Discussing the Mechanism of Dahuang Huanglian Xiexin Decoction in the Treatment of Type 2 Diabetes Mellitus via Network Pharmacology and Molecular Docking

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Research

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Abstract

Background

Type 2 diabetes mellitus (T2DM) is regarded as Pi Dan disease in traditional Chinese medicine (TCM). Dahuang Huanglian Xiexin Decoction (DHXD), a classical TCM formula, has been used for treating Pi Dan disease in clinic, its pharmacological mechanism has not been elucidated.

Methods

This study used network pharmacological analysis and molecular docking approach to explore the mechanism of DHXD on T2DM. Firstly, the compounds in DHXD were obtained from TCMSP and TCMID databases, the potential targets were determined based on TCMSP and UniProt databases. Next, Genecards, Digenet and UniProt databases were used to identify the targets of T2DM. Then, the protein-protein interaction (PPI) network was established with overlapping genes of T2DM and compounds, and the core targets in the network were identified and analyzed. Then, the David database was used for GO and KEGG enrichment analysis. Finally, the target genes were selected and the molecular docking was completed by Autodock software to observe the binding level of active components with target genes.

Results

A total of 397 related components and 128 overlapping genes were identified. After enrichment analysis, it was found that HIF-1, TNF, IL-17 and other signaling pathways, as well as DNA transcription, gene expression, apoptosis and other cellular biological processes had the strongest correlation with the treatment of T2DM by DHXD, and most of them occurred in the extracellular space, plasma membrane and other places, which were related to enzyme binding and protein binding. In addition, 42 core genes of DHXD, such as VEGFA, TP53 and MAPK1, were considered as potential therapeutic targets, indicating the potential mechanism of DHXD on T2DM. Finally, the results of molecular docking showed that HIF-1 pathway had strong correlation with the target genes INSR and GLUT4, quercetin and berberine had the strongest binding power with them respectively.

Conclusion

This study summarized the main components of DHXD in the treatment of T2DM, identified the core genes and pathways, and systematically analyzed the interaction of related targets, trying to lay the foundation for clarifying the potential mechanism of DHXD on T2DM, so as to carry out further research in the future.

1. Background

Type 2 diabetes mellitus (T2DM), as a common chronic metabolic disease globally, has brought huge burden to social finance and health, which the number of patients with T2DM will increase to 693 million by 2045[1]. Its main characteristics of persistent hyperglycemia and obesity. Due to the progress of the

disease, it will produce a series of complications to damage health, such as large or small blood vessels damage, diabetic peripheral neuropathy, diabetic foot, diabetic nephropathy, and even cardiovascular and cerebrovascular diseases[2]. It has discovered that the core pathogenesis of T2DM is insulin resistance and islet cell damage[3]. There are many therapeutic schemes in clinic, such as exercises, diet control[4], oral medication, insulin injection and stem cell replacement therapy[5]. But, due to the high costs and uncurable results, scientists accelerate the pace of exploring the mechanism of T2DM and develop new drugs and alternative therapy.

With a high safety and small side effects, Traditional Chinese Medicine (TCM) originated in ancient China, have been verified providing effective and safety methods for the treatment of T2DM. Through systematic analysis and meta-analysis, it was found that TCM could intervene T2DM by regulating intestinal flora[6]. Some studies have shown that Liuwei Dihuang pill is effective in the treatment of T2DM. The mechanism may be that quercetin and β - sitosterol act on VEGFA, MAPK1 and other targets through AGE-RAGE, TNF, NF - κ B and other signaling pathways[7]. It has been reported that berberine, quercetin and other major components in Huanglian Jiedu Decoction can act on AKT1, VEGFA and other targets, regulate apoptosis, inflammatory response and other biological processes, treat T2DM through AGE-RAGE, insulin resistance and other signaling pathways[8]. Under the action of berberine, the core component of Gegen Qinlian decoction, it can significantly improve the overall structure of intestinal flora, increase the number of butyrate-producing bacteria, so as to reduce intestinal inflammation and reduce blood glucose[9]. Some articles also mentioned that quercetin, rhein, berberine and other effective components of Da-chai-hu decoction may intervene in T2DM through TNF signaling pathway and PI3K / Akt signaling pathway[10].

DHXD is derived from *Treatise on Febrile Diseases*, it is used to treat spleen stomach excess heat syndrome, which is just to cut the pathogenesis of Pi Dan disease in TCM. It has been proved that Huanglian (coptis chinensis, HL) and Dahuang (rhubarb, DH) in DHXD play an important role in regulating the disorder of glucose and lipid metabolism, and are commonly used in the treatment of T2DM in clinic[11]. However, The mechanism of DHXD on T2DM is not clear, so this study tries to explain it from the perspective of network pharmacology. This paper used network pharmacology to establish a herb-compound-target-pathway network, carried out Gene Ontology (GO) and Kyoto Encyclopedia of genes and genomes (KEGG) analysis. It further verified the potential chemical components and core targets through molecular docking, in order to try to clarify the mechanism of DHXD on T2DM. The detailed flow chart of the research design is shown in Fig. 1.

The design flow chart of this research

2. Methods

2.1 Identification of chemical constituents

It used the Traditional Chinese Medicine System Pharmacology Database (TCMSP™, http://lsp.nwu.edu.cn/tcmsp.php)[12] for an establishment of composite component database, which is a common platform for network pharmacology research. Meanwhile, it used The Traditional Chinese Medicines Integrated Database (TCMID, http://119.3.41.228:8000/)[13], one of the largest comprehensive TCM platforms, to determine the chemical components of DHXD.

2.2 Screening of active ingredients

OB assessment

Oral bioavailability (OB) is one of the most important pharmacokinetic parameters in drug screening, which refers to the percentage of oral drug components reaching systemic circulation. it obtained the OB value from the OBioavail1.1 system of TCMSP platform[14], which has the concordancy of P450, 3A4 and P-glycoprotein information. Simultaneously, it set the OB threshold at 30% for determining the active ingredients used for the subsequent research.

DL prediction

Drug-likeness (DL) refers to the "drug like" degree of the target compound, which is used to remove the chemical inappropriate compounds, and is a qualitative indicator[12]. Comparing the target compounds with all the molecules from TCMSP platform and the DL index was calculated by Tanimoto similarity method, compounds with DL $\geq$ 0.18 were included.

2.3 Target prediction of active ingredients

The compound components in the prescription influence on the biological function of the target. Therefore, it use TCMSP platform to predict the target of active ingredients, and Uniprot Database (Uniprot, http://www.uniprot.org) [15] was used to screen the target of "Homo sapiens".

2.4 Identification of disease target genes

In Genecards Database (https://www.genecards.org)[16], DisGenet Database (https://www.disgenet.org) [17] and UniProt databases, target genes were screened with the key words "diabetes mellitus, non insulin dependent", and the selected target genes were standardized by UniProt Database.

2.5 Common targets of DHXD and T2DM

The intersection of drug target and disease target was represented by Venn diagram. It set the condition as "Homo sapiens" to construct PPI network with common targets in STRING database (STRING, http://www.string-db.org) [5].

2.6 Key Genes screening of common targets of DHXD and T2DM

All the genes were screened by CytoHubba program of Cytoscape 3.7.1 software[18], following the herb-component-target network constructed.

2.7 Biological process and pathway enrichment analysis

DAVID Bioinformatics Resources 6.8 database (DAVID, https: //david.ncifcrf.gov/) [19]was used for the GO and KEGG pathway enrichment analysis of common targets[20]. The screening was carried out under the condition of "$p < 0.01$, gene number > 8", and Bubble Plot was drawn by R Studio software. In addition, following common genes labeled, it draw the relevant path diagram according to the KEGG Mapper (https://www.genome.jp/kegg/mapper.html) [21].

2.8 Component-target molecule docking

It used Autodock 4.2.6 software for semiflexible molecular docking[22]. Firstly, the name, molecular weight and 2D structure of the compound were determined in PubChem database(https://pubchem.ncbi.nlm.nih.gov/), and its 3D structure was constructed by ChemOffice software. Under the condition of the highest degree centrality (DC), the target protein was selected from different clusters, and the 3D structure of the protein receptor was obtained in RCSB PDB database (http://www.rcsb.org/)[23], the original ligand structure of the protein was extracted by using PyMOL 2.3 software. Then, AutoDock Tools (ADT) was used to add polar hydrogen and Gasteiger charge to the processed receptor and ligand. AutoGrid tool was used to set the parameters of docking frame. Lamarckian genetic algorithm (LGA) was used to find the best docking conditions to make it flexible docking and record the docking position of receptor and ligand. Finally, PyMOL software was used to analyze and observe the docking results[8].

