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Research article

The molecular mechanism of "Dahuang-Shengjiang-Banxia decoction" in the treatment of diabetic kidney disease was verified based on network pharmacology and molecular docking

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ABSTRACT

Background: Explore the molecular mechanism of Dahuang-Shengjiang-Banxia Decoction (DSBD) in the treatment of diabetic kidney disease (DKD), using network pharmacology and molecular docking technology.
Method: The effective ingredients and targets of the DSBD were taken from the TCMSP database, while the disease targets were obtained via GeneCards, OMIM, DrugBank, TTD, and DisGeNET. Cytoscape 3.9.1 was used to create a drug-ingredient-target network diagram. STRING databases are also used to analyze the Protein-Protein Interaction (PPI) network of intersecting targets. The core targets was obtained by the intersection of the differential genes screened from the intersection target and GEO, and the core targets was enriched by Gene ontology (GO), Kyoto gene and genome (KEGG), and Gene Set Enrichment Analysis (GSEA). CIBERSORTx was used for immunoinfiltration analysis, and then the core targets was analyzed by Nephroseq V5 and KIT for clinical correlation analysis and single-cell sequencing. Lastly, AutoDock Vina was used for molecular docking of both the core targets and the top active elements.
Results: A total of 177 DSBD and 2906 DKD targets were screened. Six core targets were identified by screening, which were *IL1B, MMP9, EGF, VEGFA, HIF1A, and PTGS2*. The top 6 active ingredients are 6-gingerol, baicalin, oleic acid, β-sitosterol, linolenic acid, and aloe emodin. The core targets has good docking activity with the active ingredient.
Conclusion: DSBD may exert its therapeutic effect on DKD through multicomponent, multipath, and multi-target analyses. It is possible that *VEGFA* is a key target in therapy, and that the VEGF/PI3K/AKT signaling pathway plays a key role in therapy.

1. Introduction

Diabetes mellitus (DM) is characterized clinically by polydipsia, polyphagia, polyuria, and weight loss, which is primarily characterized by hyperglycemia. Long-term disease can cause damage to multiple systems, resulting in dysfunction. Diabetic Kidney Disease (DKD) is a chronic kidney condition that is caused by DM. According to the relevant survey [1], the prevalence rate of adult DM

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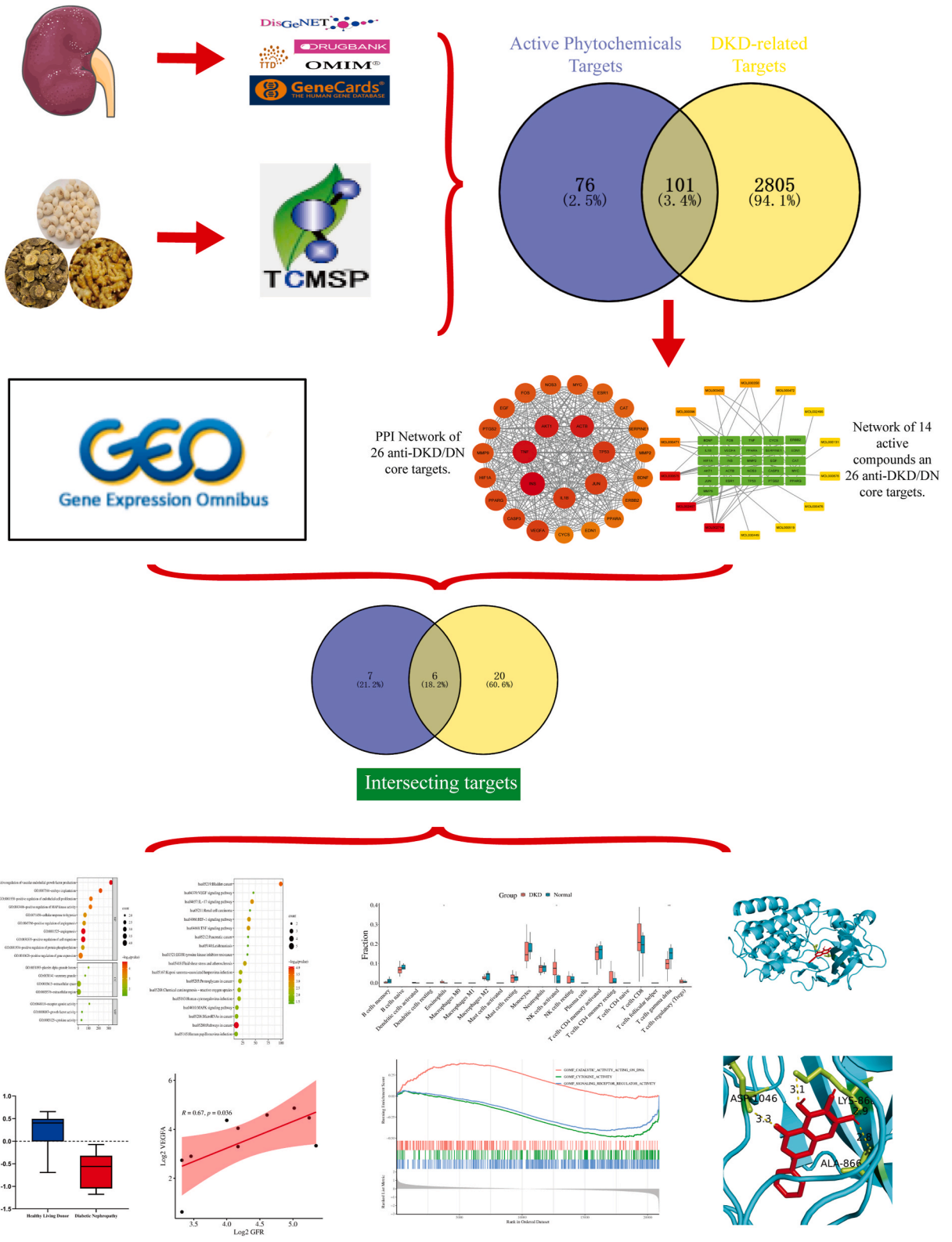


