Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Mechanistic Insights Into *Ganoderma Lucidum* for Diabetes Treatment via Network Pharmacology and Validation

Shengxiang Guo[1–3,*], Lan Yang[4,*], Jiali Zhou[1–3], Wu Luo[1–3], Beibei Nie[1–3], Xiaohong Zhong[1,5], Dongbo Liu[1–3,5], Xincong Kang[1–3]

[1]College of Horticulture, Hunan Agricultural University, Changsha, 410128, People's Republic of China; [2]State Key Laboratory of Subhealth Intervention Technology, Changsha, 410128, People's Republic of China; [3]Hunan Provincial Engineering Research Center of Medical Nutrition Intervention Technology for Metabolic Diseases, Hunan Agricultural University, Changsha, 410128, People's Republic of China; [4]School of Pharmacy, North Sichuan Medical College, Nanchong, 637000, People's Republic of China; [5]National Research Center of Engineering Technology for Utilization of Botanical Functional Ingredients, Hunan Agricultural University, Changsha, 410128, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xincong Kang; Dongbo Liu, College of Horticulture, Hunan Agricultural University, No. 1 Nongda Road, Furong District, Changsha, 410128, People's Republic of China, Tel +86-0731-8461-7068, Email kangxincong@163.com; chinasaga@163.com

Purpose: There is an urgent need to develop antidiabetic medications with minimal side effects and low toxicity. *Ganoderma lucidum*, a food-medicine homologous in China, has been used to treat diabetes. This study was aimed to explore the active ingredients and mechanism of *G. lucidum* in the treatment of diabetes.

Materials and Methods: Relevant compounds and targets of Ganoderma were collected from the TCMSP database, BATMAN-TCM database, relevant literature and PubChem. A diabetes-related target database was constructed using TTD, BATMAN-TCM, and Uniprot. A PPI network and H-C-T-P network were constructed to analyze interactions among these targets. GO and KEGG enrichment analyses were performed using WebGestalt. Molecular docking of the core compounds and key targets was carried out using AutoDock Vina. The predicted key targets were verified via qRT-PCR in PA-induced HepG2 cells, using GLAE (ethanol extract of Ganoderma lucidum) as the treatment.

Results: A total of 58 compounds were screened out in *G. lucidum*, of which 17 had predicted targets. *G. lucidum* was involved in metabolic processes, such as lipid binding, insulin secretion, and other pathways. Molecular docking results showed that the core component β-sitosterol had strong binding activity with key targets CASP3, PRKACA, and PGR. Based on the results of network pharmacology, the top 10 targets related to glucose and lipid metabolism were selected for validation. The results indicated that in a high-fat environment, glucose and lipid metabolism in HepG2 cells was improved, with decreased mRNA expression of CASP3, PRKACA, CYP19A1, NR3C1, JUN, and increased expression of PGR and RXRA.

Conclusion: Glucose and lipid metabolism are important for the anti-diabetic activity of *G. lucidum*. A strong interaction of *β-sitosterol* with CASP3, PRKACA, and PGR, which may be related to cell apoptosis, gluconeogenesis and insulin secretion, etc. This study lays the foundational groundwork for future drug development and therapeutic optimization.

Keywords: *Ganoderma lucidum*, diabetes, insulin resistant, network pharmacology, molecular docking, cell

Introduction

Diabetes mellitus is one of the most common chronic diseases worldwide, with the increase in obesity due to lifestyle changes leading to its continued rise. According to the International Diabetes Federation (IDF) report from 2021, the estimated prevalence of Diabetes mellitus in the global population aged 20–79 was 10.5% (536.6 million people) in 2021 and is projected to rise to 12.2% (783.2 million people) by 2045.[1] Currently, the main medications to treat Diabetes mellitus available on the market include rosiglitazone, liraglutide, and dapagliflozin, etc. Long-term use of these drugs

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can lead to serious side effects and complications, such as diabetic macular oedema and retinopathy.[2] There is an urgent need to develop antidiabetic medications with minimal side effects and low toxicity.

Ganoderma lucidum, a traditional Chinese medicine (TCM), is regarded as a food-medicine homologous in China since 2023.[3,4] It shows antidiabetic effect in vitro and in vivo by effectively improving insulin resistance, hyperglycemia, and oxidative stress, which are important factors in the occurrence and progression of diabetes.[5–10] Based on clinical research, *G. lucidum* can reduce plasma insulin levels and the homeostasis model assessment of insulin resistance (HOMA-IR). Additionally, it can enhance antioxidant capacity and regulate redox balance in the body, thereby improving insulin secretion.[11] Some bioactive molecules in *G. lucidum*, such as polysaccharides, glycoproteins, proteins, triterpenoids and adenosine, are reported to perform hypoglycemic activity by regulating glucose and lipid metabolism, decreasing inflammation levels, reversing gut dysbiosis and improving antioxidative effect.[9,12]

Nowadays, "one key, one lock" mode is insufficient to decipher the drug actions, especially in those complex medicines, like traditional Chinese medicine, and complex diseases, such as diabetes. Network pharmacology uses computational power to analyze drugs and drug targets in a systemic manner, attempting to understand drug actions and interactions with multiple targets.[13] The key ideas of network pharmacology, multicomponent, multichannel, and multi-objective synergies, sharing much with the basic disciplines of TCM, make it a well-suited tool for the systematic and comprehensive analysis of the mechanisms of TCM.[14] Therefore, network pharmacology is useful to discover new drug leads and targets and to repurpose existing drug molecules for different therapeutic conditions by allowing an unbiased investigation of potential target spaces.[15]

In this study, we utilized network pharmacology to predict the active ingredients and targets of *G. lucidum* treating diabetes mellitus. An in vitro model of metabolic syndrome was induced by palmitic acid (PA) in HepG2 cells, while the ethanol extract of Ganoderma lucidum (GLAE) was used as a therapeutic agent. We assessed the expression of predicted key targets after GLAE treatment. This study offers detailed insights into the active components and mechanisms of *G. lucidum*, providing a basis for targeted and effective diabetes treatments. It could also lead to new avenues for drug development and therapeutic optimization.

Material and Methods

Collection and Filtration of Bioactive Components in G. Lucidum

The components of *Ganoderma lucidum* were collected from TCMSP (TCMSP – Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (tcmsp-e.com)) databases, BATMAN-TCM (HomePage – BATMAN (ncpsb.org.cn)) databases and literatures, then were filtered according to the ADME attribute values by setting the parameters of OB (oral bioavailability) ≥30%, DL (drug likeness) ≥0.18, and Caco-2 (Caco-2 cell permeability) ≥-0.4.[16] The components meeting the screening criteria were considered as potential active components. At last, the structures of the potential bioactive components were confirmed in PubChem (PubChem (nih.gov)) Database.

Targets Collection Corresponding to Active Components of G. Lucidum

The targets corresponding to potential active components filtered above were predicted by the TTD (TTD: Therapeutic Target Database (idrblab.net))[17] and BATMAN-TCM database on account of a similarity-based target prediction method based on chemical similarity to the known drug–target interactions by setting the parameter of Score cutoff >20. Afterward, the obtained targets were matched to the UniProt database platform (UniProt) for normalization with a gene format of Homo sapiens, and redundancy and non-Homo sapiens were deleted.

Targets Screening Related to Diabetes and Putative Therapeutic Targets of G. Lucidum

Diabetes mellitus related target proteins were mined from Genecards databases (GeneCards – Human Genes | Gene Database | Gene Search) with the searching keyword "Diabetes mellitus" and then filtered by the threshold of the exceed median "Relevance score". Then, Diabetes mellitus related targets and components related targets were mapped by Venn 2.0 (Venny 2.1.0 (liuxiaoyuyuan.cn)), and the mutual targets are deemed as putative therapeutic targets of *Ganoderma lucidum* in treatment Diabetes mellitus.

The Network Construction of Protein-Protein Interaction (PPI)

Protein–protein interaction (PPI) gets involved in various cellular biochemical reaction processes such as bio-signal transmission, gene expression regulation, energy and substance metabolism and cell cycle regulation. PPI network and transcriptional regulation network are of great significance for regulating cells and their signals. Systematic analysis of the PPI is of great significance for understanding the biological response processes of specific pathological state of disease altering by drugs, furtherly elucidating the mechanism of treatment. The interaction of 67 potential therapeutic targets of *Ganoderma lucidum* in Diabetes mellitus treatment was analyzed and visualized on Gene MANIA online platform (GeneMANIA).

Gene Function and Pathway Enrichment Analysis

The Web Gestalt (WebGestalt (WEB-based GEne SeT AnaLysis Toolkit)) is a popular analysis web tool for the interpretation of gene lists and functional enrichment. It can provide multiple types of interactive and result visualizations of publication-ready figures freely and user-friendly.[18] Gene function and pathway enrichment analysis of the therapeutic target proteins and genes of *Ganoderma lucidum* in Diabetes mellitus treatment were performed on the Web Gestalt online platform with the newest version. The relevant parameters were set as follows, Organism: homo sapiens, Method: Over-Representation Analysis (ORA), Functional Database: Gene Ontology (GO) and the Kyoto Genetic and Genomics Encyclopedia (KEGG) database, Select reference set: genome protein-coding, significance level: FDR with 0.05. Keep the other default values and then submit.

Construction of "Herb-Bioactive Compound-Target-Pathway" Network

In order to further understand the intricate relationship among herb, bioactive components, targets and disease, a "herb-bioactive components-target-pathway network" (H-C-T-P) construction was performed as follows: firstly, pairing of *Ganoderma lucidum* and filtered bioactive components, bioactive components and corresponding targets, the targets and their related enrichment pathways were linked together and listed in a worksheet sequentially. Secondly, the "H-C-T-P" network of *Ganoderma lucidum* was constructed and visualized using the software Cytoscape 3.6.0.[19] Cytoscape is an open-source software platform for complex network construction, analysis and visualization. It can present the relations between elements intuitively via the form of connection, nodes and network on the whole. In addition, it can provide different algorithms to analyze the constructed network, and more important nodes in the network can be screened from multi-angles via setting related parameter. Degree is an index which represents the number of edges between a node and another node in the network. In the present work, degree was applied to evaluate every node in the "H-C-T-P" network, Network Topological Feature is the larger the degree value, then larger node icon.

Filtration of Key Targets

In order to further understand the target proteins that play a major role in the protein–protein network, a total of 12 different algorithms and related indices were applied to filter key targets among the 67 putative therapeutic targets using Cytoscape software plugins cytoHubba. The 12 indices are "maximum clique centrality" (MCC), "the density of the maximum neighbor component" (DMNC), "maximum neighbor component" (MNC), Degree (The connection degree of node), Closeness, "Edge permeate component" (EPC), BottleNeck, EcCentricity, Radiality, Betweenness, "Stress and Clustering Coefficient", respectively. The level of these 12 indices had a positive association with the importance of node in the network and so can be used to filter the hub targets in the PPI network. The top 10 targets were selected by each algorithm and regarded as candidate key targets from protein–protein network, then 120 key targets under 12 algorithms were obtained, counted and sorted by frequency.

Through Comparison of the top 10 key targets filtered from "H-C-T-P" network by degree value and the PPI network by 12 different algorithms, the intersection of the two groups of candidate key protein targets was taken as the key therapeutic targets of *Ganoderma lucidum* in Diabetes mellitus treatment.

Molecular Docking

From the "H-C-T-P", core components were obtained. The key targets were identified from the intersection of the top 10 targets from "H-C-T-P" and "PPI". The molecular docking was conducted on the core components and key targets through "Autodock Vina". The 3D structure of β-sitosterol was obtained from the PubChem database. The 3D structures of CASP3, PRKACA, and PGR were retrieved from the Protein Data Bank (PDB). The β-sitosterol molecule was subjected to the removal of water molecules and the addition of hydrogen atoms, followed by an analysis of the resulting structure.