3. Results

3.1 Active compounds and target genes of DHXD

From TCMSP and TCMID databases, 397 related compounds were identified in DHXD. Among them, there are 287 components (72.3%) in HL and 110 components (27.7%) in DH. After screening by ADME threshold (OB ≥ 30%, DL ≥ 0.18 and Caco-2 > 0), 30 compounds were obtained (Table 1). 197 genes were obtained in this research, including 177 genes from HL, 70 genes from DH.

Table 1
Active compounds of DHXD

Herb	Mol ID	Molecule Name	OB (%)	DL	Caco-2
Coptis chinensis	MOL001454	Berberine	4	0.57	36.86
Coptis chinensis	MOL013352	Obacunone	7	-0.43	43.29
Coptis chinensis	MOL002894	Berberrubine	4	0.17	35.74
Coptis chinensis	MOL002897	epiberberine	4	0.4	43.09
Coptis chinensis	MOL002903	(R)-Canadine	5	0.57	55.37
Coptis chinensis	MOL002904	Berlambine	6	0.17	36.68
Coptis chinensis	MOL002907	Corchoroside A_qt	6	-1.31	104.95
Coptis chinensis	MOL000622	Magnograndiolide	4	-0.24	63.71
Coptis chinensis	MOL000762	Palmidin A	8	-1.47	35.36
Coptis chinensis	MOL000785	palmatine	4	0.37	64.6
Coptis chinensis	MOL000098	quercetin	7	-0.77	46.43
Coptis chinensis	MOL001458	coptisine	4	0.32	30.67
Coptis chinensis	MOL002668	Worenine	4	0.24	45.83
Coptis chinensis	MOL008647	Moupinamide	5	-0.51	86.71
rhubarb	MOL000096	(-)-catechin	6	-0.78	49.68
rhubarb	MOL000358	beta-sitosterol	1	0.99	36.91
rhubarb	MOL000471	aloe-emodin	5	-1.07	83.38
rhubarb	MOL000554	gallic acid-3-O-(6'-O-galloyl)-glucoside	14	-2.76	30.25
rhubarb	MOL002235	EUPATIN	8	-0.26	50.8

Herb	Mol ID	Molecule Name	OB (%)	DL	Caco-2
rhubarb	MOL002251	Mutatochrome	1	0.84	48.64
rhubarb	MOL002259	Physciondiglucoside	15	-3.43	41.65
rhubarb	MOL002260	Procyanidin B-5,3'-O-gallate	16	-2.88	31.99
rhubarb	MOL002268	rhein	6	-0.99	47.07
rhubarb	MOL002276	Sennoside E_qt	9	-1.56	50.69
rhubarb	MOL002280	Torachrysone-8-O-beta-D-(6'-oxayl)-glucoside	12	-1.84	43.02
rhubarb	MOL002281	Toralactone	5	0.37	46.46
rhubarb	MOL002288	Emodin-1-O-beta-D-glucopyranoside	10	-2	44.81
rhubarb	MOL002293	Sennoside D_qt	9	-1.46	61.06
rhubarb	MOL002297	Daucosterol_qt	1	1.07	35.89
rhubarb	MOL002303	palmidin A	8	-1.47	32.45

3.2 Disease target acquisition

With the key words of "Diabetes Mellitus, Non-Insulin-Dependent", it integrated the disease-related genes obtained from multi-source databases (including GeneCards, DisGenet, UniProt). At last, it identified 3272 related genes, and UniProt database was used to standardize the selected targets.

3.3 Network construction and analysis

Taking the intersection of drug target and diabetes target, 128 overlapping targets were obtained, which were represented by Venn diagram(Fig. 2a). Through the interaction of T2DM, HL and DH targets, it was found that HL alone had 90 related targets for T2DM, suggesting that the main effective components of DHXD in the prevention and treatment of T2DM mostly came from HL. As shown in Fig. 2b, red represented T2DM, yellow represented DH, and blue represented HL. The composition and target information of DH and HL and the related target information of T2DM were made into herb-component-target network diagram, as shown in Fig. 2c, yellow nodes represented HL and DH, green represented active ingredient, purple represents central target, and lines represent their interaction. According to the network analysis, quercetin in HL was considered to be the most effective compound interacting with target genes. Input target genes into the STRING database for PPI network analysis, as shown in Fig. 3a. In this network, there were 126 nodes and 1888 edges in total. When the setting conditions were degree ≥ 17, closeness ≥ 0.459 and betweenness ≥ 0.002, the PPI network with 69 nodes and 1110 edges would be obtained in Fig. 3b. Under the condition of degree ≥ 28, closeness ≥ 0.532 and betweenness ≥ 0.004, the PPI network with 42 nodes and 632 edges remained would be build in Fig. 3c after clustering analysis of these related targets ,in this network, the color and size change of all nodes is based on the pertinency

from high to low, the most influential target was VEGFA. The detailed information of 42 genes was shown in Table 2.

Venn diagram and herb-component-target network. **a** 128 intersection genes; **b** red, yellow and blue areas represented T2DM, DH and HL respectively; **c** a complete herb-component-target network

Table 2
Information of 42 targets

Uniprot ID	Gene Symbol	Description
P05231	IL6	Interleukin-6
P04637	TP53	Cellular tumor antigen p53
P15692	VEGFA	Vascular endothelial growth factor A
P01375	TNF	Tumor necrosis factor
P42574	CASP3	Caspase-3
P28482	MAPK1	Mitogen-activated protein kinase 1
P00533	EGFR	Epidermal growth factor receptor
P01133	EGF	Pro-epidermal growth factor
P35354	PTGS2	prostaglandin-endoperoxide synthase 2
P14780	MMP9	Matrix metalloproteinase-9
P03372	ESR1	Estrogen receptor
P01584	IL1B	Interleukin-1 beta
P01100	FOS	Proto-oncogene c-Fos
P13500	CCL2	C-C motif chemokine 2
P29474	NOS3	Nitric oxide synthase
P60484	PTEN	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN
P22301	IL10	Interleukin-10
P37231	PPARG	Peroxisome proliferator-activated receptor gamma
P09601	HMOX1	Heme oxygenase 1
P05121	SERPINE1	Plasminogen activator inhibitor 1
P05362	ICAM1	Intercellular adhesion molecule 1
P04626	ERBB2	Receptor tyrosine-protein kinase erbB-2
Q04206	RELA	Transcription factor p65
P60568	IL2	Interleukin-2
P10451	SPP1	Osteopontin
P42224	STAT1	Signal transducer and activator of transcription 1-alpha/beta

Uniprot ID	Gene Symbol	Description
P05164	MPO	Myeloperoxidase
Q16665	HIF1A	Hypoxia-inducible factor 1-alpha
Q07817	BCL2L1	Bcl-2-like protein 1
P35968	KDR	Vascular endothelial growth factor receptor 2
P42771	CDKN2A	Cyclin-dependent kinase inhibitor 2A
P02741	CRP	C-reactive protein
P08684	CYP3A4	Cytochrome P450 family 3 subfamily A member 4
Q9UNQ0	ABCG2	ATP binding cassette subfamily G member 2
P00734	F2	Prepro-coagulation factor II
P00441	SOD1	Superoxide dismutase 1
P35869	AHR	Aryl hydrocarbon receptor
P06401	PGR	Progesterone receptor
Q16236	NFE2L2	Nuclear factor erythroid 2-related factor 2
Q03135	CAV1	Caveolin 1
P10275	AR	Androgen receptor
Q14790	CASP8	Caspase 8

3.4 Screening and analysis of key genes

Based on 42 genes in Fig. 3c, a target-pathway network was built in Fig. 3d which showed central targets as purple nodes and 6 related pathways as red nodes. There were 11 targets in this network, contained EGFR, MMP9, TP53, MAPK1, TNF, EGF, IL6, CASP3, PTGS2, IL10, VEGFA. In the 6 pathways in the figure, C-type lectin receptor signaling pathway (hsa04625), AGE-RAGE signaling pathway (hsa04933), HIF-1 signaling pathway (hsa04066) were associated with 5 genes respectively, TNF signaling pathway (hsa04668) was associated with 6 genes, IL-17 signaling pathway (hsa04657) was associated with 7 genes, VEGF signaling pathway (hsa04370) was associated with 3 genes.

