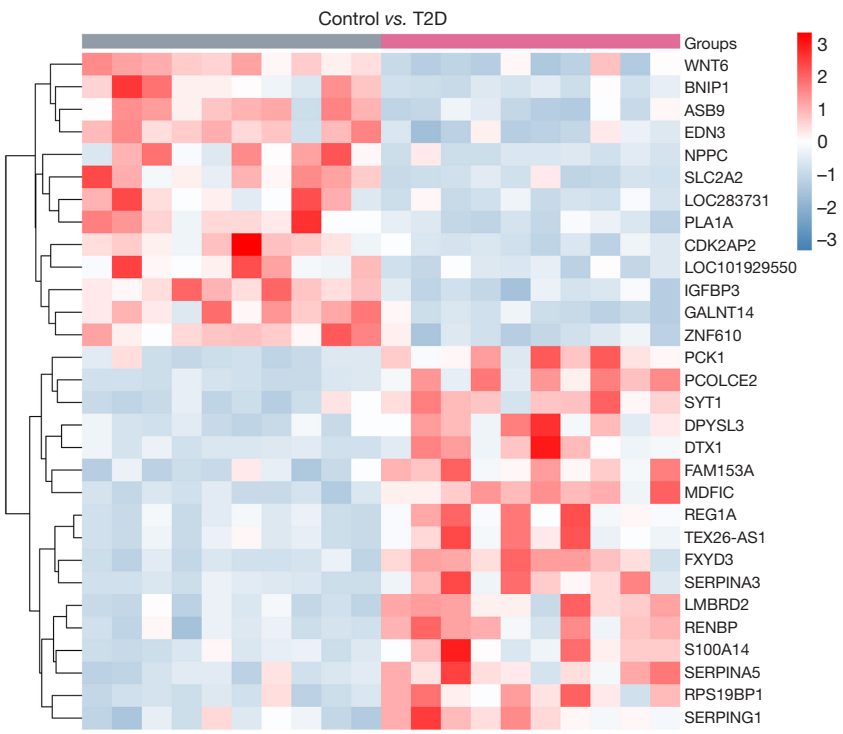

Figure 3 Heat map of the top 30 differential genes in ascending P value order. T2D, type 2 diabetes.

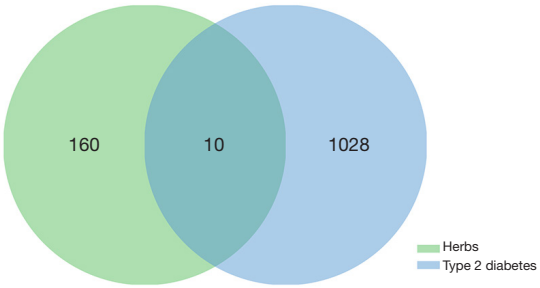

Figure 4 Venn diagram of drug and disease target genes.

Table 3 Compound-target gene-disease relationship list

Drugs	Target
Empagliflozin	SLC5A2
Empagliflozin	SLC5A1
Empagliflozin	SLC5A4
Empagliflozin	SLC5A11
Empagliflozin	ADK
Empagliflozin	ADORA2A