Cell Cultures

Human hepatic HepG2 cells (Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM/high glucose supplement with 10% FBS, penicillin G (100 U/mL), and streptomycin (100μg/mL) at 37°C in a humidified atmosphere with 5% CO_2. The cells were subcultured and seeded into 6/24/96-well plates when the cell density in the T25 culture flasks reached 85%–95%. The experimental units were randomly allocated to the control and treatment groups, and then the principle of experimental blinding was applied. After the cells have adhered to the wells, the treatment groups are administered with the corresponding concentrations of GLAE and pre-treated for 24 hours. Following this, the induction is carried out by adding 0.2 mm PA for another 24 hours according to the group assignments. The experimental groups were as follows: Control, Model (0.2mM PA), and low, medium, high concentration (20 μg/mL, 40 μg/mL, 80 μg/mL) GLAE treatment groups.

Cell Viability Assay

HepG2 cells are seeded into a 96-well plate and are allowed to attach, reaching a density of 50–60%. Then, the cells are treated individually with PA (0.1–1.6 mm) and GLAE (1–100 μg/mL), as well as pre-treated with GLAE for 24 hours. Following the instructions of the CCK8 kit, 10 μL of 10% CCK8 solution is added to each well and incubated at 37°C with 5% CO_2 for 30 minutes. The absorbance at 450 nm is measured using a microplate reader to determine cell viability. Cell viability is calculated using the following formula: Cell Viability (%) = (treated group/control group) × 100%.

Glucose Consumption Assay

Cells were grouped according to the experimental design and treated with the corresponding drugs for a specified period. Afterward, the cell culture medium was collected, and the glucose content in the medium was measured using a glucose detection kit (Nanjing Jiancheng, Cat#A154-1-1). The remaining amount of glucose in the medium was calculated following the instructions provided with the kit. The original glucose content was subtracted from the remaining amount to determine the amount of glucose consumed by the cells. Use the CCK8 assay to assess cell viability in a 96-well plate, calibrating by dividing the amount of glucose consumed by the CCK8 readings.

Determination of Total Triglyceride and Cholesterol

For TG/TC measurement, the TG and TC assay kits (Nanjing Jiancheng, Cat#A110-1-1/A111-1-1) are used. Cells were seeded into 6-well plates and treat them according to the model establishment and treatment steps described above, five groups of cell samples are collected: control group, model group, and low/medium/high-dose treatment groups. The samples are then prepared according to the instructions provided, and the concentrations of TG and TC in the samples are measured. The results obtained will be compared with the control group to calculate the differences between each group and the control group.

Immunofluorescence Assays

Immunofluorescence staining was used to observe the total amount of GLUT2 protein and its translocation on the membrane of HepG2 cells. HepG2 cells were plated on 10 mm glass coverslips in a 24-well plate. The cells were modeled and treated according to the method described in "Cell cultures". Upon completion, the cells were gently washed 3 times with PBS. Cells were fixed in freshly prepared 4% paraformaldehyde at room temperature for 25 min. Coverslip was rinsed with PBS 3 times for 3 min each. For 30 min, 10% goat serum was used to block at room temperature, and then the goat serum was discarded.

Anti-GLUT2 antibody (PTM Bio, Cat#PTM-6209) was added, and the cells were incubated at 37°C for 1 h protected from light. After washing, cells were incubated with the Alexa Fluor 488-conjugated Donkey anti-Goat IgG (H+L) at 37°C for 1 h protected from light. The coverslips were mounted on microscope slides and sealed with an antifade solution, and nuclei were stained with DAPI. Images were captured using a fluorescence microscope (Leica DM 4000, Germany).[20]

RNA Extraction and qRT-PCR Analysis

Cells were inoculated in a 6-well plate with an appropriate density were modeled and treated according to the method described in "Cell cultures". Total RNA was extracted from the treated cells using an RNA extraction kit, and complementary cDNA was synthesized using a reverse transcription kit according to the manufacturer's instructions. Quantitative detection of gene expression was performed using the BIO-RAD CFX96 Real-Time PCR Detection System. The PCR amplification process was as follows: 95°C for 30 seconds, followed by 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds. Data were analyzed using the $2^{-\Delta\Delta Ct}$ relative expression method.

Statistical Analysis

All experiments were performed with at least three replicates per sample. The data analysis was performed using GraphPad Prism version 9.4.1. First, the data was subjected to the Shapiro–Wilk normality test. Data that conformed to a normal distribution were expressed as mean ± SD. One-way analysis of variance (ANOVA) was used to compare differences between sample groups, and Tukey's post-hoc test was conducted for pairwise comparisons between groups. A p-value of less than 0.05 ($P<0.05$) was considered to indicate statistical significance.

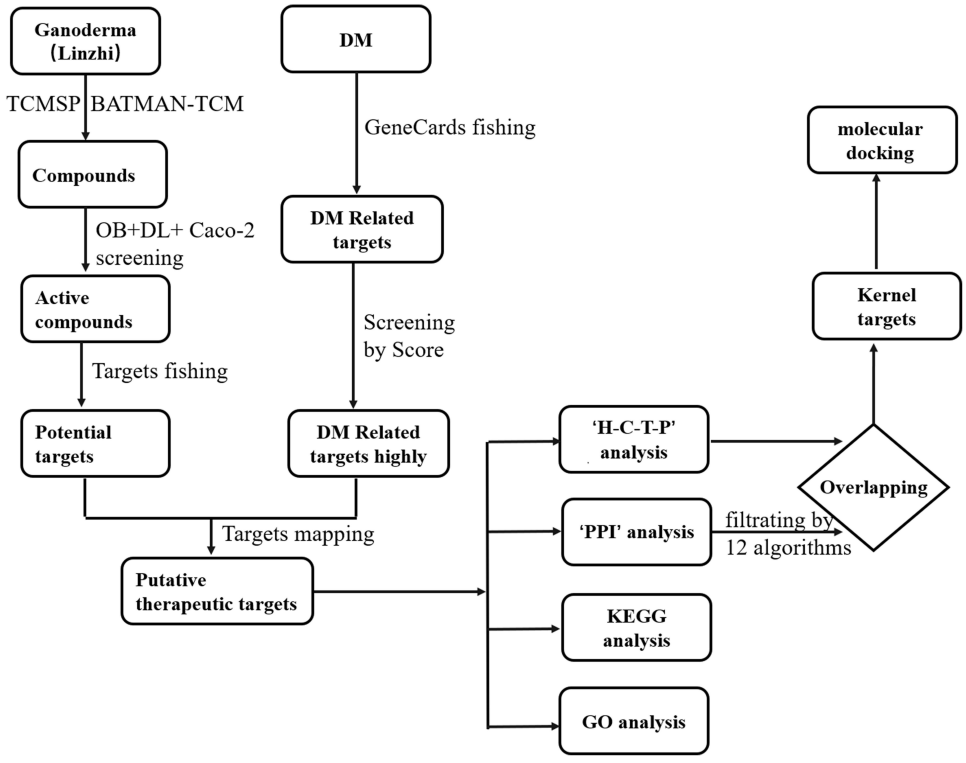

Figure 1 The workflow of present network pharmacology and molecular docking.

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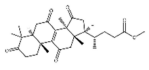
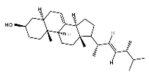
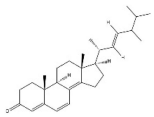
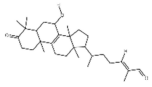
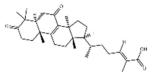
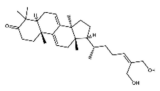
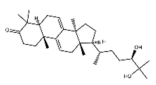
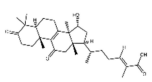
Results

Bioactive Compounds and Corresponding Targets of G. Lucidum in Diabetes Treatment

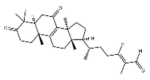
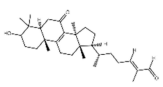
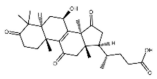
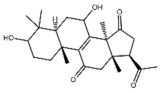
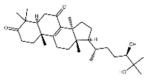
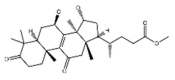
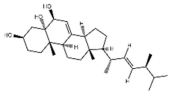
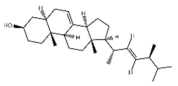
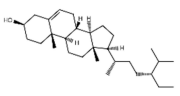
The data collection, analysis, and processing for the network pharmacology and molecular docking study of *Ganoderma lucidum* in the treatment of Diabetes mellitus were carried out according to the experimental process shown in Figure 1. A total of 60 compounds of *Ganoderma lucidum* were screened out by the threshold of filtration criteria, of which, 2

Table 1 The Attribute Values of Potential Active Components of Ganoderma Lucidum

Mol ID	PubChemCID	Molecule Name	Structure	OB (%)	DL	Caco-2
MOL011129	21633085	Methyl lucidenate F		32.67	0.81	−0.21
MOL011137	5283669	Campesta-7,22E-dien-3beta-ol		43.51	0.72	1.33
MOL011159	56676695	Ergosta-4,6,8 (14),22-tetraene-3-one		48.32	0.75	1.51
MOL011171	14015440	Ganoderal B		42.56	0.81	0.43
MOL011189	11784642	Ganoderic acid DM		38.8	0.83	0.36
MOL011235	471008	Ganoderiol F		38.12	0.82	0.35
MOL011241	73294	Ganodermanondiol		37.64	0.8	0.46
MOL011256	15602283	Ganolucidic acid E		32.85	0.82	−0.15

(*Continued*)

Table 1 (Continued).

Mol ID	PubChemCID	Molecule Name	Structure	OB (%)	DL	Caco-2
MOL011267	10343868	Lucialdehyde B		43.12	0.81	0.53
MOL011268	10366713	Lucialdehyde C/(24E)-3 beta-hydroxy-7-oxo-5 alpha-lanosta -8,24-dien-26-al		42.26	0.81	0.5
MOL011270	14109375	Lucidenic acid A		30.34	0.79	−0.2
MOL011287	71453988	Lucidone A		37.22	0.64	−0.13
MOL011290	475410	Lucidumol A (Lanost-8-ene-3,7-dione, 24.25-dihydroxy-, (24S)-)		34.75	0.8	0.28
MOL011309	11271456	Methyl (4R)-4-[(5R,7S,10S,13R,14R,15S,17R)-7,15-dihydroxy -4,4,10,13,14-pentamethyl-3,11-dioxo-2,5,6,7,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl] pentanoate (Methyl lucidenate Q)		30.19	0.81	−0.36
MOL000279	10181133	Cerevisterol		37.96	0.77	0.28
MOL000282	5283628	Stellasterol		43.51	0.72	1.32
MOL000358	222,284	β-sitosterol		36.91	0.75	1.32

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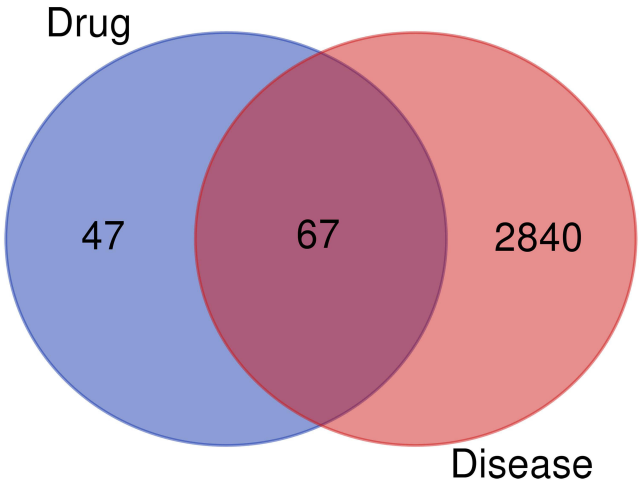

Figure 2 The Venn diagram of targets both in Diabetes mellitus highly associated targets and Ganoderma lucidum active components targets.

compounds which cannot be confirmed in PubChem Database then was deleted. Among the 58 confirmed compounds, 17 compounds are predicted to interacted with 114 targets (Table 1), while other 41 components have no related targets. Among the 17 compounds, there were 11 triterpenoids and 6 alcohols.