PPI networks and their analysis. **a** a PPI network of 128 targets; **b** a PPI network of 69 targets; **c** a PPI network of 42 targets; **d** the target-pathway network based on 42 key targets; **e** the bubble chart of KEGG enrichment based on 42 key targets

3.5 Enrichment analysis of GO

Biological processes (BP) were sorted in ascending order of logP value, we found that in the top 10 positive regulation of BP were concentrated in vasoconstriction (GO: 0042310), transcription, DNA-templated (GO: 0045893), transcription from RNA polymerase II promoter (GO: 0045944), superoxide anion generation (GO: 0032930), protein phosphorylation (GO: 0006468), nitric oxide biosynthetic process (GO: 0045429), gene expression (GO: 0010628), ERK1 and ERK2 cascade (GO: 0070374), cell proliferation (GO: 0008284) and angiogenesis (GO: 0045766) as shown in Fig. 4a, among these BP, gene expression (GO: 0010628) was the most important. In the top 10 negative regulation of BP were concentrated in transcription from RNA polymerase □ promoter (GO: 0010553), smooth muscle cell proliferation (GO: 0048662), neuron apoptotic process (GO: 0043524), lipid storage (GO: 0019915), gene expression (GO: 0010629), extrinsic apoptotic signaling pathway via death domain receptors (GO: 1902042), endothelial cell apoptotic process (GO: 2000352), cell proliferation (GO: 0008285), cell growth (GO: 0030308) and the most significant BP: apoptotic process (GO: 0006915) as shown in Fig. 4b. About cell composition (CC), the most influential was extracellular space (GO: 0005615), as shown in Fig. 4c, others in the top 10 were receptor complex (GO: 00043235), plasma membrane (GO: 0005886), perinuclear region of cytoplasm (GO: 0048471), membrane raft (GO: 0045121), extracellular region (GO: 0005576), extracellular matrix (GO: 0031012), extracellular exosome (GO: 0070062), cytosol (GO: 0005829) and caveola (GO: 0005901). In terms of molecular function (MF), enzyme binding (GO: 0019899) was most significant, others in the top 10 were transcription factor binding (GO: 0008134), steroid hormone receptor activity (GO: 0003707), steroid binding (GO: 0005496), RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding (GO: 0004879), protein homodimerization activity (GO: 0042803), protein binding (GO: 0005515), identical protein binding (GO: 0042802), heme binding (GO: 0020037) and drug binding (GO: 0008144) (Fig. 4d).

The bubble chart about whole 128 genes. In these bubble charts, the larger the bubble, the more genes were enriched in the pathway; the redder the color, the smaller the P value, the more significant the result is; Rich factor referred to the ratio of the number of genes belonging to the pathway in the target gene set to the number of genes belonging to the pathway in the background gene set, and the higher the value, the higher the enrichment degree. **a** Top 10 of positive regulation of GO-BP; **b** Top 10 of negative regulation of GO-BP; **c** Top 10 of GO-CC; **d** Top 10 of GO-MF; **e** Top 10 of of KEGG-Pathway

3.6 Enrichment analysis of KEGG pathway

By enrichment analysis of KEGG pathway in 128 genes, top 10 pathways were found to be significantly correlated with T2DM, PI3K-Akt signaling pathway (hsa04151) had the most genes and HIF-1 signaling pathway (hsa04066) was the most significant pathway, others were VEGF signaling pathway (hsa04370), Toll-like receptor signaling pathway (hsa04620), TNF signaling pathway (hsa04668), Sphingolipid signaling pathway (hsa04071), p53 signaling pathway (hsa04115), FoxO signaling pathway (hsa04068), Estrogen signaling pathway (hsa04915) and Calcium signaling pathway (hsa04020) (Fig. 4e). After clustering analysis, there were most relevant 6 pathways in 42 genes, especially IL-17 signaling pathway (hsa04657), others were VEGF signaling pathway (hsa04370), TNF signaling pathway (hsa04668), HIF-1

signaling pathway (hsa04066), C-type lectin receptor signaling pathway (hsa04625), AGE-RAGE signaling pathway in diabetic complications (hsa04933) (Fig. 3e).

After labeling the common genes with KEGG-MAPPER, insulin resistance signaling pathway (hsa04931) and HIF-1 signaling pathway (hsa04066) were the most correlated pathways with DHXD, according to KEGG-MAPPER, their most related targets were shown in Fig. 5 and Fig. 6 as red nodes.

HIF-1 signaling pathway influenced by DHXD. The red nodes represented the hub genes.

Insulin resistance signaling pathway influenced by DHXD. The red nodes represented the hub genes.

3.7 Component-target molecule docking of DHXD

INSR and GLUT4, two target genes, showed strong association with other targets, pathways and active components, in this study, we combined them with 5 putative components to test their binding ability as shown in Table 3. According to the binding energy (Δ gbind) of molecular docking results, their binding activities were good (Δ gbind < − 5kj · mol − 1). The docking results of INSR with 5 active components were shown in Fig. 7, Fig. 7a-e represented their action mode, and the highly relevant target-pathway network was presented in Fig. 7f, Among them, HIF-1 signaling pathway (hsa04066) were the most correlated pathway (Fig. 7g) and quercetin (MOL000098) had the strongest binding force with INSR (Fig. 7h). Figure 8a-e meant the action mode of GLUT4 with 5 active ingredients, INSR,VEGFA,PPARG and other targets had been proved to be related again(Fig. 8f), HIF-1 signaling pathway (hsa04066) was also the most significant related pathway(Fig. 8g), berberine (MOL001454) had a strong interaction with GLUT4 (Fig. 8h).

Table 3
Compounds- targets docking score of DHXD

Mol ID	Compounds	GLUT4	INSR	Herb
MOL000098	Quercetin	-8.9	-7.6	Coptis chinensis
MOL000358	Beta-sitosterol	-6.7	-7.4	rhubarb
MOL000471	Aloe-emodin	-8.5	-7.1	rhubarb
MOL002904	Berlambine	-9.3	-7.3	Coptis chinensis
MOL001454	Berberine	-10.6	-7.5	Coptis chinensis

Molecular docking results of 5 active compounds with INSR. **a** action mode of quercetin (MOL000098) with target INSR; **b** action mode of berberine (MOL001454) with target INSR; **c** action mode of berlambine (MOL002904) with target INSR; **d** action mode of beta-sitosterol (MOL000358) with target INSR; **e** action mode of aloe-emodin (MOL000471) with target INSR; **f** the relevant target-pathway network; **g** the bubble chart of KEGG enrichment based on target INSR; **h** a bar chart of comparison on binding force

Molecular docking results of 5 active compounds with GLUT4. **a** action mode of berberine (MOL001454) with target GLUT4; **b** action mode of berlambine (MOL002904) with target GLUT4; **c** action mode of quercetin (MOL000098) with target GLUT4; **d** action mode of aloe-emodin (MOL000471) with target GLUT4R; **e** action mode of beta-sitosterol (MOL000358) with target GLUT4; **f** the relevant target-pathway network; **g** the bubble chart of KEGG enrichment based on target GLUT4; **h** a bar chart of comparison on binding force

4. Discussion

T2DM has become a worldwide disease, threatening people's lives and property. TCM has significant advantages in the comprehensive regulation of glucose and lipid metabolism disorders, prevention and treatment of complications. In recent years, network pharmacology has become an advanced method to analyze the complex mechanism of TCM by knowing their effective components or formulas. This study found that DHXD could treat T2DM by regulating multiple targets of multiple components.

The mechanism of DHXD on T2DM was analyzed by network pharmacology and related databases, Studies have shown that berberine, berberrubine, epiberberine, EUPATIN, beta-sitosterol, rhein and other components play a major role in the treatment of T2DM and its complications. At the same time, we analyzed the MF, BP and CC of DHXD in T2DM, found that the BP of gene expression and apoptosis process were most affected, these processes occur mainly in the extracellular space, plasma membrane and so on. the MF shows that these components can bind to enzyme and protein well to maintain the activity of cells and receptors, we further examined 42 target genes and top 10 key pathways about DHXD on T2DM. Studies have shown that berberine can regulate glucose and lipid metabolism by regulating IL-6, TNF and other targets for anti-oxidation, reducing inflammatory response and increasing serum SOD activity[24], some researches have also confirmed this result of promoting insulin secretion and improving insulin resistance (IR) by regulating AMPK, MAPK and NF - κ B pathways [25]. In addition, other alkaloids such as epiberberine, magnoflorine, palmatine, jatrorrhizine and coptisine also intervene T2DM and its complications through related mechanisms[26]. Some network pharmacological studies have found that β - sitosterol may resist diabetic retinopathy by regulating key targets such as VEGFA in HIF-1 pathway[27], in addition to insulin secretagogue, β - sitosterol also has insulin-like activity, regulating PI3K / Akt and GLUT4[28]. Rhein can play an anti-inflammatory role by regulating NF - κ B and reducing the expression of pro-inflammatory factors TNF - α and IL-6, which is closely related to T2DM; clinically, oral rhubarb rhizome extract can significantly reduce glycosylated hemoglobin, fasting blood glucose and body weight of patients with T2DM[29].