Fig. 1. A flow chart exploring DSBD against DKD based on network pharmacology.

in China is 11.9 %; taking 27 % of DM patients worldwide, and our country has the highest number of DM patients. As the leading cause of death from DM-induced end-stage kidney disease (ESRD), early intervention and treatment of DKD is of particular importance. At present, the treatment of DKD mainly includes the drug therapy, blood purification, kidney transplantation, etc [2]. Although drugs can effectively control blood sugar and improve glomerular circulation, when patients with DKD progress to the azotemia or even uremia stage, guanidine and peptide substances accumulate in the body and cannot be metabolized, causing nausea, vomiting, and other gastrointestinal dysfunction complications [3]. At this time, traditional drug treatment is not effective. Therefore, we urgently need to find a treatment to improve gastrointestinal complications.

Professor Zheng Nan, a Chinese Medical Master, has been in medicine for more than 50 years and has rich experience in treating DKD. While DSBD is the leading decoction used in the clinical treatment of DKD by the professor [4,5]. *Radix Rhei Et Rhizome* (Dahuang) is the common name for perennial plants in the *Polygonaceae* family from three *rhubarb* species. Its main components are rhein, emodin, and aloe emodin, which has lipid-lowering, hemostasis, and anti-tumor properties [6]. Rhein can inhibit TGF-1 expression, reduce renal fibrosis, antioxidant stress, and other factors to achieve DKD therapy [7]. In DKD mice, emodin can improve the pathological lesion of kidney tissue by increasing urinary albumin, serum creatinine, and blood urea nitrogen [8]. Aloe emodin can delay the progression of DKD by inhibiting IRF4, Notch1, and *p*-AKT signaling pathways [9]. *Zingiberis Rhizoma Recens* (Shengjiang), which is a member of the *ginger* family, is the fresh rhizome of *Zingiber Officinale Roscoe* and contains the major components of the volatile oil, gingerol, and has the effects of anti-vomiting, antitumour, blood lipids, etc [10]. Some studies have shown that *Rhizomes* can enhance liver glycolysis and thus reduce oxidative stress, inflammation, and cell apoptosis caused by DKD [11,12]. Meanwhile, the antiemetic effect of *Rhizomes* can also improve the gastrointestinal symptoms in the late stages of DKD. The dry tuber of *Pinellia ternata(Thunb.)Breit.* is *Pinelliae Rhizoma* (Banxia), which belongs to the *Araceae* family. Its main components include alkaloids, volatile oils, organic acids, and so on [13]. According to the research, *Pinellia* has a significant effect of preventing vomiting [14], improving gastrointestinal motility [15], and treating other gastrointestinal complications. β-sitosterol, the active ingredient in rhubarb, ginger and pinellia, can enhance insulin sensitivity and lower blood glucose levels by increasing RRARγ and GLUT4 expression [16,17].