Table 2 67 Putative Therapeutic targets of Ganoderma Lucidum in Diabetes Mellitus Treatment

No	Gene Name	Uniprot ID	Protein Name	No	Gene Name	Uniprot ID	Protein Name
1	FADS1	O60427	Acyl-CoA (8-3)-desaturase	35	SLC8A1	P32418	Sodium/calcium exchanger 1
2	ATP1A3	P13637	Sodium/potassium-transporting ATPase subunit alpha-3	36	SLC6A4	P31645	Sodium-dependent serotonin transporter
3	SHBG	P04278	Sex hormone-binding globulin	37	BCL2	P10415	Apoptosis regulator Bcl-2
4	PON1	P27169	Serum paraoxonase/ arylesterase 1	38	RXRA	P19793	Retinoic acid receptor RXR-alpha
5	FXYD2	P54710	Sodium/potassium-transporting ATPase subunit gamma	39	ATP1A1	P05023	Sodium/potassium-transporting ATPase subunit alpha-1
6	RARB	P10826	Retinoic acid receptor beta	40	NR3C1	P04150	Glucocorticoid receptor
7	ADRA1A	P35348	Alpha-1A adrenergic receptor	41	CASP8	Q14790	Caspase-8
8	KCNH2	Q12809	Potassium voltage-gated channel subfamily H member 2	42	NR1I3	Q14994	Nuclear receptor subfamily 1 group I member 3
9	RDH5	Q92781	Retinol dehydrogenase 5	43	FADS2	O95864	Acyl-CoA 6-desaturase
10	NOTCH2	Q04721	Neurogenic locus notch homolog protein 2	44	PPIA	P62937	Peptidyl-prolyl cis-trans isomerase A
11	CYP19A1	P11511	Aromatase	45	AGTR1	P30556	Type-1 angiotensin II receptor
12	PTGS2	P35354	Prostaglandin G/H synthase 2	46	ADRB2	P07550	Beta-2 adrenergic receptor
13	BMP2	P12643	Bone morphogenetic protein 2	47	CASP3	P42574	Caspase-3
14	PDE3A	Q14432	cGMP-inhibited 3′,5′-cyclic phosphodiesterase A	48	ELOVL4	Q9GZR5	Elongation of very long chain fatty acids protein 4
15	ESR1	P03372	Estrogen receptor	49	VDR	P11473	Vitamin D3 receptor

(*Continued*)

Table 2 (Continued).

No	Gene Name	Uniprot ID	Protein Name	No	Gene Name	Uniprot ID	Protein Name
16	PTGER4	P35408	Prostaglandin E2 receptor EP4 subtype	50	GABRA1	P14867	Gamma-aminobutyric acid receptor subunit alpha-1
17	TRPV1	Q8NER1	Transient receptor potential cation channel subfamily V member 1	51	RXRG	P48443	Retinoic acid receptor RXR-gamma (Nuclear receptor subfamily 2 group B member 3) (Retinoid X receptor gamma)
18	TSPO	P30536	Translocator protein	52	CASP9	P55211	Caspase-9
19	JUN	P05412	Transcription factor AP-1	53	RDH12	Q96NR8	Retinol dehydrogenase 12
20	NR3C2	P08235	Mineralocorticoid receptor	54	MAP2	P11137	Microtubule-associated protein 2
21	SRD5A1	P18405	3-oxo-5-alpha-steroid 4-dehydrogenase 1	55	OPRM1	P35372	Mu-type opioid receptor
22	PGR	P06401	Progesterone receptor	56	NOX1	Q9Y5S8	NADPH oxidase 1
23	CYP17A1	P05093	Steroid 17-alpha-hydroxylase /17,20 lyase	57	RAD51	Q06609	DNA repair protein RAD51 homolog 1
24	PIK3CG	P48736	Phosphatidylinositol 4.5-bisphosphate 3-kinase catalytic subunit gamma isoform	58	PDE8B	O95263	High affinity cAMP-specific and IBMX-insensitive 3′,5′-cyclic phosphodiesterase 8B
25	GABRA5	P31644	Gamma-aminobutyric acid receptor subunit alpha-5	59	HSD11B1	P28845	Corticosteroid 11-beta-dehydrogenase isozyme 1
26	PRKACA	P17612	cAMP-dependent protein kinase catalytic subunit alpha	60	PTGS1	P23219	Prostaglandin G/H synthase 1
27	RBP3	P10745	Retinol-binding protein 3	61	TGFB1	P01137	Transforming growth factor beta-1 proprotein
28	CHRM3	P20309	Muscarinic acetylcholine receptor M3	62	RBP1	P09455	Retinol-binding protein 1
29	HTR2A	P28223	5-hydroxytryptamine receptor 2A	63	AR	P10275	Androgen receptor
30	LRAT	O95237	Lecithin retinol acyltransferase	64	BAX	Q07812	Apoptosis regulator BAX
31	DRD1	P21728	D(1A) dopamine receptor	65	PRKCA	P17252	Protein kinase C alpha type
32	TGFB2	P61812	Transforming growth factor beta-2 proprotein	66	SCN5A	Q14524	Sodium channel protein type 5 subunit alpha
33	ANXA1	P04083	Annexin A1	67	S100A9	P06702	Protein S100-A9
34	RLBP1	P12271	Retinaldehyde-binding protein 1				

Targets Screening Related to Diabetes and Putative Therapeutic Targets of Bioactive Components of G. Lucidum

A total of 2907 targets related to Diabetes mellitus were obtained through searching from GeneCards and filtering by median score. By mapping these targets with 114 predicted targets of *Ganoderma lucidum*, a total of 67 mutual targets were obtained as putative therapeutic targets of *Ganoderma lucidum* in Diabetes mellitus treatment. The 67 putative therapeutic targets were showed in Figure 2 and listed in Table 2.

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Protein–Protein Interaction Network Analysis

In the present work, a list of the 67 putative therapeutic targets was inputted into the search bar on GeneMANIA within the selected datasets of Homo sapiens. The protein–protein interaction network was constructed, collated and analyzed. Among the 67 targets and their interacting proteins, 39.29% displayed similar co-expression characteristics, 17.10% showed co-localization and 12.33% exerted physical interactions, etc. (Figure 3).

Gene Function and Pathway Enrichment Analysis

To investigate the potential mechanisms by which *Ganoderma lucidum* may improve Diabetes mellitus, GO functional enrichment analysis and KEGG pathway analysis were conducted using a Web Gestalt database. The results indicate that *Ganoderma lucidum* is involved in biological regulation, metabolic process, multicellular organismal process, developmental process, cellular component organization, cell proliferation, and reproduction, and other Biological Processes (Figure 4). It also participates in the regulation of cellular components such as membrane, protein-containing structure, nucleus, cell projection, mitochondria, etc. Molecular functions including protein binding and lipid binding, among others.

A total of 71 pathways were enriched significantly with FDR-value less than or equal to 0.05 and of which 50 pathways were most significantly enriched with FDR-value less than or equal to 0.01 (Figure 5, Supplementary Table 1). The therapeutic effect of *Ganoderma lucidum* in Diabetes mellitus treatment were achieved through AGE-RAGE

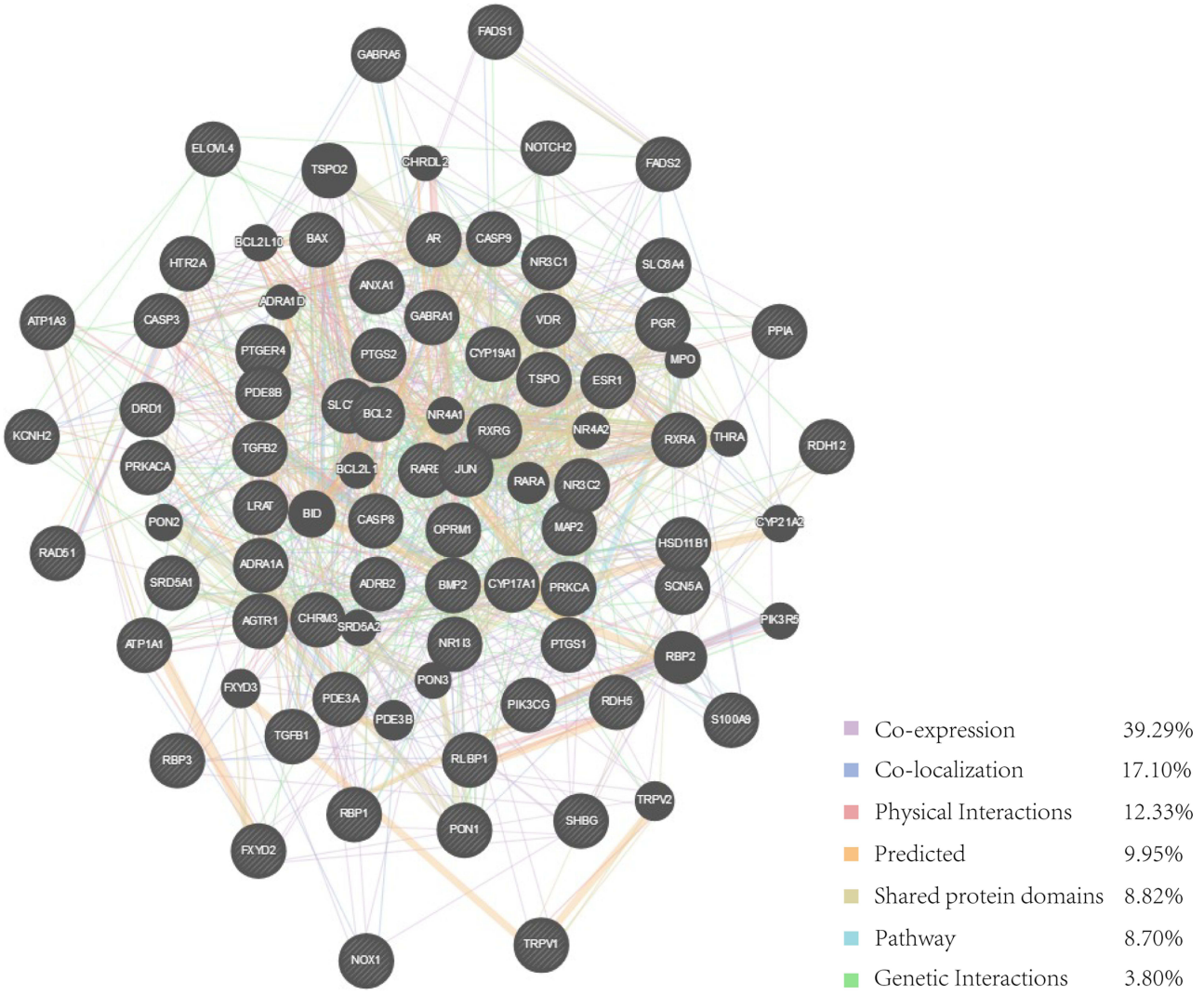

Figure 3 The network diagram of protein–protein interaction.