HIF-1 signaling pathway is closely related to hypoxia, HIF-1 is a heterodimer composed of HIF-1α and HIF-1β[30]. Both HIF-1α and HIF-1β play a regulatory role in metabolic diseases. HIF-1α may inhibit or increase obesity and IR, play an important role in islet β cells, and HIF1β has a separate function in metabolic abnormalities[31]. In this paper, KEGG enrichment analysis based on 128 overlapping genes and 42 core targets, as well as molecular docking results showed that HIF-1 signaling pathway played an important role, which might be the potential mechanism of DHXD in the treatment of T2DM. Some

experiments had shown that the activation of HIF-1α could control the weight and blood glucose of the model mice, the insulin sensitivity, lipid metabolism level and albuminuria of the model mice were improved, which might suggest that HIF-1 signaling pathway has a potential impact on diabetes and its complications[32]. Some studies found that HIF-1α gene expression in T2DM patients with poor metabolic control was decreased[33]. However, some studies showed that the level of HIF-1α in T2DM patients was significantly higher than that in the control group, and insulin secretion increased when hypoxia was inhibited[34]. Therefore, the effect of HIF-1 signaling pathway on T2DM is not completely clear, which needs to be further confirmed. This paper also laid a research foundation for its elucidation.

INSR is a protein coding gene, which encodes two isoforms of INSR-A and INSR-B, the former is beneficial to prenatal development and tissue growth, expressed in undifferentiated cells; the latter is responsible for the systemic metabolism of insulin, growing in differentiated cells and post-mitotic cells, INSR plays an important role in maintaining glucose homeostasis and insulin sensitivity[35]. It was found that INSR increased in IR rats, and the increase of soluble INSR might be an early sign of metabolic syndrome[36]. This may suggest that the increase of INSR could be found in the early stage of T2DM. There was a research reported that the activation of INSR in liver and skeletal muscle of LEPR $^{db/db}$ mice can improve insulin sensitivity and reduce blood glucose[37]. An article also showed that up regulation of INSR can reduce blood glucose and increase insulin level in mice[38].

GLUT4, a member of glucose transporter family, is a major insulin regulated glucose transporter. The decrease of GLUT4 content will affect glucose uptake in adipose tissue and fat formation[39], activating the expression of GLUT4, promoting the fusion of GLUT4 plasma membrane and subsequent glucose uptake could effectively improve hyperglycemia and protect islet function[40]. It has been proved that GLUT4 can stay on the plasma membrane for a long time in order to maximize glucose absorption[41]. There was a study showed that berberine could reduce blood glucose, total cholesterol, low density lipoprotein by increasing the expression of GLUT4 in rat skeletal muscle to reduce IR[42]. In this paper, the results of molecular docking showed that berberine had the strongest binding capacity with GLUT4.

5. Conclusion

DHXD is effective in the treatment of T2DM, but the specific active ingredients and mechanisms of intervention have not been clarified. Therefore, using network pharmacology and molecular docking verification to establish a herbs-components- targets-pathways network and observe the potential targets with the highest correlation will help to clarify the direction of further research and provide the basis for explaining the exact mechanism. It was explored in this paper that berberine and other components might be the reason for the efficacy of DHXD in the treatment of T2DM, gene expression and apoptosis process had the greatest impact on it, INSR and GLUT4 might be the most important targets of DHXD on T2DM, and the effect of HIF-1 signaling pathway was the most significant. Nevertheless, more in vivo and in vitro experiments are needed to confirm the availability of these data and materials.

Abbreviations

T2DM: Type 2 diabetes mellitus; TCM: Traditional Chinese medicine; DHXD: Dahuang Huanglian Xiexin Decoction; PPI: Protein-protein interaction; HL: Huanglian; DH: Dahuang; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of genes and genomes; OB: Oral bioavailability; DL: Drug-likeness; DC: Degree centrality; IR: insulin resistance.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

XC, XDZ and WZ all conceived the idea of this paper and supervised the research. XC performed the research, analyzed the data and wrote the manuscript. YW reviewed the data and improved the manuscript. LLZ, XXX and YW carried out target prediction and molecule docking as well as related enrichment process. All authors reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are available in public databases from TCMSP, TCMID, UniProt, Genecards, DAVID, STRING, Digenet, PubChem and RCSB PDB.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Cho NH. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. diabetes research and clinical practice. 2018;:11.
2. World Health Organization, editor. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva, Switzerland: World Health Organization; 2009.
3. Zang L, Hao H, Liu J, Li Y, Han W, Mu Y. Mesenchymal stem cell therapy in type 2 diabetes mellitus. Diabetol Metab Syndr. 2017;9:36.
4. Taheri S, Chagoury O, Zaghloul H, Elhadad S, Ahmed SH, Omar O, et al. Diabetes Intervention Accentuating Diet and Enhancing Metabolism (DIADEM-I): a randomised controlled trial to examine the impact of an intensive lifestyle intervention consisting of a low-energy diet and physical activity on body weight and metabolism in early type 2 diabetes mellitus: study protocol for a randomized controlled trial. Trials. 2018;19:284.
5. Wang L. Mesenchymal stromal cells ameliorate oxidative stress-induced islet endothelium apoptosis and functional impairment via Wnt4-β-catenin signaling. 2017;:13.
6. Zheng Y, Ding Q, Zhang L, Gou X, Wei Y, Li M, et al. The effect of traditional Chinese medicine on gut microbiota in adults with type 2 diabetes: A protocol for systematic review and meta-analysis. Medicine. 2020;99:e22233.
7. He D, Huang J, Zhang Z, Du Q, Peng W, Yu R, et al. A Network Pharmacology-Based Strategy For Predicting Active Ingredients And Potential Targets Of LiuWei DiHuang Pill In Treating Type 2 Diabetes Mellitus. DDDT. 2019;Volume 13:3989–4005.
8. Yin B, Bi Y-M, Fan G-J, Xia Y-Q. Molecular Mechanism of the Effect of Huanglian Jiedu Decoction on Type 2 Diabetes Mellitus Based on Network Pharmacology and Molecular Docking. Journal of Diabetes Research. 2020;2020:1–24.
9. Xu X, Gao Z, Yang F, Yang Y, Chen L, Han L, et al. Antidiabetic Effects of Gegen Qinlian Decoction via the Gut Microbiota Are Attributable to Its Key Ingredient Berberine. Genomics, Proteomics & Bioinformatics. 2020. doi:10.1016/j.gpb.2019.09.007.
10. Ren B, Tan L, Xiong Y, Ji W, Mu J, Pei Y, et al. Integrated Analysis of the Mechanisms of Da-Chai-Hu Decoction in Type 2 Diabetes Mellitus by a Network Pharmacology Approach. Evidence-Based Complementary and Alternative Medicine. 2020;2020:1–21.
11. Peng W. Efficacy of Chinese herbal medicine for stroke modifiable risk factors: a systematic review. 2017;:29.
12. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform. 2014;6:13.
13. Sorokina M. Review on natural products databases: where to find data in 2020. 2020;:51.

14. Yi F. In silico approach in reveal traditional medicine plants pharmacological material basis. 2018;:20.
15. Jain E, Bairoch A, Duvaud S, Phan I, Redaschi N, Suzek BE, et al. Infrastructure for the life sciences: design and implementation of the UniProt website. BMC Bioinformatics. 2009;10:136.
16. Dahary D, Golan Y, Mazor Y, Zelig O, Barshir R, Twik M, et al. Genome analysis and knowledge-driven variant interpretation with TGex. BMC Med Genomics. 2019;12:200.
17. Coulet A. Learning from biomedical linked data to suggest valid pharmacogenes. 2017;:12.
18. Shannon P. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Research. 2003;13:2498–504.
19. Gu C, Lhamo T, Zou C, Zhou C, Su T, Draga D, et al. Comprehensive analysis of angiogenesis-related genes and pathways in early diabetic retinopathy. BMC Med Genomics. 2020;13:142.
20. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Research. 2009;37:1–13.
21. Porollo A. EC2KEGG: a command line tool for comparison of metabolic pathways. Source Code Biol Med. 2014;9:19.
22. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2009;:NA-NA.
23. Gao X, Li S, Cong C, Wang Y, Xu L. A Network Pharmacology Approach to Estimate Potential Targets of the Active Ingredients of Epimedium for Alleviating Mild Cognitive Impairment and Treating Alzheimer's Disease. Evidence-Based Complementary and Alternative Medicine. 2021;2021:1–15.
24. Pang B, Zhao L-H, Zhou Q, Zhao T-Y, Wang H, Gu C-J, et al. Application of Berberine on Treating Type 2 Diabetes Mellitus. International Journal of Endocrinology. 2015;2015:1–12.
25. Meng F-C. Coptidis rhizoma and its main bioactive components: recent advances in chemical investigation, quality evaluation and pharmacological activity. 2018;:18.
26. Wang J. Cellular stress response mechanisms of Rhizoma coptidis: a systematic review. 2018;:14.
27. Wang L, Li S, Wang L, Lin K, Du J, Miao W, et al. Uncovering the protective mechanism of Taohong Siwu decoction against diabetic retinopathy via HIF-1 signaling pathway based on network analysis and experimental validation. BMC Complement Med Ther. 2020;20:298.
28. Chai J-W, Lim S-L, Kanthimathi MS, Kuppusamy UR. Gene regulation in β-sitosterol-mediated stimulation of adipogenesis, glucose uptake, and lipid mobilization in rat primary adipocytes. Genes Nutr. 2011;6:181–8.
29. Xiang H, Zuo J, Guo F, Dong D. What we already know about rhubarb: a comprehensive review. Chin Med. 2020;15:88.
30. Zhang Z, Yao L, Yang J, Wang Z, Du G. PI3K/Akt and HIF-1 signaling pathway in hypoxia-ischemia (Review). MOLECULAR MEDICINE REPORTS. 2018;:8.
31. Gonzalez FJ, Xie C, Jiang C. The role of hypoxia-inducible factors in metabolic diseases. Nat Rev Endocrinol. 2019;15:21–32.