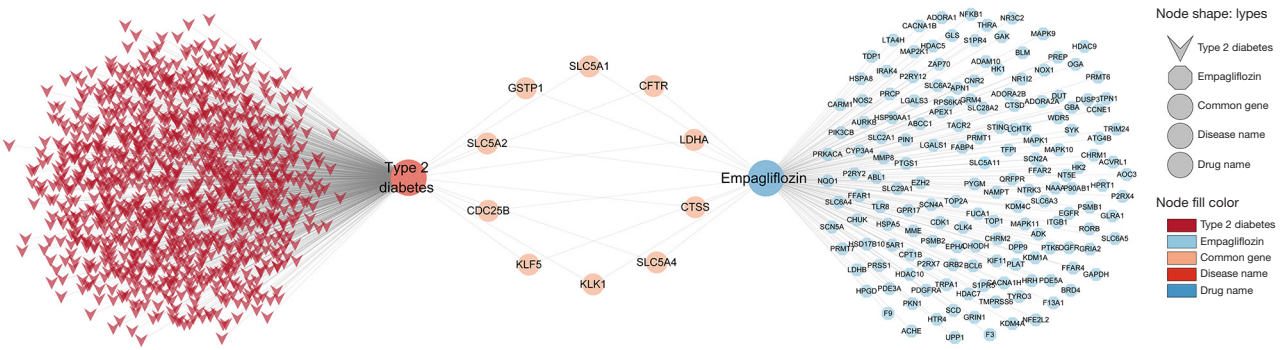

Figure 5 Compound-target gene network diagram.

GO enrichment analysis

The enriched GO data were screened according to P<0.05. Cellular components, biological processes, and molecular functions were all included in the GO process. The GO enrichment analysis bubble chart and GO enrichment analysis chord diagram are shown in *Figures 6*,*7*. The key parts of the GO summary map included chemical reactions, membrane organelles, protein binding, etc. The KEGG pathway summary map included the AMP-activated protein kinase (*AMPK*) pathway, insulin resistance, the mitogen-activated protein kinase (*MAPK*) pathway, longevity-related pathway regulation, and so on.

KEGG pathway enrichment analysis

The enriched KEGG pathway data were screened according to P<0.05. The KEGG enrichment analysis results showed that there were 168 pathways involved in 58 potential target genes. The top 10 pathways included endocrine resistance, the nuclear factor-kappa B (*NF-κB*) signaling pathway, the hypoxia-inducible factor-1 (*HIF-1*) signaling pathway, the apoptosis pathway, the cell senescence pathway, the Ras pathway, the mitogen-activated protein kinase (*MAPK*) signal pathway, the FoxO signaling pathway, the Phosphatidylinositol-3-kinase (*P13K/Akt*) pathway, and the p53 signaling pathway (*Figure 8*).

Molecular docking results

The binding of active compounds to key proteins was verified based on the Swiss Dock database, and the molecular docking of 193 bioactive compounds was finally verified. Among them, *SLC5A2*, *SLC5A1*, *LDHA*, *KLK1*, *KLF5*, and *GSTP1* had better binding to protein molecules (*Figure 9*).

Discussion

Diabetes is a chronic disease characterized by hyperglycemia. There are several types of diabetes, among which T2DM is the most common. In recent years, the prevalence and incidence of this disease have increased every year. Moreover, T2DM causes irreversible damage to