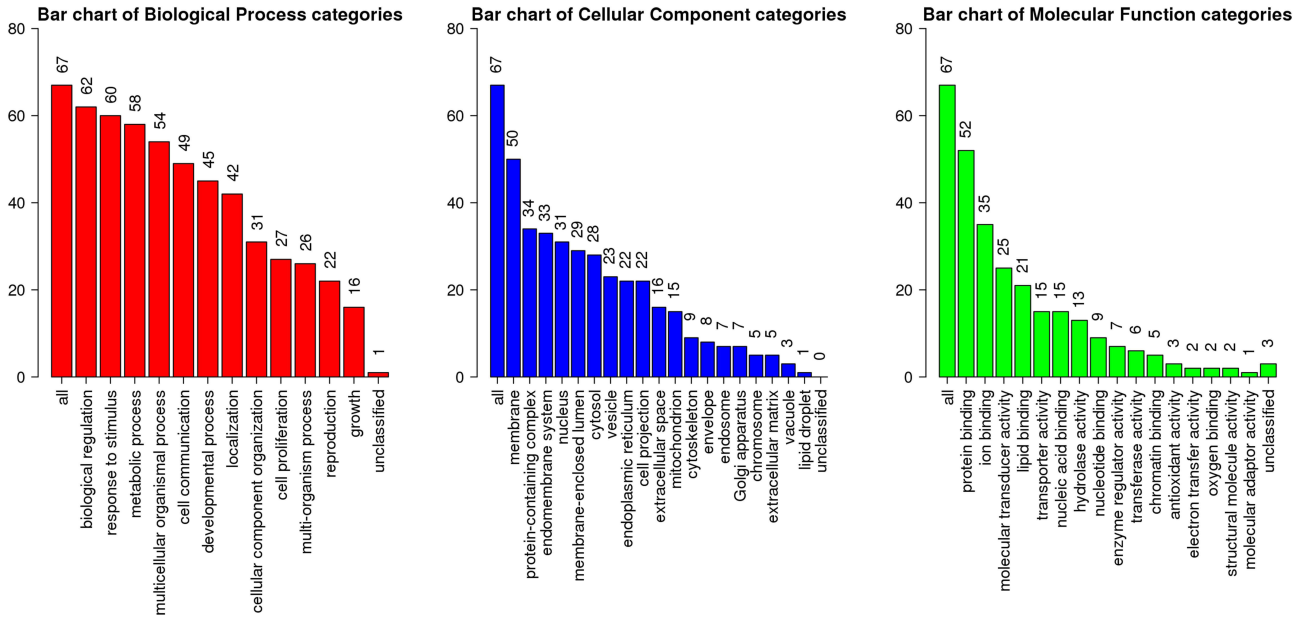

Figure 4 GO enrichment analysis of Ganoderma lucidum-related therapeutic target genes in the treatment of Diabetes mellitus. Y-axis represents the Ganoderma lucidum-related therapeutic target gene count of each GO term, and the X-axis represents the categories in the Biological Process (red bar), Cellular Component (blue bar) and Molecular Function (green bar) respectively. All the categories were screened using the criteria of FDR value < 0.01.

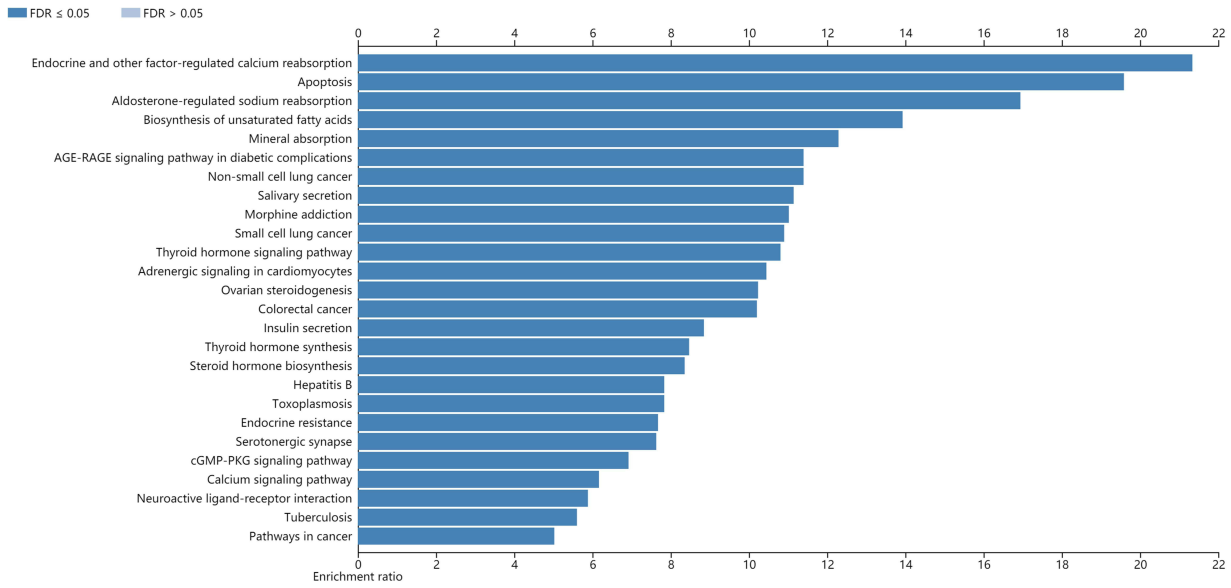

Figure 5 The Bar chart of part most significantly enriched pathways.

signaling pathway in diabetic complications, Insulin secretion, Calcium Signaling Pathway, Endocrine Resistance, Serotonergic Synapse, and Adrenergic Signaling in Cardiomyocytes. Mapping of Pathways in "Insulin secretion" regulated by *Ganoderma lucidum* in Diabetes mellitus was shown in Figure 6. In pancreatic β-cells, *Ganoderma lucidum* may promote glucose entry into cells by regulating GLUT proteins, facilitating the transfer of glucose to the mitochondria for the TCA cycle. This increases intracellular ATP levels, leading to the closure of ion channels and resultant cellular depolarization. This process modulates CAMK to enhance insulin release. The calcium signaling pathway also targets the endoplasmic reticulum, activating CAMK to stimulate insulin secretion. Additionally, PKA is activated by cAMP and then promotes insulin secretion through CREB and PDX-1.

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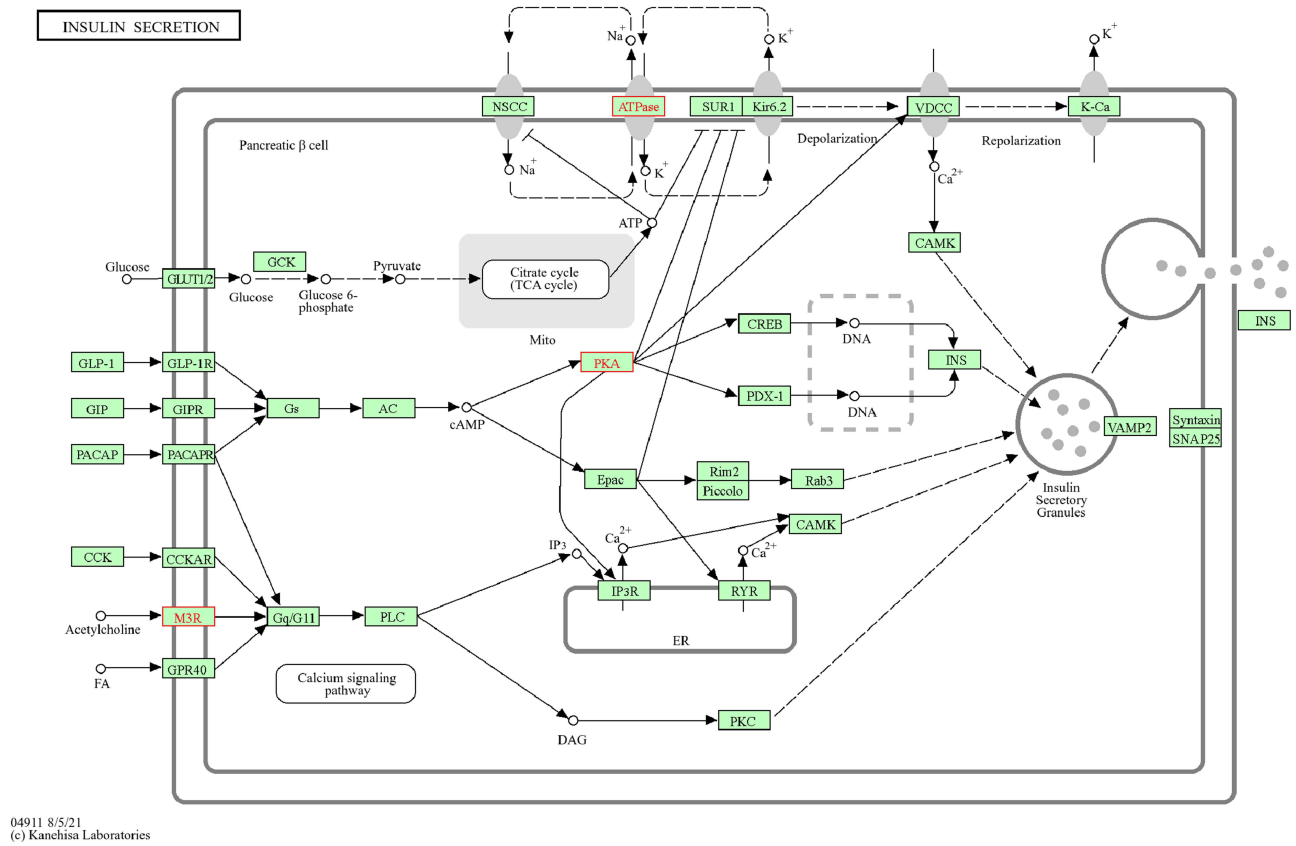

Figure 6 Targets labeling diagram of Ganoderma lucidum in regulating Diabetes mellitus related pathways. The targets regulated by Ganoderma lucidum in Diabetes mellitus treatment were labeled by red mark. Distribution of target proteins of Ganoderma lucidum on pathway.

"Herb-Bioactive Components-Target-Pathway" Network Analysis

The "H-C-T-P" network included 175 nodes and 665 edges and showed that the components of β-sitosterol, lucidone A, Lucialdehyde C, Ganoderiol F, lucidumol A, Ganodermanondiol, ganoderic acid DM and Lucialdehyde B had much higher degree value, and may be the main bioactive components of *Ganoderma lucidum* for treatment of Diabetes mellitus (Figure 7). The most important target proteins were PRKCA, PRKACA, CASP3, BAX, BCL2, CASP9, ATP1A1, ATP1A3, NR3C2, PGR and so on.

Key Targets Analysis

The top 10 key targets in each group filtered by the corresponding algorithm were listed in Supplementary Table 2. The frequency detail of total 120 candidate key targets filtered by 12 algorithms from the protein–protein interaction network was showed in Figure 8. It showed that the most important top 10 key candidate targets are PTGS2 (targeted by components of MOL011189, MOL011268, MOL011290 and MOL000358), NR3C1, JUN (targeted by compound of MOL000358), CASP3, PRKACA, CYP19A1 (targeted by compound of MOL011267), AR, ESR1, PGR, RXRA (targeted by compound of MOL011235) in order of importance. By comparing the candidate key targets as described in "material and method", three targets of PGR, PRKACA and CASP3 were filtered to be the key therapeutic targets of *Ganoderma lucidum* in Diabetes mellitus treatment.

Molecular Docking Analysis

β-sitosterol was conjugated with CASP3, PRKACA, and PGR by Autodock Vina. The docking analysis (Table 3) revealed that the binding energies of β-sitosterol with CASP3, PRKACA, and PGR were −9.3 kcal/mol, −9.3 kcal/mol, and −8.8 kcal/mol, respectively, all of which are less than −5.0 kcal/mol, indicating strong binding activity.[21] Its binding