32. Sugahara M, Tanaka S, Tanaka T, Saito H, Ishimoto Y, Wakashima T, et al. Prolyl Hydroxylase Domain Inhibitor Protects against Metabolic Disorders and Associated Kidney Disease in Obese Type 2 Diabetic Mice. 2020;:26.
33. López-Cano C, Gutiérrez-Carrasquilla L, Barbé F, Sánchez E, Hernández M, Martí R, et al. Effect of Type 2 Diabetes Mellitus on the Hypoxia-Inducible Factor 1-Alpha Expression. Is There a Relationship with the Clock Genes? JCM. 2020;9:2632.
34. Fang Z, Duan X, Zhao J, Wu Y, Liu M. Novel Polysaccharide H-1-2 from Pseudostellaria Heterophylla Alleviates Type 2 Diabetes Mellitus. Cell Physiol Biochem. 2018;49:1037–47.
35. Cignarelli A, Genchi VA, Perrini S, Natalicchio A, Laviola L, Giorgino F. Insulin and Insulin Receptors in Adipose Tissue Development. International Journal of Molecular Sciences. 2019;20. doi:10.3390/ijms20030759.
36. Hiriart-Urdanivia M, Sánchez-Soto C, Velasco M, Ortiz-Huidobro RI. El receptor soluble de insulina y el síndrome metabólico. Gaceta Médica de México. :5.
37. Lan Z-J, Lei Z, Yiannikouris A, Yerramreddy TR, Li X, Kincaid H, et al. Non-peptidyl small molecule, adenosine, 5'-Se-methyl-5'-seleno-, 2',3'-diacetate, activates insulin receptor and attenuates hyperglycemia in type 2 diabetic Leprdb/db mice. Cell Mol Life Sci. 2020;77:1623–43.
38. Tian S, Wang M, Liu C, Zhao H, Zhao B. Mulberry leaf reduces inflammation and insulin resistance in type 2 diabetic mice by TLRs and insulin Signalling pathway. BMC Complement Altern Med. 2019;19:326.
39. Smith U, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. Journal of Internal Medicine. 2016;280:465–75.
40. Song G, Huang Y, Xiong M, Yang Z, Liu Q, Shen J, et al. Aloperine Relieves Type 2 Diabetes Mellitus via Enhancing GLUT4 Expression and Translocation. Front Pharmacol. 2021;11. doi:10.3389/fphar.2020.561956.
41. Mukaida S, Sato M, Öberg AI, Dehvari N, Olsen JM, Kocan M, et al. BRL37344 stimulates GLUT4 translocation and glucose uptake in skeletal muscle via β2-adrenoceptors without causing classical receptor desensitization. :12.
42. Mi J, He W, Lv J, Zhuang K, Huang H, Quan S. Effect of berberine on the HPA-axis pathway and skeletal muscle GLUT4 in type 2 diabetes mellitus rats. Diabetes Metab Syndr Obes. 2019;12:1717–25.