In recent years, studies on DKD have emerged in an endless stream, and researchers have analyzed the therapeutic effects of DKD from different molecular mechanisms. Xianglong Meng [18] said that the therapeutic effect of Bekhogainsam Decoction is achieved primarily through inhibition of iNOS and COX-2 expression. The mechanism of this inhibition is through activation of the PI3K/AKT pathway due to inhibition of I-κB/NF-κB signaling pathway. Tingting Zhao et al. [19] found that the mechanism of Yishen Huashi Granules to treat DKD is mainly to enhance renal fibrosis and maintain the integrity of the renal filtration barrier through inhibition of PI3K/AKT/mTOR phosphorylation so as to delay the onset of DKD. Jing Chang et al. [20] discovered that TangShenWeiNing Formula could regulate the SIRT1/HIF-1 pathway in renal podocytes, promote SIRT1, and inhibit HIF-1 to protect podocyocytes after intervening with TangShenWeiNing Formula in DKD mice, reducing the effect of DKD. Xiaoyuan Guo et al. [21] used ZiShenWan to intervene in db/db mice and discovered that blood sugar levels and pathological injury were improved in db/db mice. It is suggested that ZhiShenWan can treat DKD by through inhibition of inflammatory cytokine overexpression and regulation of PI3K/AKT and MAPK signaling pathways. Many years of clinical experience by Professor Nan Zheng proved that there is a clear therapeutic effect of DSBD on DKD, but the mechanism of action is not clear. Therefore, the molecular mechanisms of DSBD in the treatment of DKD will be analyzed and investigated in this study.

Traditional Chinese Medicine(TCM) is developed by ancient Chinese people in the course of fighting against diseases for thousands of years. In terms of treatment, TCM and its compounds are characterized by multiple components, multiple pathways, and multiple targets, which makes the mechanism of action unclear [22]. Before the development of network pharmacology, analytical chemistry and chemical biology were the most commonly used methods to understand the active components of Chinese medicines and their formulations. However, due to the large number of components in the formulations, this approach could not well elucidate the synergistic effects of their components [23]. In contrast, traditional pharmacology only studies single diseases and drugs in isolation [24]. With the emergence of network pharmacology, researchers have a feasible and efficient method for addressing this issue. In the era of big data, network pharmacology is an emerging discipline with the goal of understanding the molecular mechanisms between drugs and diseases. By constructing a "disease-target-drug" network, the system is able to systematically observe the drug intervention on the disease network. Its diversity and complexity are consistent with TCM research. It can provide new scientific basis for studying complex Chinese medicine system and promote the development of TCM [25]. The aim of this study was to use the network pharmacology method to analyze DSBD active components and targets in the treatment of DKD and investigate the molecular mechanism of its treatment. Fig. 1 shows a flow chart. We used the online database to search the targets of drugs and diseases, screened their core targets, and then analyzed their prognosis and clinical relevance.

2. Methods

2.1. Acquisition of active components and target of DSBD

The chemical compositions of Dahuang, Shengjiang, and Banxia were queried by the database TCMSP (https://tcmsp-e.com/), and oral availability (OB) ≥ 20 % and drug-likeness (DL) ≥ 0.10 were set as conditions for screening of the active ingredients and collection of their corresponding drug targets. The resulting UniProt database (https://www.uniprot.org/) was then used to convert the targets to gene names and normalize the protein target data. The potential target of DSBD can be obtained after removing the repeated target. A “drugs-ingredients-targets” network map was made using Cytoscape 3.9.1 software.

2.2. DKD related target acquired

With "Diabetic Kidney Diseases" and "Diabetic Nephropathy" as keywords in GeneCards (https://www.genecards.org/), OMIM (https://omim.org/), DrugBank (https://go.drugbank.com/), TTD (http://db.idrblab.net/ttd/), and DisGeNET (https://www.disgenet.org/) database DKD-related target retrievals, targets for DKD disease were obtained by the removal of duplicate targets.