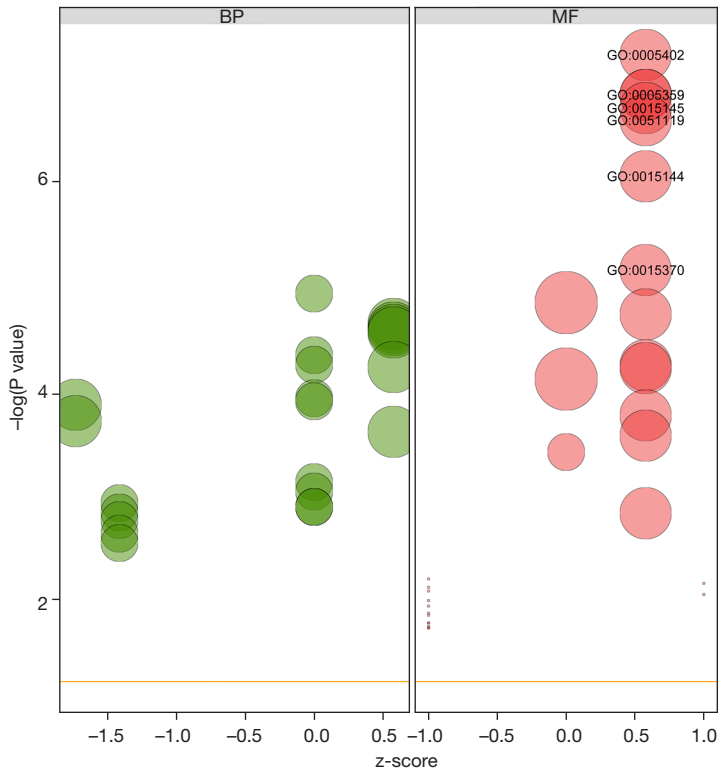

Figure 6 GO enrichment analysis bubble chart. BP, biological progress; MF, molecular function; GO, Gene Ontology.

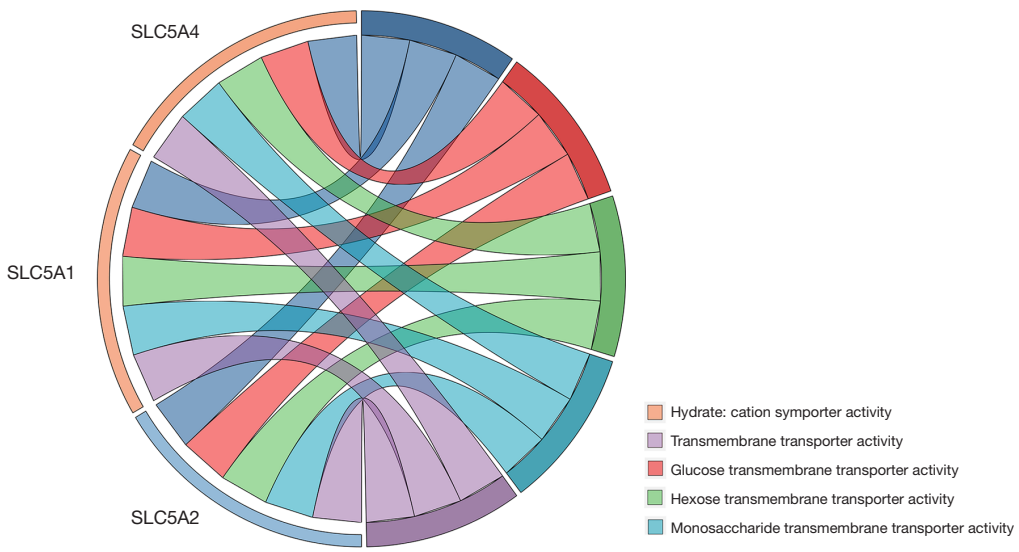

Figure 7 Chord diagram of GO enrichment. GO, Gene Ontology.