Figures

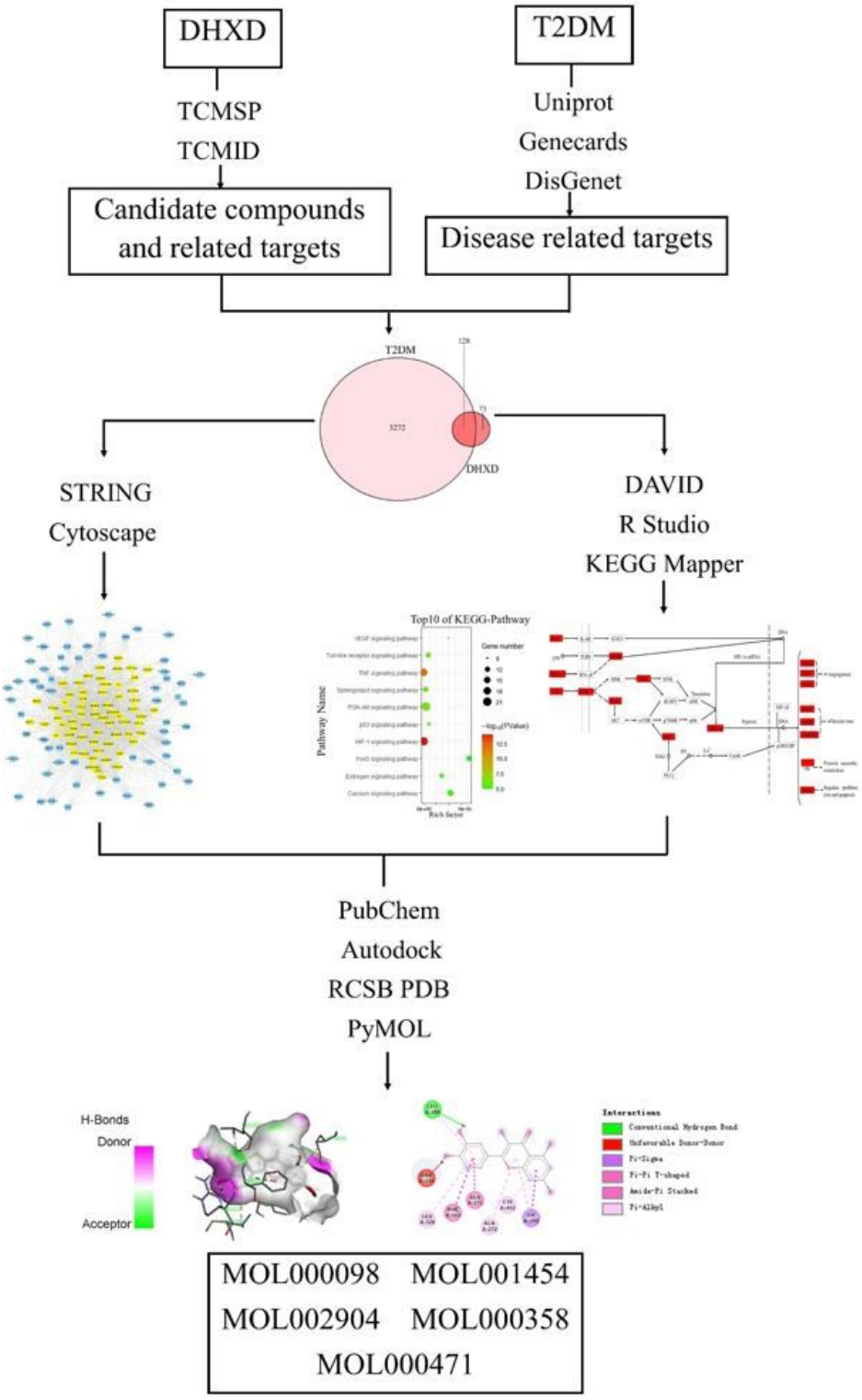

Figure 1

The design flow chart of this research

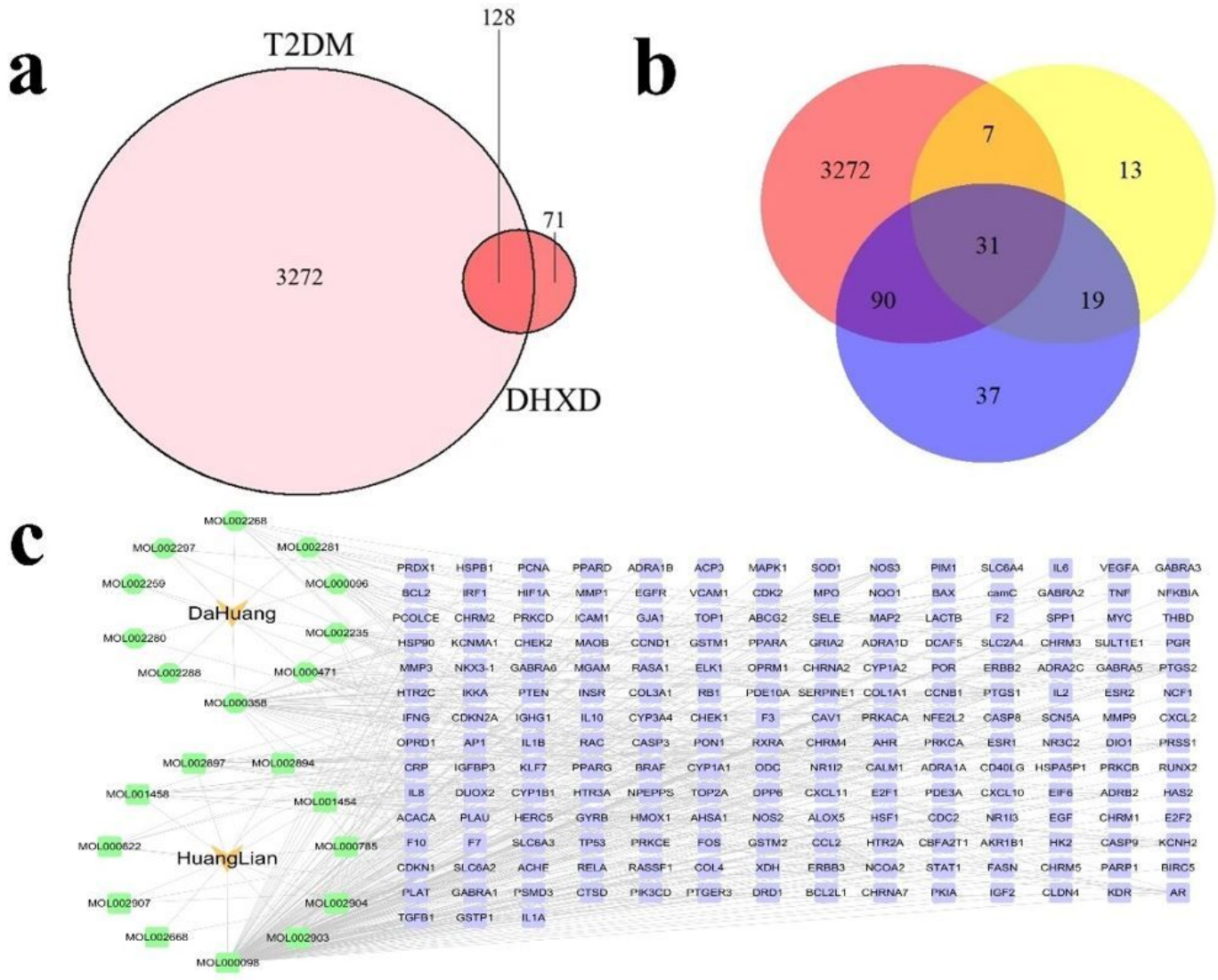

Figure 2

Venn diagram and herb-component-target network. a 128 intersection genes; b red, yellow and blue areas represented T2DM, DH and HL respectively; c a complete herb-component-target network

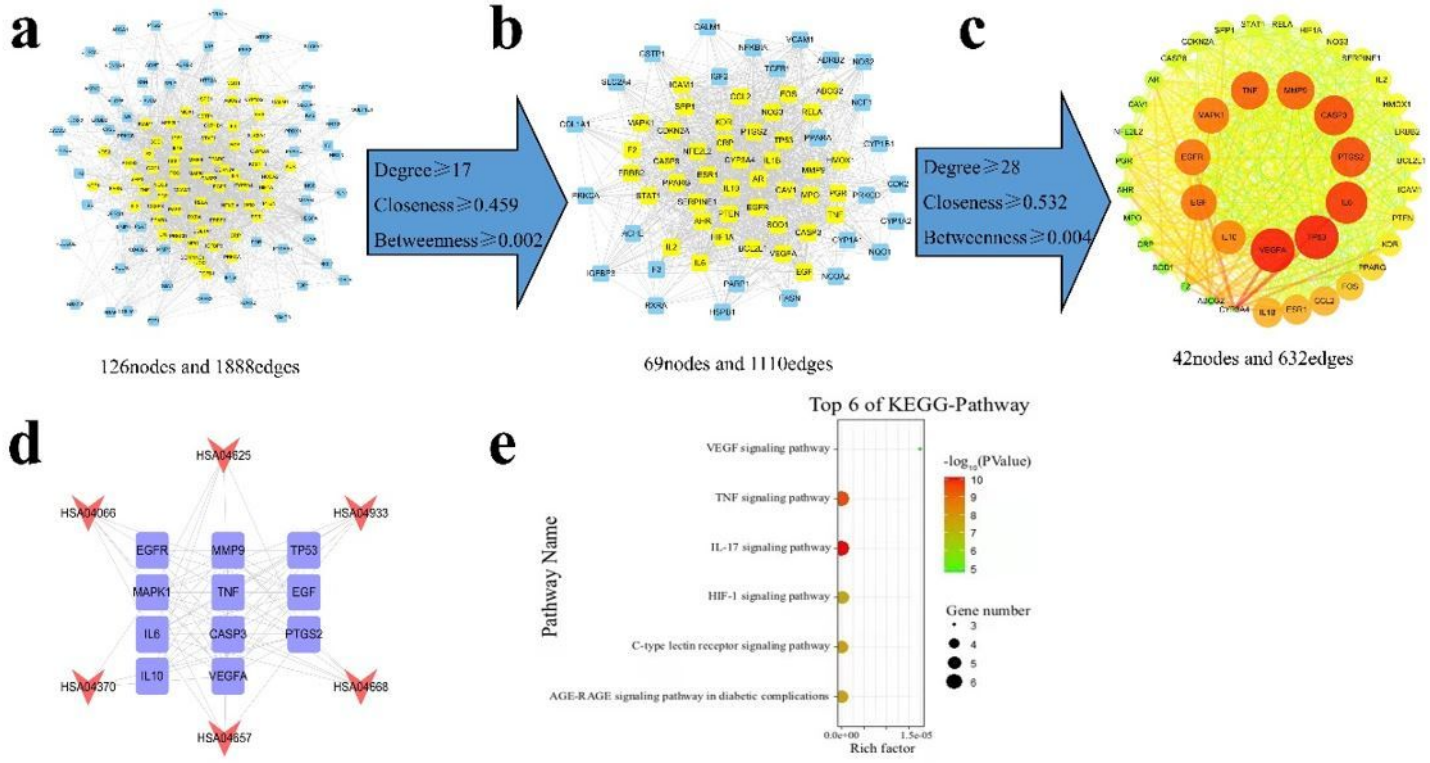

Figure 3

PPI networks and their analysis. a a PPI network of 128 targets; b a PPI network of 69 targets; c a PPI network of 42 targets; d the target-pathway network based on 42 key targets; e the bubble chart of KEGG enrichment based on 42 key targets