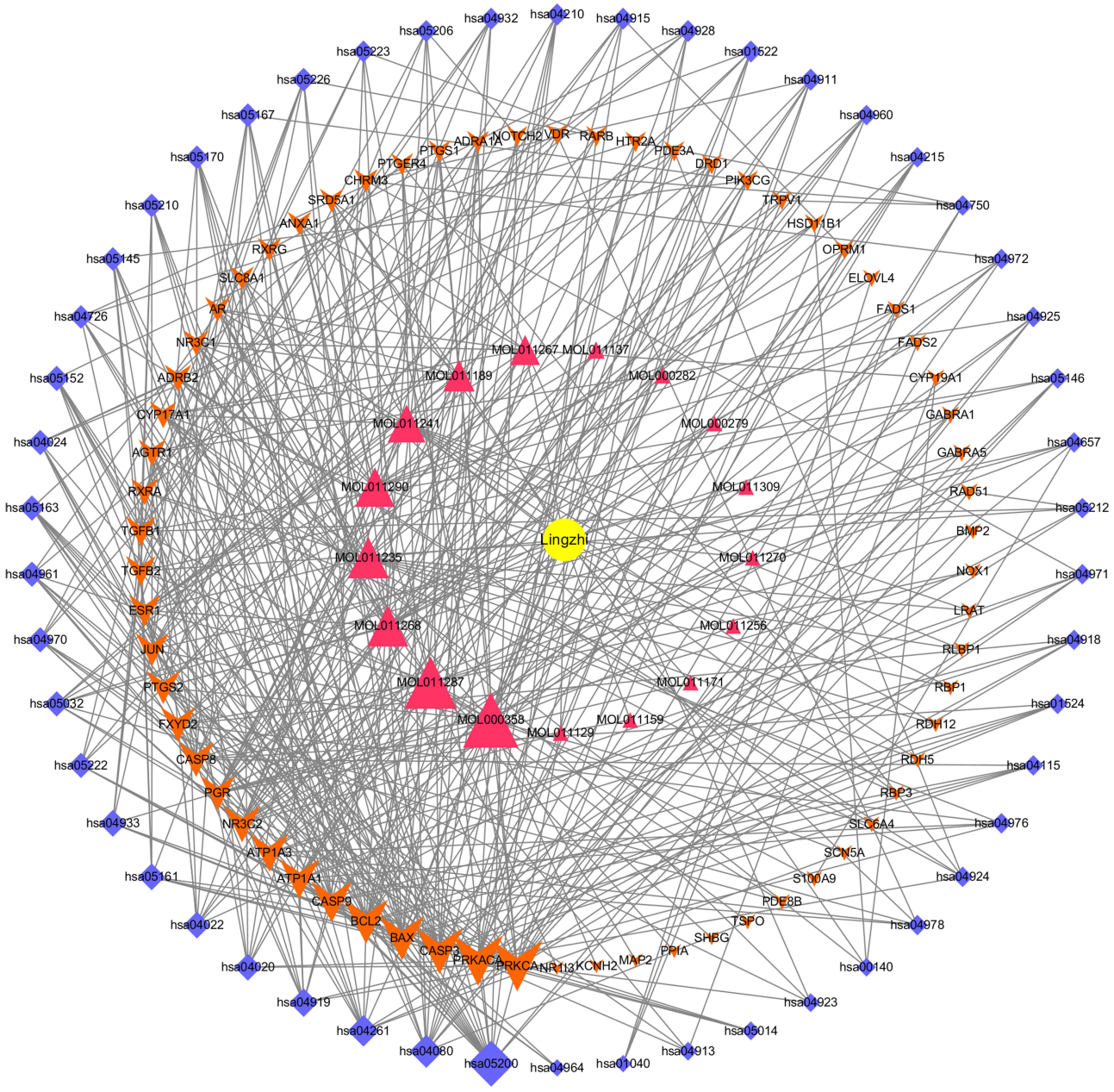

Figure 7 The network diagram of "H-C-T-P" of Ganoderma lucidum in Diabetes mellitus treatment.
Notes: golden yellow circle represent Ganoderma lucidum, magenta triangle represent bioactive compound, aurantia bold V represent putative therapeutic target, and the blue diamond significant enrichment pathways.

with PRKACA and PGR was primarily through Alkyl and Pi-Alkyl interactions. The binding with CASP3 was also mainly via Alkyl and Pi-Alkyl interactions (Figure 9C–9F), but it also involved other interactions, including Pi-Sigma interaction with TYR200 and Carbon Hydrogen Bond interaction with LYS140 (Figure 9A and 9B).

Effects of Different Concentrations of PA and GLAE on the Viability of HepG2 Cells

The effects of different concentrations of PA and GLAE on the viability of HepG2 cells were individually assessed. PA with the concentration of 0.1–0.2 mm had no significant effect on cell viability (Figure 10A), and GLAE with the concentration from 1 μg/mL to 100 μg/mL showed no significant effect on cell viability as well (Figure 10B). To minimize the influence of drug-induced inhibition of cell viability under the PA-induced condition, we further studied the

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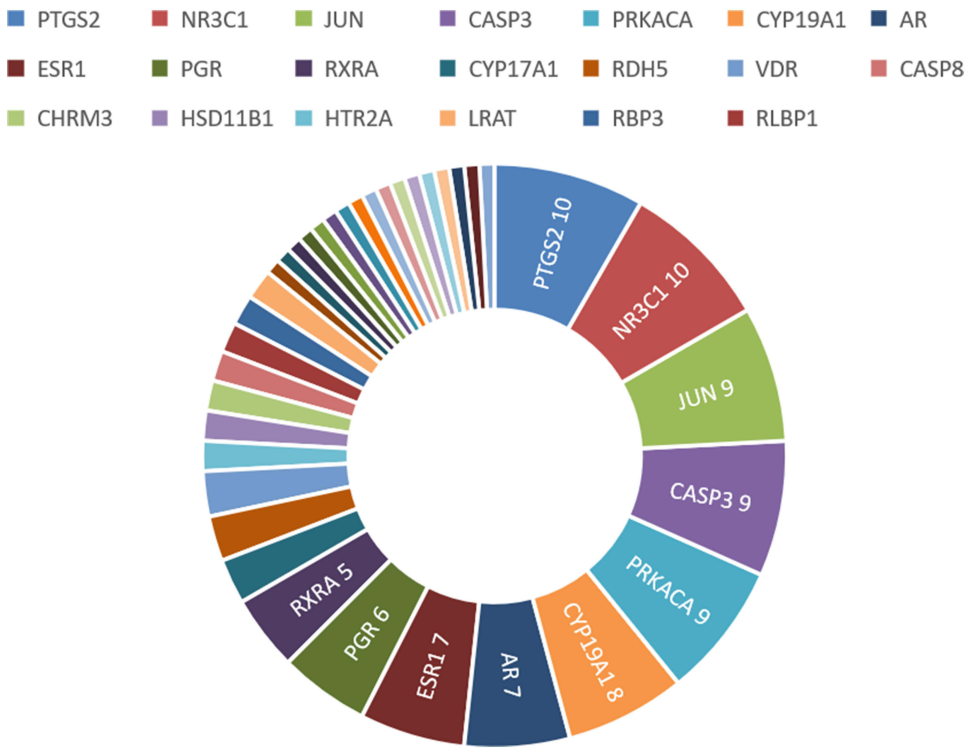

Figure 8 The frequency distribution diagram of important targets filtered by twelve different parameters.

effect of 20 μg/mL, 40 μg/mL, and 80 μg/mL (as low, medium and high dose) of GLAE on the cell viability when the cells were treated with 0.2 mm PA (Figure 10C). The results showed that GLAE with low, medium and high dose had no effect on the cell viability.

GLAE Increases Glucose Consumption in PA-Induced HepG2 Cells

To investigate whether GLAE improves glucose uptake by promoting the translocation of GLUT2, thereby alleviating hyperglycemia, we examined the expression of GLUT2 using immunofluorescence. As shown in Figure 11A, compared to the model group, the green fluorescence was more pronounced in the control and treatment groups, and the cell edge outlines were clearer, indicating that the protein was aggregated on the cell membrane. The fluorescent quantitative analysis revealed that the intensity of the PA-induced group was significantly reduced, and this phenomenon was markedly alleviated in the medium- and high-dose GLAE treatment groups (Figure 11B). The glucose consumption in the PA-induced group was much lower than that in the control group, while that in the medium- and high-dose GLAE treatment group was significantly increased compared to the model group (Figure 11C). Therefore, it was concluded that high-fat conditions inhibit the expression of GLUT2 protein on the cell membrane of HepG2 and cause its translocation from the membrane to the cytoplasm. GLAE treatment significantly improved this phenomenon, thereby restoring glucose uptake in HepG2 cell.

Table 3 β-Sitosterol with Corresponding Key Targets

Compound	Key Targets	PDB ID	Binding Energy (kcal/mol)
β-sitosterol	CASP3	7JL7	−9.3
	PRKACA	7Y1G	−9.3
	PGR	9ENN	−8.8

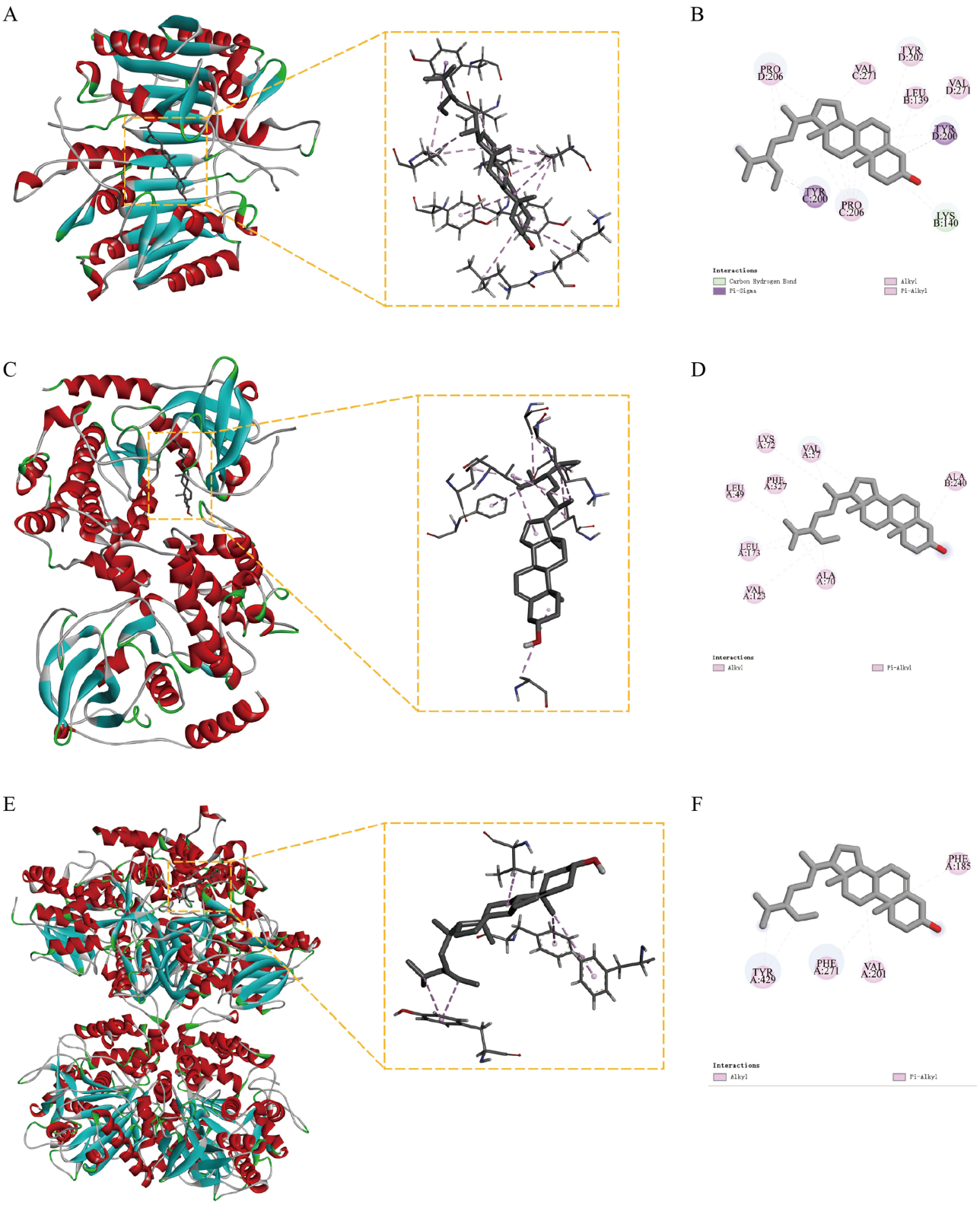

Figure 9 Molecular models of β-sitosterol binding to its predicted protein target. (**A**) 3D model of CASP3 crystal structure docking. (**B**) CASP3 docking 2D model. (**C**) 3D model of PRKACA crystal structure docking. (**D**) PRKACA docking 2D model. (**E**) 3D model of PGR crystal structure docking. (**F**) PGR docking 2D model.