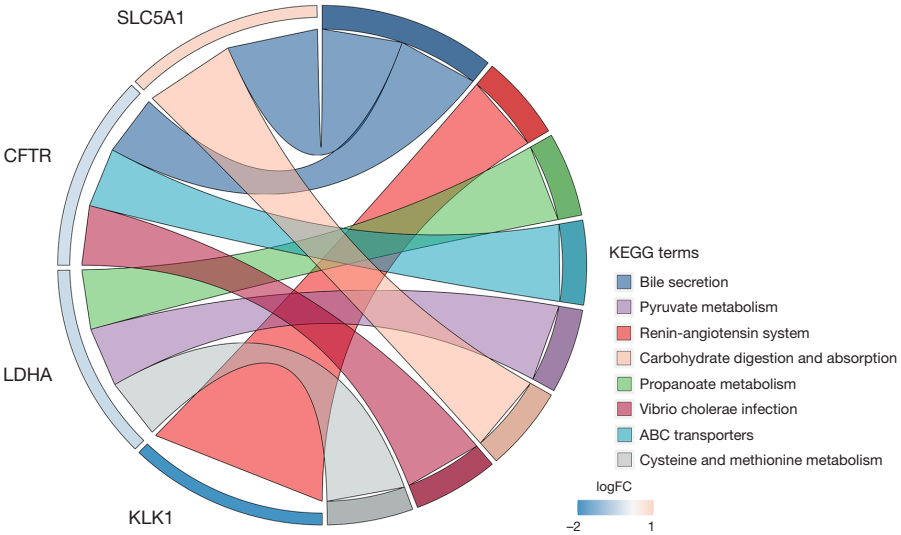

Figure 8 Chord diagram of KEGG enrichment. KEGG, Kyoto Encyclopedia of Genes and Genomes; ABC, ATP-binding cassette; FC, fold change.

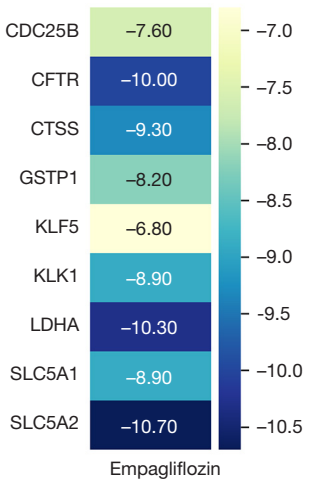

Figure 9 The heap map of molecular docking binding energy.

the cardio-cerebrovascular system, kidneys, and eyes, which has seriously endangered the lives and health of patients. In the past decade, a large number of basic and clinical studies on hypoglycemic drugs have been carried out both in China and abroad, with promising results. Given the variable etiology of T2DM, its pathogenesis is highly complex and there are many complications; thus, more attention should be paid to the treatment of this disease and its complications. At present, conventional hypoglycemic drugs, such as sulfonylureas, glinides, and biguanides, are used to treat T2DM. However, all of these drugs have therapeutic limitations, including hypoglycemia, digestive tract reactions, etc., and the long-term use of drugs can easily result in a reduced curative effect (22). In addition, since most conventional drugs require secondary use, patient compliance can be easily diminished. Therefore, there is an urgent need for new hypoglycemic drugs or treatments to strengthen the control of blood sugar.

In addition to insulin, the main drugs used in the treatment of T2DM include biguanides, thiazolidinediones, dipeptidyl peptidase-IV inhibitors, sulfonylureas, α-glucosidase inhibitors, glinides, and glucagon-like peptide-1 receptor agonists. However, in controlling blood sugar, most drugs also result in inevitable side effects, such as hypoglycemia and weight gain (23), and some may even increase the incidence of cardiovascular disease in patients with T2DM (24). In the past few years, the *SGLT-2* inhibitor, as a new type of hypoglycemic drug, has attracted widespread attention worldwide owing to its application in T2DM. Empagliflozin is a kind of inhibitor; the *SGLT-2* protein is mainly distributed in the S1 segment of the renal proximal tubule, which mainly inhibits the special mechanism of glucose reabsorption in the renal proximal convoluted tubule and promotes urinary glucose excretion to achieve a hypoglycemic effect (25,26). Therefore, the problem of poor blood glucose control caused by

conventional oral hypoglycemic drugs in T2DM patients can also be resolved. In 2017, empagliflozin was approved as a therapeutic drug for T2DM by the State Food and Drug Administration. At present, *SGLT-2* inhibitors have shown good results in treating T2DM, and their clinical efficacy with or without complications has been confirmed (27). In addition to reducing blood sugar, empagliflozin can also improve islet function and affect blood lipids. Weight loss and blood pressure reduction play a role in multiple organ protection, including in the cardiovascular system (28) and the kidneys (29-32), which play a role in delaying the occurrence and development of T2DM complications. Some studies (30-32) have shown that *SGLT-2* inhibitors have a lower incidence of major cardiovascular adverse events, heart failure, and mortality in patients with T2DM during clinical use. Due to these significant effects, *SGLT-2* inhibitors are also recommended as first-line oral hypoglycemic agents for T2DM complicated with coronary heart disease (30). Currently, this kind of drug has also become a particular focus of research; however, its treatment mechanism in T2DM is not clear. However, common side effects of empagliflozin include: urinary tract infection, vulvovaginal candidiasis, cervicitis, genital candidiasis, genitourinary infection, vaginal infection, vulvitis, and vulvovaginitis. Continue reading for a comprehensive list of adverse effects.