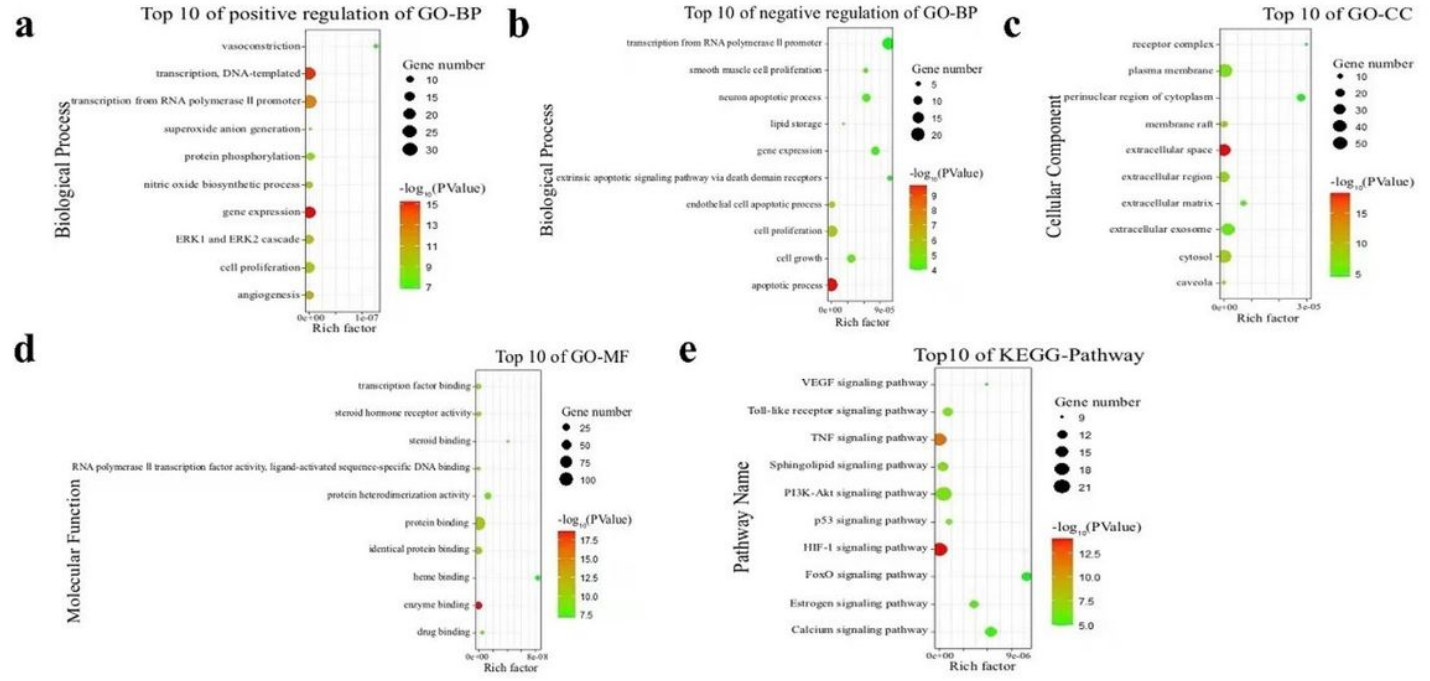

Figure 4

The bubble chart about whole 128 genes. In these bubble charts, the larger the bubble, the more genes were enriched in the pathway; the redder the color, the smaller the P value, the more significant the result is; Rich factor referred to the ratio of the number of genes belonging to the pathway in the target gene set to the number of genes belonging to the pathway in the background gene set, and the higher the value, the higher the enrichment degree. a Top 10 of positive regulation of GO-BP; b Top 10 of negative regulation of GO-BP; c Top 10 of GO-CC; d Top 10 of GO-MF; e Top 10 of of KEGG-Pathway

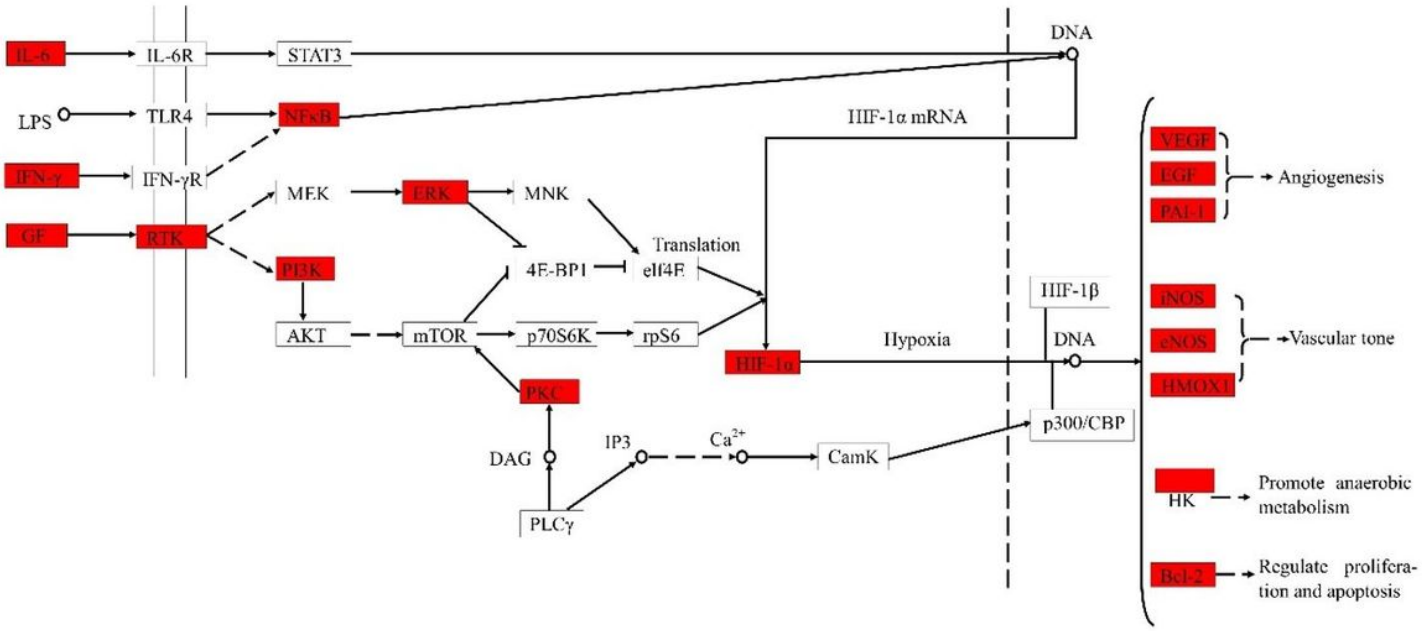

Figure 5

HIF-1 signaling pathway influenced by DHXD. The red nodes represented the hub genes.

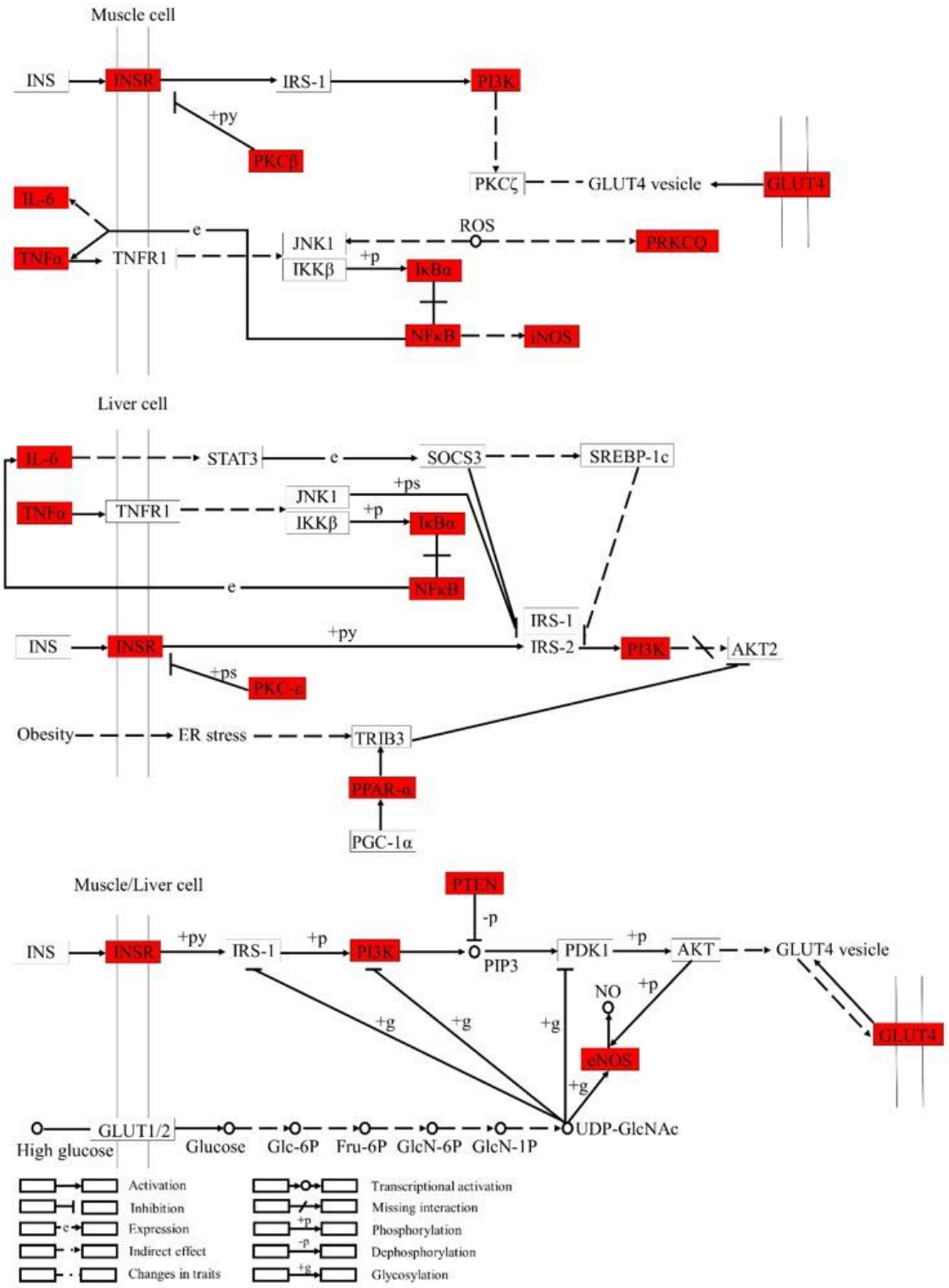

Figure 6

Insulin resistance signaling pathway influenced by DHXD. The red nodes represented the hub genes.