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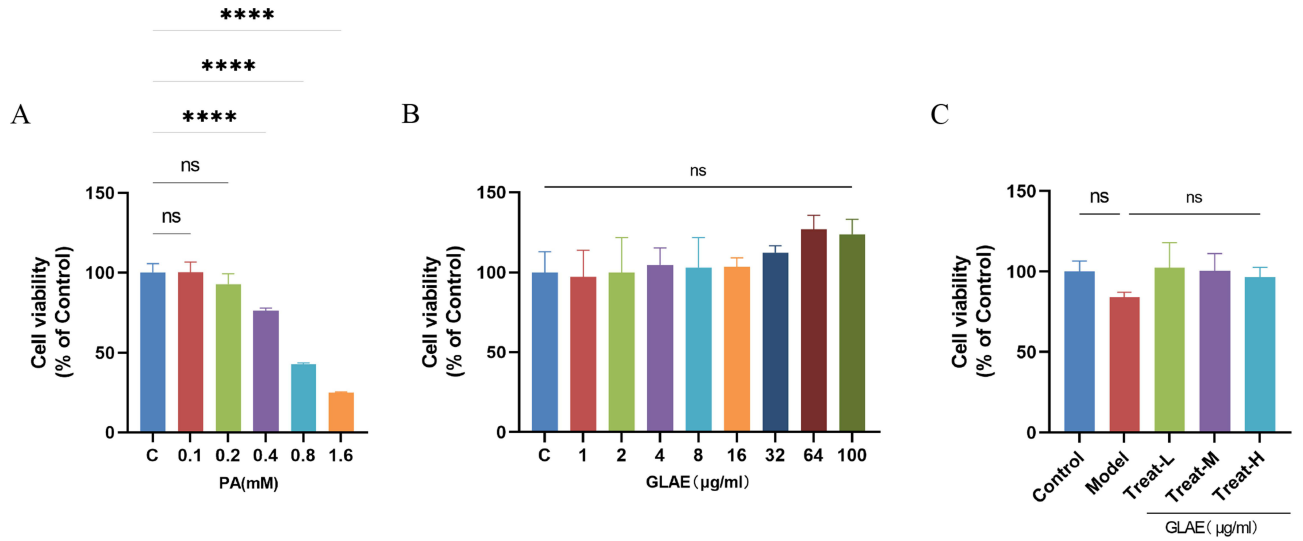

Figure 10 The effects of GLAE and PA on the viability of HepG2 cells. (**A**) The impact on cell viability after treating HepG2 cells with different concentrations of PA for 24 hours, normalized against the control group (%). When the PA concentration reaches 0.4 mm, cell viability begins to decrease significantly compared to the control group. (**B**) The effect on HepG2 cell viability of various concentrations of GLAE, normalized against the control group (%). When the GLAE concentration ranges from 1 to 100 μg/mL, cell viability is not affected compared to the control group. (**C**) After pre-treating HepG2 cells with GLAE for 24 hours, the influence of GLAE on the cell viability of HepG2 induced by PA was assessed, normalized against the control group (%). After GLAE treatment, cell viability shows no significant difference compared to the model group.
Notes: Data are presented as the means ± SD. ns $p > 0.05$, **** $p < 0.0001$.

GLAE Improves PA-Induced Lipid Accumulation

Studies have shown that excessive accumulation of body fat can mediate insulin resistance.[22] Lipid accumulation in HepG2 cells was induced by high fat. Figure 12A and 12B indicate that, after treatment with GLAE, there was a significant reduction in TG/TC content compared to the model group. GLAE is capable of alleviating lipid deposition in HepG2 cells induced by high fat.

GLAE Treatment Ameliorated Key Target Gene Expression in PA-Induced HepG2 Cells

Combining the results of network pharmacology, we used qPCR to detect the mRNA levels of 10 key genes. As shown in Figure 13, compared with the control group, PA led to the abnormally elevated expression of five genes: CASP3, PRKACA, CYP19A1, NR3C1, and JUN, which improved after treatment with GLAE. The expression of PGR and RXRA decreased after PA treatment, and GLAE restored their mRNA expression. The three gene tests PTGS2, AR and ESR1 showed no expression. The primers used are listed in the Supplementary Information (Supplementary Table 3).

Discussion

G. lucidum is a medicinal food homologous herb that does not produce toxic side effects on the human body.[3,4,23] It is reported that *G. lucidum* could protect pancreatic β cells from damage and apoptosis induced by oxidative stress and maintain the activity of pancreatic β cells. It ensures adequate insulin production, which may fundamentally assist in controlling blood sugar levels.[24] In addition, *G. lucidum* is reported to improve glucose metabolism, and reduce lipid accumulation in the liver.[5,11,25] In this study, a total of 17 anti-diabetic compounds were screened with 114 predicted targets from the TCMSP database, among which 11 were triterpenoids. A few reports suggest that *G. lucidum* triterpenoids possess inhibitory activity against aldose reductase and alpha-glucosidase.[26–28] Based on our GO and KEGG enrichment results, it appears that the treatment of Diabetes mellitus with *G. lucidum* is closely related to metabolic processes,[29] mitochondria,[30] lipid binding,[31] the biosynthesis of unsaturated fatty acids,[32] the AGE-RAGE signaling pathway in Diabetes mellitus complications,[33] and insulin secretion.[34–36] These functions and pathways are closely related to the regulation of metabolic disorders, which are important factors in the onset of Diabetes mellitus.

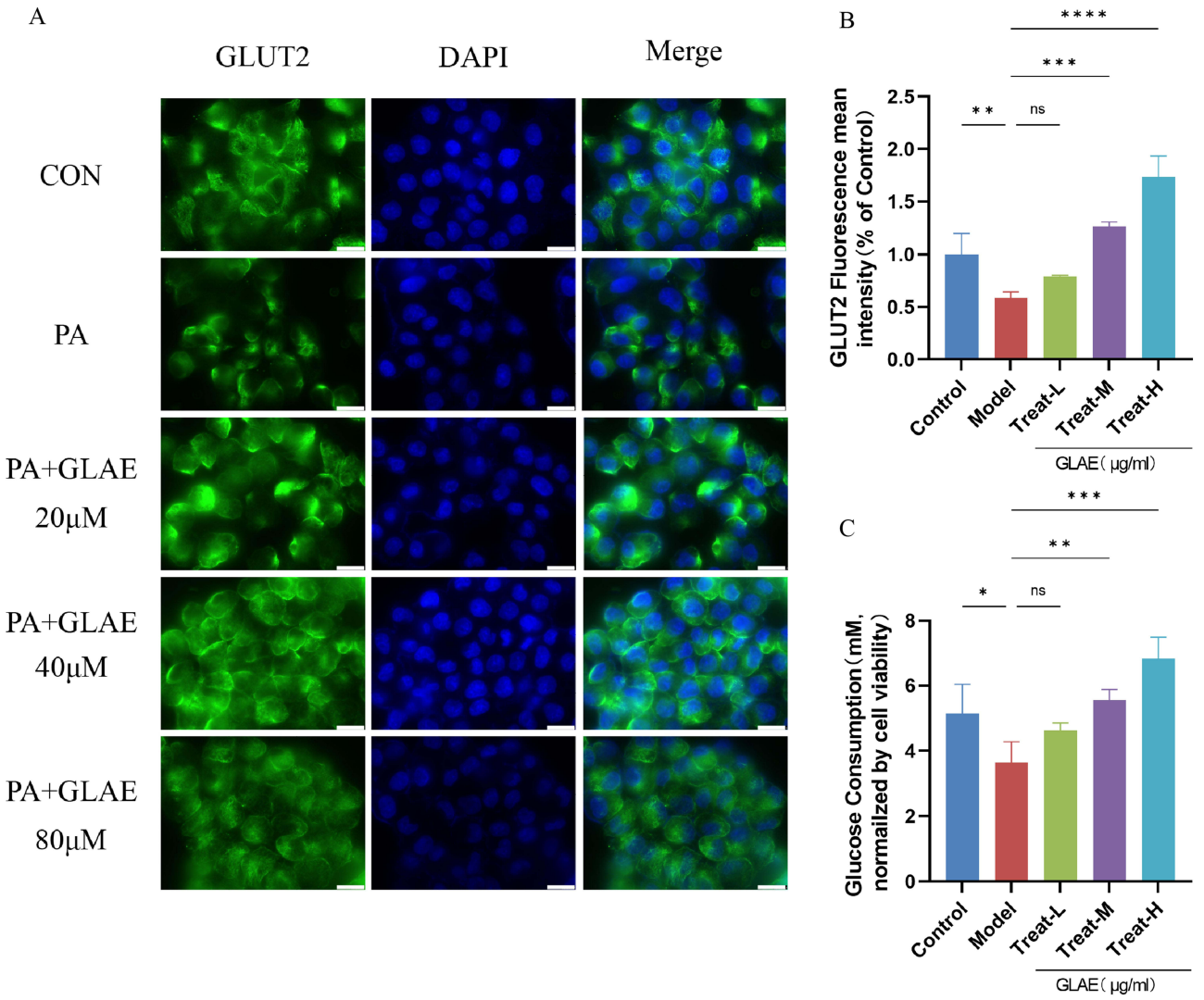

Figure 11 GLAE increases glucose consumption in HepG2 cells induced by PA. (**A**) Immunofluorescence staining was used to detect GLUT2 (green), cell nuclei were stained with DAPI (blue). Scale bar, 16μm. (**B**) GLUT2 Fluorescence Quantitative Analysis. (**C**) Glucose consumption. After PA stimulation, GLUT2 fluorescence on the cell membrane and glucose consumption significantly decreased. Following Ganoderma treatment, both the membrane green fluorescence and glucose consumption significantly increased compared to the model group.

Notes: Data are presented as the means ± SD. ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, compared to the model group.

This also provides us with a new direction for research: Whether Diabetes mellitus treated by *G. lucidum* is through the improvement of glucose and lipid metabolism disorders in the liver.

To validate this hypothesis, we used GLAE as a drug in a model of metabolic disorder and insulin resistance induced by palmitic acid (PA) in HepG2 cells.[37] The research findings indicate that GLAE can effectively improve glucose consumption disorders in HepG2 cells induced by PA, as well as lipid accumulation caused by excess TG/TC. Following GLAE treatment, the fluorescence intensity of GLUT2 and glucose consumption in HepG2 cells increased, and GLUT2 translocation reduced. TG/TC levels significantly decreased in a concentration-dependent manner, thereby improving insulin resistance to treat Diabetes mellitus. Glucose and lipids are essential nutrients for humans and serve as primary sources of energy.[38,39] This study addresses Diabetes mellitus by focusing on restoring hepatic glucose consumption and improving hepatic lipid accumulation. It aims to enhance the liver's glucose utilization, reduce hepatic gluconeogenesis, mitigate glucolipotoxicity, and restore insulin sensitivity.

Through the screening and analysis of key targets and ingredients, we found that the active component TOP1 β-sitosterol (MOL000358) in H-C-T-P can simultaneously and strongly interact with CASP3, PRKACA, and PGR, which

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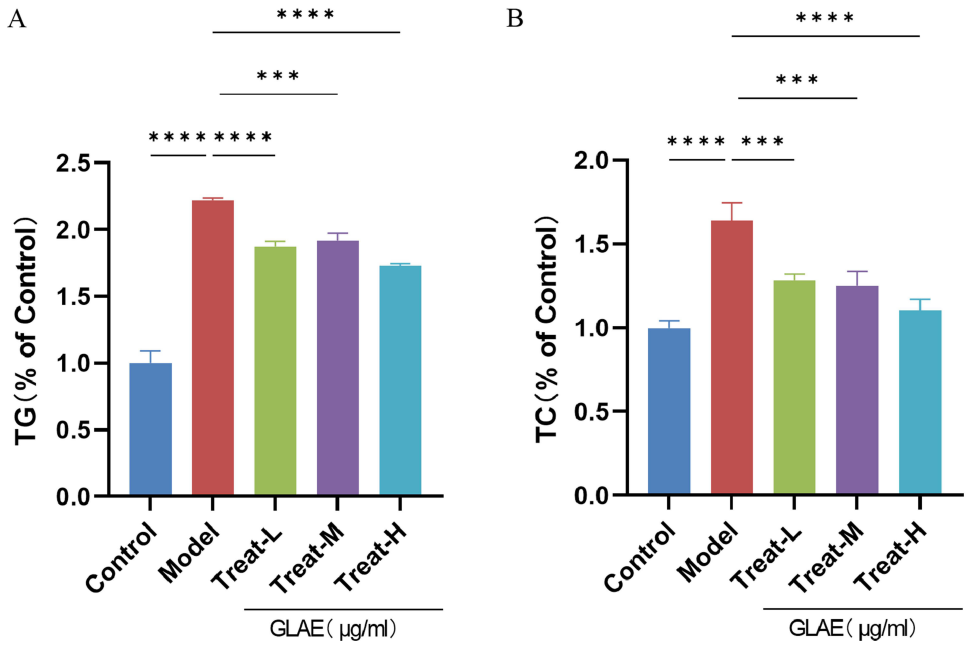