In this study, the compounds of T2DM treatment were analyzed using a network pharmacology method. The activated targets of the compounds were also obtained. After combining the results, the target genes were identified as SLC5A2, SLC5A1, SLC5A4, SLC5A11, ADK, and ADORA2A. In total, 1,038 potential disease targets were identified. Python was utilized to draw a Wayne map of 170 drug targets and 1,038 disease targets. After intersecting the two, 10 drug-disease common targets were identified, including 160 drug-specific target genes and 10 overlapping target genes. A "compound-target gene-disease" network map was built, in which *SLC5A2*, *SLC5A1*, *SLC5A4*, *SLC5A11*, *ADK*, and *ADORA2A* were the core genes of T2DM. The KEGG pathway summary map included the AMPK pathway, insulin resistance, the *MAPK* pathway, longevity-related pathway regulation, and so on. Moreover, there were 168 pathways associated with 58 possible target genes according to the KEGG enrichment analysis. The top 10 pathways included endocrine resistance as well as the *NF-kappa B*, *HIF-1HIF-1*, *apoptosis*, *cell senescence*, *Ras*, *MAPK*, *FoxO*, *P13K/Akt*, and *p53* signaling pathways. The binding of active compounds to key proteins was verified based on the Swiss Dock database, and the molecular docking of 193 bioactive compounds was finally verified. Among them, *SLC5A2*, *SLC5A1*, *LDHA*, *KLK1*, *KLF5*, and *GSTP1* had better binding to the protein molecules.

In the GO enrichment analysis results, we found that it was mainly involved in endothelial cell apoptosis, DNA transcription, RNA polymerase II promoter transcription, major histocompatibility complex (MHC) class II biosynthesis, the process of peptide serine phosphorylation, and the lipopolysaccharide-mediated signaling pathway. Diabetic angiopathy, including macrovascular and microvascular lesions, is the main cause of death and disability in patients with diabetes. Vascular endothelial dysfunction is an important factor leading to diabetic angiopathy. Apoptosis is an active method of programmed cell death, which is the main cause of vascular endothelial injury in diabetes. The KEGG pathway analysis results showed that the key prediction targets were mainly concentrated in the advanced glycation end products (AGE-RAGE), AMPK, and vascular endothelial growth factor (VEGF) pathways. These pathways primarily play a positive role in oxidative stress, inflammation, and lipid metabolism, and the above pathways are consistent with the current research results, which confirms that the predicted results of this study are reliable. Empagliflozin may regulate the *NF-kappa B*, *HIF-1HIF-1*, *apoptosis*, *cell senescence*, *Ras*, *MAPK*, *FoxO*, *P13K/Akt*, and *p53* signaling pathways (among others) by regulating the targets of *SLC5A2*, *SLC5A1*, *LDHA*, *KLK1*, *KLF5*, and *GSTP1*.

Conclusions

In summary, the regulation of endothelial cell apoptosis, DNA transcription, and RNA polymerase II promoter transcription are the potential mechanisms of empagliflozin in the treatment of T2DM. Its molecular function is mainly related to the activity of cytokines. In addition, some items have also shown that it is related to inflammation and angiogenesis, which provides a scientific basis for elucidating the mechanism of empagliflozin in the treatment of T2DM.

However, there were some limitations in this study that should be noted. Primarily, the predicted active components, key targets, and related pathways have not been verified experimentally. To further explore its complex process and mechanism, more in-depth experimental studies will be carried out in the future, combined with the above results.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6406/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6406/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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