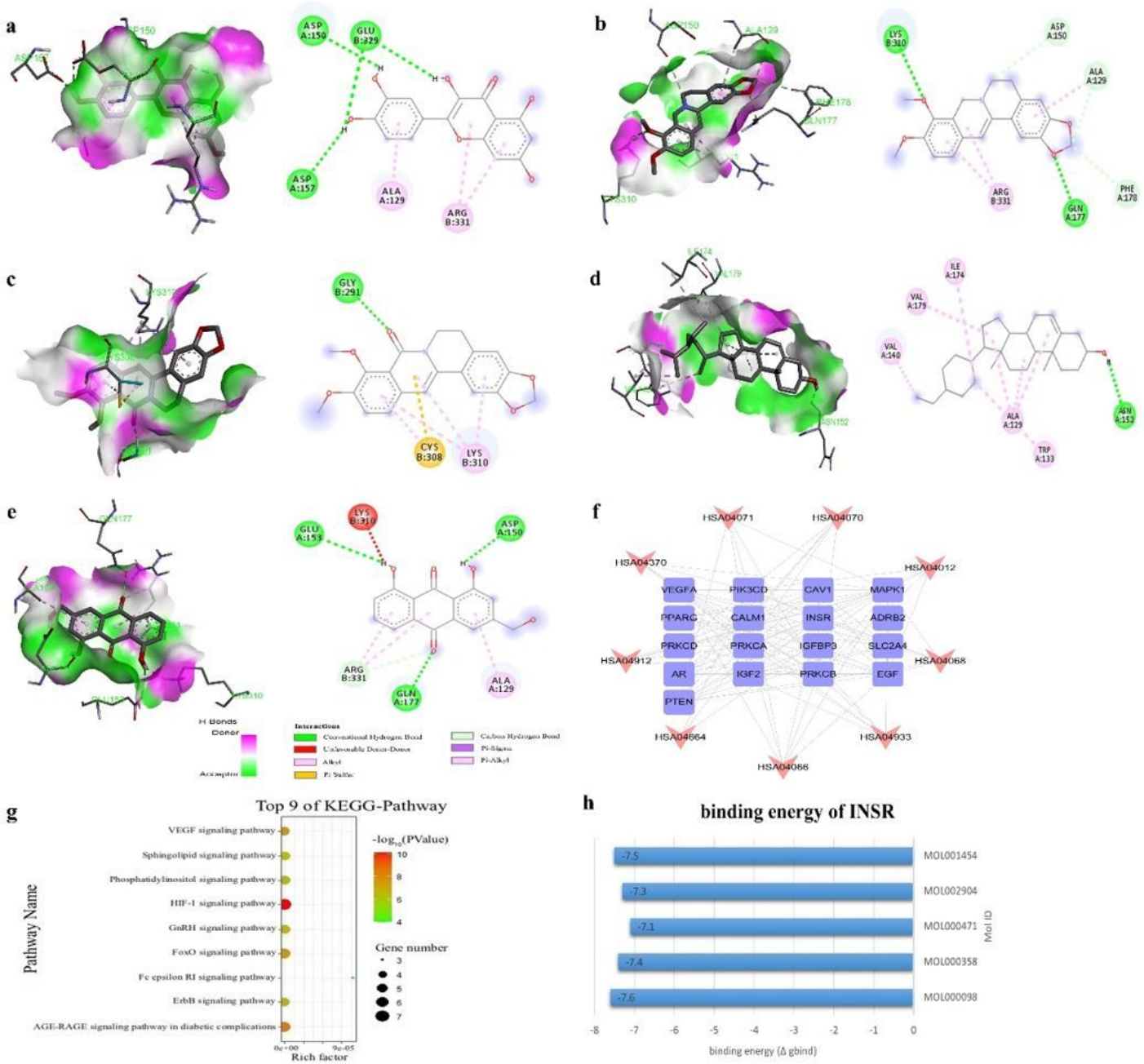

Figure 7

Molecular docking results of 5 active compounds with INSR. a action mode of quercetin (MOL000098) with target INSR; b action mode of berberine (MOL001454) with target INSR; c action mode of berlambine (MOL002904) with target INSR; d action mode of beta-sitosterol (MOL000358) with target INSR; e action mode of aloe-emodin (MOL000471) with target INSR; f the relevant target-pathway network; g the bubble chart of KEGG enrichment based on target INSR; h a bar chart of comparison on binding force

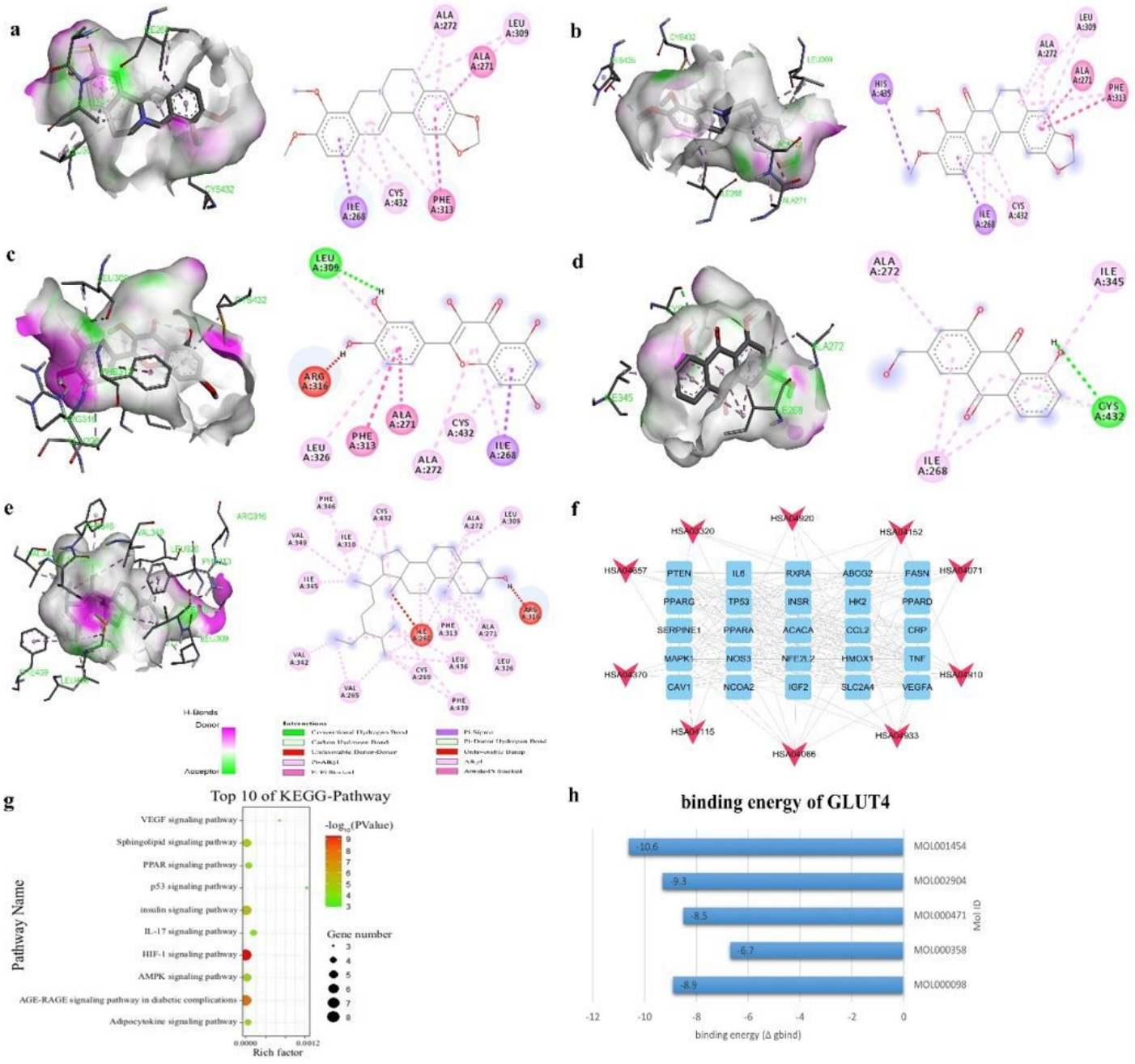

Figure 8

Molecular docking results of 5 active compounds with GLUT4. a action mode of berberine (MOL001454) with target GLUT4; b action mode of berlambine (MOL002904) with target GLUT4; c action mode of quercetin (MOL000098) with target GLUT4; d action mode of aloe-emodin (MOL000471) with target GLUT4R; e action mode of beta-sitosterol (MOL000358) with target GLUT4; f the relevant target-pathway network; g the bubble chart of KEGG enrichment based on target GLUT4; h a bar chart of comparison on binding force

Supplementary Files

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- initialdata.xlsx