Figure 12 The effect of GLAE on alleviating lipid accumulation in HepG2 cells induced by PA. (**A**) Triglyceride accumulation. (**B**) Cholesterol accumulation. After PA stimulation, TG/TC significantly increased. Following GLAE treatment, TG/TC significantly decreased compared to the model group.
Notes: Data are presented as the means ± SD. *** $p < 0.001$, **** $p < 0.0001$, compared with the model cells.

are in the TOP10 of the Protein–Protein Interaction (PPI) network. CASP3 is a caspase that regulates apoptosis through both intrinsic and extrinsic pathways.[40] Apoptosis occurs more frequently in pancreatic islets and liver of diabetic patients, with higher baseline levels of CASP3 in their plasma. This may be due to mitochondrial dysfunction and downstream activation of CASP3 caused by glucotoxicity.[41,42] In this study, after treatment with *G. lucidum*, the expression level of CASP3 was downregulated, accordingly glucose consumption increased, glucotoxicity reduced in the blood. The PRKACA gene encodes one of the catalytic subunits of Protein Kinase A (PKA). PKA, dependent on cAMP for protein phosphorylation, plays an important role in various cellular processes such as differentiation, proliferation, and apoptosis, consequently regulates the body's energy metabolic response processes,[43–45] such as hepatic gluconeogenesis.[46] Activation of the cAMP-dependent Ser PKA phosphorylates the cAMP-response element (CRE)-binding protein (CREB) serine 133, leading to the transcriptional activation of gluconeogenic genes. In this study, PA led to upregulation of PRKACA expression in HepG2 cells. After treatment with *G. lucidum*, the expression of PRKACA was significantly reduced, thereby alleviating gluconeogenesis to achieve therapeutic effects on diabetes. As a progesterone activated transcription factor, PGR mediates the physiological of progesterone, playing critical effects on the endocrine system and immune system. Progesterone modulates glucose homeostasis by increasing insulin secretion and processing, enhancing hepatic insulin signaling, and reducing gluconeogenesis. The expression of PGR can be effectively regulated by *G. lucidum*, which may result from a certain substance acting on this target, thereby improving Diabetes mellitus through this pathway.[47–53]

Studies have shown that,[54] under conditions of glucotoxicity and lipotoxicity, NR3C1 is activated in pancreatic β-cells, leading to harmful excessive autophagy, which ultimately results in Diabetes mellitus. JUN is a transcription factor AP-1 that plays a significant role in type 2 diabetes. AP-1 is crucial for regulating insulin resistance and lipid metabolism. Studies have indicated that PA increases JUN expression in HepG2 cells, which may be a result of the inflammatory response.[55–57] Mutations or abnormal expression of the CYP19A1 gene may lead to an imbalance in sex hormone levels, and changes in sex hormone levels can affect insulin sensitivity. Its genetic polymorphism is also related to insulin resistance.[58] RXRA is involved in the regulation of energy balance, including food intake and energy expenditure. Imbalances in energy metabolism can lead to weight gain and obesity, which are important risk factors for type 2 diabetes.[59] These genes are closely associated with the occurrence and progression of Diabetes mellitus, and *G. lucidum* has shown to effectively improve their expression.

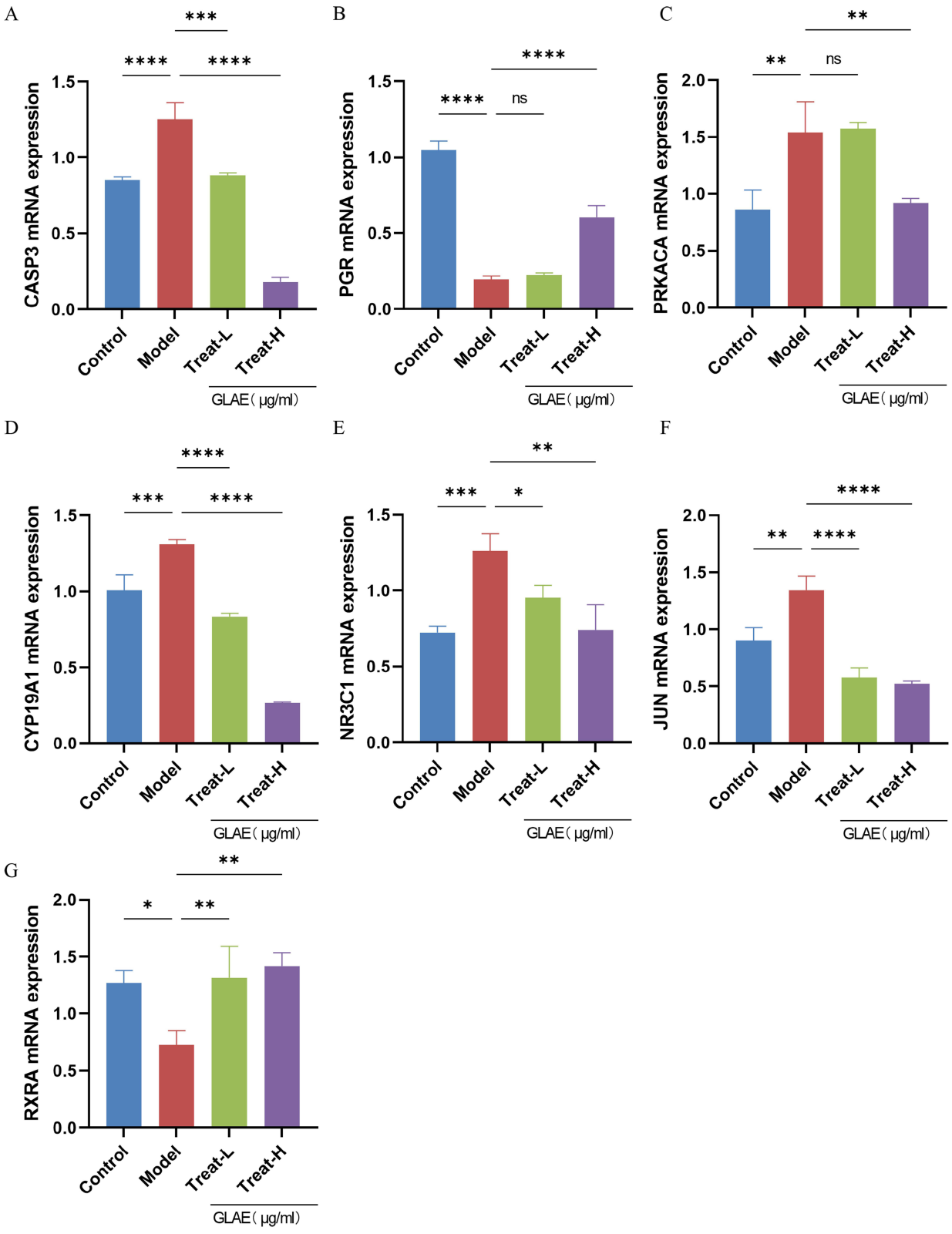

Figure 13 GLAE increases glucose consumption in HepG2 cells induced by PA. (**A**) Caspase3 mRNA expression. (**B**) PGR mRNA expression. (**C**) PRKACA mRNA expression. (**D**) CYP19A1 mRNA expression. (**E**) NR3C1 mRNA expression. (**F**) JUN mRNA expression. (**G**) RXRA mRNA expression. After PA stimulation, the mRNA expression of CASP3, PRKACA, CYP19A1, NR3C1, and JUN significantly increased. Following GLAE treatment, their mRNA expression significantly decreased compared to the model group. Additionally, the mRNA expression of PGR and RXRA significantly decreased after PA stimulation but was significantly increased after GLAE treatment.
Notes: Data are presented as the means ± SD. ns p >0.05, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, compared to the model group.

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Conclusion

Our results suggested that glucose and lipid metabolism are important for the anti-diabetic activity of *G. lucidum* by using an integrated approach based on network pharmacology combined with experimental validation. Molecular docking showed a possible interaction of β-sitosterol with CASP3, PRKACA, and PGR, which may be related with cell apoptosis, gluconeogenesis and insulin secretion, etc. In PA-induced HepG2 cells, glucose and lipid metabolism were improved by *G. lucidum via* increasing hepatic glucose utilization, reducing gluconeogenesis and mitigating glucolipotoxicity, with decreased mRNA expression of CASP3, PRKACA, CYP19A1, NR3C1, JUN, and increased expression of PGR and RXRA. In conclusion, *G. lucidum* is predicted to target a variety of pathways to form a network of systemic pharmacological effects to regulate glucose and lipid metabolism in diabetes intervention. The significant benefits for health of *G. lucidum*, coupled with its low toxicity and minimal side effects, make it suitable for consumption as a dietary supplement in daily life. However, the targets and pathways were only explored in vitro in this study, and it will be verified in animal experiments and clinical trials in the future. This study provides detailed insights into the active components and mechanisms of *G. lucidum* in diabetes intervention. It lays the foundational groundwork for future drug development and therapeutic optimization for diabetes and its complications.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; participated in the drafting, revising, or critical review of the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Sun H, Saeedi P, Karuranga S, et al. IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabet Res Clin Pract*. 2022;183:109119. doi:10.1016/j.diabres.2021.109119
2. Ntentakis DP, Correa V, Ntentaki AM, et al. Effects of newer-generation anti-diabetics on diabetic retinopathy: a critical review. *Graefe's Arch Clin Exp Ophthalmol*. 2024;262(3):717–752. doi:10.1007/s00417-023-06236-5
3. Zhao Y, Qin Y, Hu X, et al. Sporoderm-removed Ganoderma lucidum spores ameliorated early depression-like behavior in a rat model of sporadic Alzheimer's disease. *Front Pharmacol*. 2024;15:1406127. doi:10.3389/fphar.2024.1406127
4. Luo W, Zhou J, Yang X, et al. A Chinese medical nutrition therapy diet accompanied by intermittent energy restriction alleviates type 2 diabetes by enhancing pancreatic islet function and regulating gut microbiota composition. *Food Res Int*. 2022;161:111744. doi:10.1016/j.foodres.2022.111744
5. Lee HA, Cho JH, Afinanisa Q, et al. Ganoderma lucidum Extract Reduces Insulin Resistance by Enhancing AMPK Activation in High-Fat Diet-Induced Obese Mice. *Nutrients*. 2020;12(11):3338. doi:10.3390/nu12113338
6. Zhang N, Han Z, Zhang R, et al. Ganoderma lucidum Polysaccharides Ameliorate Acetaminophen-Induced Acute Liver Injury by Inhibiting Oxidative Stress and Apoptosis along the Nrf2 Pathway. *Nutrients*. 2024;16(12):1859. doi:10.3390/nu16121859
7. Jiao J, Yong T, Huang L, et al. A Ganoderma lucidum polysaccharide F31 alleviates hyperglycemia through kidney protection and adipocyte apoptosis. *Int J Biol Macromol*. 2023;226:1178–1191. doi:10.1016/j.ijbiomac.2022.11.231
8. Rubel R, Dalla Santa HS, Fernandes LC, et al. Hypolipidemic and antioxidant properties of Ganoderma lucidum (Leyss: fr) Karst used as a dietary supplement. *World J Microbiol Biotechnol*. 2011;27(5):1083–1089. doi:10.1007/s11274-010-0554-9
9. Ma HT, Hsieh JF, Chen ST. Anti-diabetic effects of Ganoderma lucidum. *Phytochemistry*. 2015;114:109–113. doi:10.1016/j.phytochem.2015.02.017

10. Liu Q, Tie L. Preventive and Therapeutic Effect of Ganoderma (Lingzhi) on Diabetes. *Adv Exp Med Biol*. 2019;1182:201–215. doi:10.1007/978-981-32-9421-9_8
11. Pan Y, Yuan S, Teng Y, et al. Antioxidation of a proteoglycan from Ganoderma lucidum protects pancreatic β-cells against oxidative stress-induced apoptosis in vitro and in vivo. *Int J Biol Macromol*. 2022;200:470–486. doi:10.1016/j.ijbiomac.2022.01.044
12. Chang CJ, Lin CS, Lu CC, et al. Ganoderma lucidum reduces obesity in mice by modulating the composition of the gut microbiota. *Nat Commun*. 2015;6(1):7489. doi:10.1038/ncomms8489
13. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol*. 2008;4(11):682–690. doi:10.1038/nchembio.118
14. Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Medi*. 2013;11(2):110–120. doi:10.1016/s1875-5364(13)60037-0
15. Kibble M, Saarinen N, Tang J, Wennerberg K, Mäkelä S, Aittokallio T. Network pharmacology applications to map the unexplored target space and therapeutic potential of natural products. *Nat Prod Rep*. 2015;32(8):1249–1266. doi:10.1039/c5np00005j
16. Wan Y, Xu L, Liu Z, et al. Utilising network pharmacology to explore the underlying mechanism of Wumei Pill in treating pancreatic neoplasms. *BMC Complement Alternat Med*. 2019;19(1):158. doi:10.1186/s12906-019-2580-y
17. Wang Y, Zhang S, Li F, et al. Therapeutic target database 2020: enriched resource for facilitating research and early development of targeted therapeutics. *Nucleic Acids Res*. 2020;48(D1):D1031–D1041. doi:10.1093/nar/gkz981
18. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res*. 2019;47(W1):W199–w205. doi:10.1093/nar/gkz401
19. Demchak B, Hull T, Reich M, et al. Cytoscape: the network visualization tool for GenomeSpace workflows. *F1000Research*. 2014;3:151. doi:10.12688/f1000research.4492.2
20. Liu H, Luo W, Liu J, et al. The glucotoxicity protecting effect of honokiol in human hepatocytes via directly activating AMPK. *Front Nutr*. 2022;9:1043009. doi:10.3389/fnut.2022.1043009
21. Li Y, Wu F, Zhang J, et al. Mechanisms of Action of Potentilla discolor Bunge in Type 2 Diabetes Mellitus Based on Network Pharmacology and Experimental Verification in Drosophila. *Drug Des Devel Ther*. 2024;18:747–766. doi:10.2147/dddt.S439876
22. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89(6):2548–2556. doi:10.1210/jc.2004-0395
23. Yang X, Zhou J, Shao H, et al. Effect of an Intermittent Calorie-restricted Diet on Type 2 Diabetes Remission: a Randomized Controlled Trial. *J Clin Endocrinol Metab*. 2023;108(6):1415–1424. doi:10.1210/clinem/dgac661
24. Liang H, Pan Y, Teng Y, et al. A proteoglycan extract from Ganoderma Lucidum protects pancreatic beta-cells against STZ-induced apoptosis. *Biosci. Biotechnol. Biochem.* 2020;84(12):2491–2498. doi:10.1080/09168451.2020.1805718
25. Pan Y, Zhang Y, Li J, et al. A proteoglycan isolated from Ganoderma lucidum attenuates diabetic kidney disease by inhibiting oxidative stress-induced renal fibrosis both in vitro and in vivo. *J Ethnopharmacol*. 2023;310:116405. doi:10.1016/j.jep.2023.116405
26. Fatmawati S, Shimizu K, Kondo R. Ganoderic acid Df, a new triterpenoid with aldose reductase inhibitory activity from the fruiting body of Ganoderma lucidum. *Fitoterapia*. 2010;81(8):1033–1036. doi:10.1016/j.fitote.2010.06.025
27. Fatmawati S, Shimizu K, Kondo R. Inhibition of aldose reductase in vitro by constituents of Ganoderma lucidum. *Planta Med*. 2010;76(15):1691–1693. doi:10.1055/s-0030-1249782
28. Fatmawati S, Shimizu K, Kondo R. Ganoderol B: a potent α-glucosidase inhibitor isolated from the fruiting body of Ganoderma lucidum. *Phytomedicine*. 2011;18(12):1053–1055. doi:10.1016/j.phymed.2011.03.011
29. Luan Y, Luan Y, He H, et al. Glucose metabolism disorder: a potential accomplice of SARS-CoV-2. *Int J Obes Lond*. 2023;47(10):893–902. doi:10.1038/s41366-023-01352-y
30. Cheng H, Gang X, Liu Y, Wang G, Zhao X, Wang G. Mitochondrial dysfunction plays a key role in the development of neurodegenerative diseases in diabetes. *Am J Physiol Endocrinol Metab*. 2020;318(5):E750–e764. doi:10.1152/ajpendo.00179.2019
31. Emanuelsson F, Benn M. LDL-Cholesterol versus Glucose in Microvascular and Macrovascular Disease. *Clin Chem*. 2021;67(1):167–182. doi:10.1093/clinchem/hvaa242
32. Huang L, Lin JS, Aris IM, Yang G, Chen WQ, Li LJ. Circulating Saturated Fatty Acids and Incident Type 2 Diabetes: a Systematic Review and Meta-Analysis. *Nutrients*. 2019;11(5):998. doi:10.3390/nu11050998
33. Kay AM, Simpson CL, Stewart Jr JA. The Role of AGE/RAGE Signaling in Diabetes-Mediated Vascular Calcification. *J Diabet Res*. 2016;2016:6809703. doi:10.1155/2016/6809703
34. Abbasi F, Lamendola C, Harris CS, et al. Statins Are Associated With Increased Insulin Resistance and Secretion. *Arterioscler Thromb Vasc Biol*. 2021;41(11):2786–2797. doi:10.1161/atvbaha.121.316159
35. Johnson JD. On the causal relationships between hyperinsulinaemia, insulin resistance, obesity and dysglycaemia in type 2 diabetes. *Diabetologia*. 2021;64(10):2138–2146. doi:10.1007/s00125-021-05505-4
36. Vazquez Arreola E, Hanson RL, Bogardus C, Knowler WC. Relationship Between Insulin Secretion and Insulin Sensitivity and its Role in Development of Type 2 Diabetes Mellitus: beyond the Disposition Index. *Diabetes*. 2021;71(1):128–141. doi:10.2337/db21-0416
37. Yan C, Chen J, Chen N. Long noncoding RNA MALAT1 promotes hepatic steatosis and insulin resistance by increasing nuclear SREBP-1c protein stability. *Sci Rep*. 2016;6(1):22640. doi:10.1038/srep22640
38. Kramer DA, Quiroga AD, Lian J, Fahlman RP, Lehner R. Fasting and refeeding induces changes in the mouse hepatic lipid droplet proteome. *J Proteom*. 2018;181:213–224. doi:10.1016/j.jprot.2018.04.024
39. Feldstein AE. Novel insights into the pathophysiology of nonalcoholic fatty liver disease. *Semin Liver Disease*. 2010;30(4):391–401. doi:10.1055/s-0030-1267539
40. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. 1999;6(2):99–104. doi:10.1038/sj.cdd.4400476
41. Veluthakal R, Arora DK, Goalstone ML, Kowluru RA, Kowluru A. Metabolic Stress Induces Caspase-3 Mediated Degradation and Inactivation of Farnesyl and Geranylgeranyl Transferase Activities in Pancreatic β-Cells. *Cellular Physiol Biochem*. 2016;39(6):2110–2120. doi:10.1159/000447907
42. Tomita T. Immunocytochemical localisation of caspase-3 in pancreatic islets from type 2 diabetic subjects. *Pathology*. 2010;42(5):432–437. doi:10.3109/00313025.2010.493863
43. Zhao Y, Ding S. A high-throughput siRNA library screen identifies osteogenic suppressors in human mesenchymal stem cells. *Proc Natl Acad Sci USA*. 2007;104(23):9673–9678. doi:10.1073/pnas.0703407104

44. Siddappa R, Mulder W, Steeghs I, et al. cAMP/PKA signaling inhibits osteogenic differentiation and bone formation in rodent models. *Tissue Eng Part A*. 2009;15(8):2135–2143. doi:10.1089/ten.tea.2008.0512
45. Wang W, Zhang X, Zheng J, Yang J. High glucose stimulates adipogenic and inhibits osteogenic differentiation in MG-63 cells through cAMP/protein kinase A/extracellular signal-regulated kinase pathway. *mol Cell Biochem*. 2010;338(1–2):115–122. doi:10.1007/s11010-009-0344-6
46. Zhao Y, Li S, Chen Y, et al. Histone phosphorylation integrates the hepatic glucagon-PKA-CREB gluconeogenesis program in response to fasting. *Molecular Cell*. 2023;83(7):1093–1108.e8. doi:10.1016/j.molcel.2023.02.007
47. Picard F, Wanatabe M, Schoonjans K, Lydon J, O'Malley BW, Auwerx J. Progesterone receptor knockout mice have an improved glucose homeostasis secondary to beta -cell proliferation. *Proc Natl Acad Sci USA*. 2002;99(24):15644–15648. doi:10.1073/pnas.202612199
48. Wada T, Hori S, Sugiyama M, et al. Progesterone inhibits glucose uptake by affecting diverse steps of insulin signaling in 3T3-L1 adipocytes. *Am J Physiol Endocrinol Metab*. 2010;298(4):E881–8. doi:10.1152/ajpendo.00649.2009
49. Straub SG, Sharp GW, Meglasson MD, De Souza CJ. Progesterone inhibits insulin secretion by a membrane delimited, non-genomic action. *Biosci Rep*. 2001;21(5):653–666. doi:10.1023/a:1014773010350
50. Nunes VA, Portioli-Sanches EP, Rosim MP, et al. Progesterone induces apoptosis of insulin-secreting cells: insights into the molecular mechanism. *J Endocrinol*. 2014;221(2):273–284. doi:10.1530/joe-13-0202
51. Kuznetsova NV, Pal'chikova NA, Kuzminova OI, Selyatitskaya VG. Production of corticosteroid hormones in vitro by adrenals in rats with streptozotocin-induced diabetes. *Bull. Exp. Biol. Med.* 2014;157(1):28–31. doi:10.1007/s10517-014-2484-2
52. Hofmann A, Peitzsch M, Brunssen C, et al. Elevated Steroid Hormone Production in the db/db Mouse Model of Obesity and Type 2 Diabetes. *Hormone Metab Res*. 2017;49(1):43–49. doi:10.1055/s-0042-116157
53. Yang X, Wang Y. Correlation analysis of serum progesterone with clinical indicators and common traditional Chinese medicine syndrome types in male type 2 diabetic patients. *J South Med Univ*. 2019;39(5):586–590. doi:10.12122/j.issn.1673-4254.2019.05.14
54. Wu T, Shao Y, Li X, et al. NR3C1/Glucocorticoid receptor activation promotes pancreatic β-cell autophagy overload in response to glucolipotoxicity. *Autophagy*. 2023;19(9):2538–2557. doi:10.1080/15548627.2023.2200625
55. Sun Y, Wang J, Guo X, et al. Oleic Acid and Eicosapentaenoic Acid Reverse Palmitic Acid-induced Insulin Resistance in Human HepG2 Cells via the Reactive Oxygen Species/JUN Pathway. *Genomics Proteomics Bioinf*. 2021;19(5):754–771. doi:10.1016/j.gpb.2019.06.005
56. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444(7121):860–867. doi:10.1038/nature05485
57. Zenz R, Eferl R, Scheinecker C, et al. Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease. *Arthritis Res Ther*. 2008;10(1):201. doi:10.1186/ar2338
58. Wang X, Liang Y, Liu Q, et al. Association of CYP19A1 Gene, Plasma Zinc, and Urinary Zinc with the Risk of Type 2 Diabetes Mellitus in a Chinese Population. *Biol Trace Elem Res*. 2023;201(9):4205–4215. doi:10.1007/s12011-022-03502-1
59. Nam SH, Kim M, Kim YJ, et al. Risk of New-Onset Diabetes Mellitus Associated with Antirheumatic Drugs in Patients with Rheumatoid Arthritis: a Nationwide Population Study. *J Clin Med*. 2022;11(8):2109. doi:10.3390/jcm11082109