2.3. Acquired the common targets for DSBD and DKD

To obtain the joint targets of DSBD and DKD, we used the Venny 2.1.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/) to intersect the potential targets of DSDB with the disease targets of DKD.

2.4. Construction of PPI network between active components of DSBD and DKD targets

Screen out the common targets of DSBD and DKD, and use STRING 11.5 (https://cn.string-db.org/) to build a PPI network diagram. The biological species is set to "Homo sapiens", and all settings are set to default. Data on protein interaction relationships were obtained and the results visualized. Download the TSV file of the PPI network and import into Cytoscape 3.9.1 software to use the CytoNCA plugin to select core targets in the PPI network based on the degree of the nodes.

2.5. Mapping the network of components and targets

Cytoscape 3.9.1 software was used to build a network between selected PPI network targets and and active components of the DSBD. The CytoNCA plug-in was used to analyze the main components and targets of DSBD in the treatment of DKD according to the degree of the node, and the results were visually displayed.

2.6. GEO chip difference analysis

In the GEO database (https://www.ncbi.nlm.nih.gov/), "Diabetic Kidney Disease" is used as the keyword for screening. The GSE142153 dataset file has been obtained. The documents included 10 healthy control patients, 23 DKD patients, and 7 ESRD patients. A gene chip data set can be divided into two groups (CONTROL and DKD) using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/). For differentially expressed genes (DEGs), set the P-value <0.05 and the change in expression acuity to 1.5 times ($|\log_2 FC| \geq 0.58$). $\log_2 FC \geq 0.58$ was defined as up-regulated genes, and $\log_2 FC \leq -0.58$ was defined as down-regulated genes. Venn diagrams of DEGs and DSBD targets were made using the Bioinformatics online platform (http://www.bioinformatics.com.cn/), and a bar diagram visualization of intersection differential genes was made. The intersection of DEGs and PPI core targets was carried out by exploiting the Venny 2.1.0 platform to ultimately filter out the core targets, heat maps were created using the ChiPlot online platform (https://www.chiplot.online/), and volcano plot was produced using GraphPad Prism 8.

2.7. GO and KEGG enrichment analysis

We performed enrichment analysis to analyze relevant functions of the central targets. GO provides an understanding of three biological aspects of the core targets, including the biological process (BP), the molecular function (MF), and the cellular composition (CC). KEGG encyclopedia also allows us to discover the biological pathways that mediate the central target. The enrichment process is performed through the DAVID database (https://david.ncifcrf.gov/). P-value<0.05 is the condition, and the entries that meet the condition are screened. The results were visualized using Bioinformatics online platform.

2.8. Gene Set Enrichment Analysis (GSEA)

Due to many shortcomings of traditional enrichment methods, for example, GO and KEGG enrichment analysis is amenable to thresholded gene filtering, but there are large differences among the genes and this method of screening will result in the loss of a great deal of gene information. The goal of GSEA enrichment analysis is to analyze an entire gene set, which compensates for the shortcomings of traditional enrichment methods. Gene information from the GSE142153 dataset was analyzed using the online Bioinformatics platform.

2.9. Immune infiltration analysis

Using CIBERSORTx (https://cibersortx.stanford.edu/) analysis of the GEO probe file of immune cells, the results using the Bioinformatics platform are shown in the box plot. The correlations between immune cells were then visualized using the ChiPlot online platform. Correlations between core targets and immune cells were also visualized using the ChiPlot online platform.

2.10. Correlation analysis of core targets with disease and clinical indexes

Using the Nephroseq V5 online database (https://www.http://v5.nephroseq.org/), the core targets expression level in patients

with DKD was compared with the normal control group. P-value <0.05 was considered to be statistically significant, and visualization was performed using GraphPad Prism 8. In parallel, the estimated glomerular filtration rate (eGFR) was used to analyze the correlation with maize targets and visualized the online platform Bioinformatics.

2.11. Single-cell sequencing analysis of core targets

Select the Human Diabetic Kidney data set for single-corn targets sequencing analysis from the KIT online database (http://humphreyslab.com/SingleCell/).

2.12. Molecular docking

The 3D structures of the top active ingredients and core targets of DSBD were downloaded from PubChem (https://pubchem.ncbi.

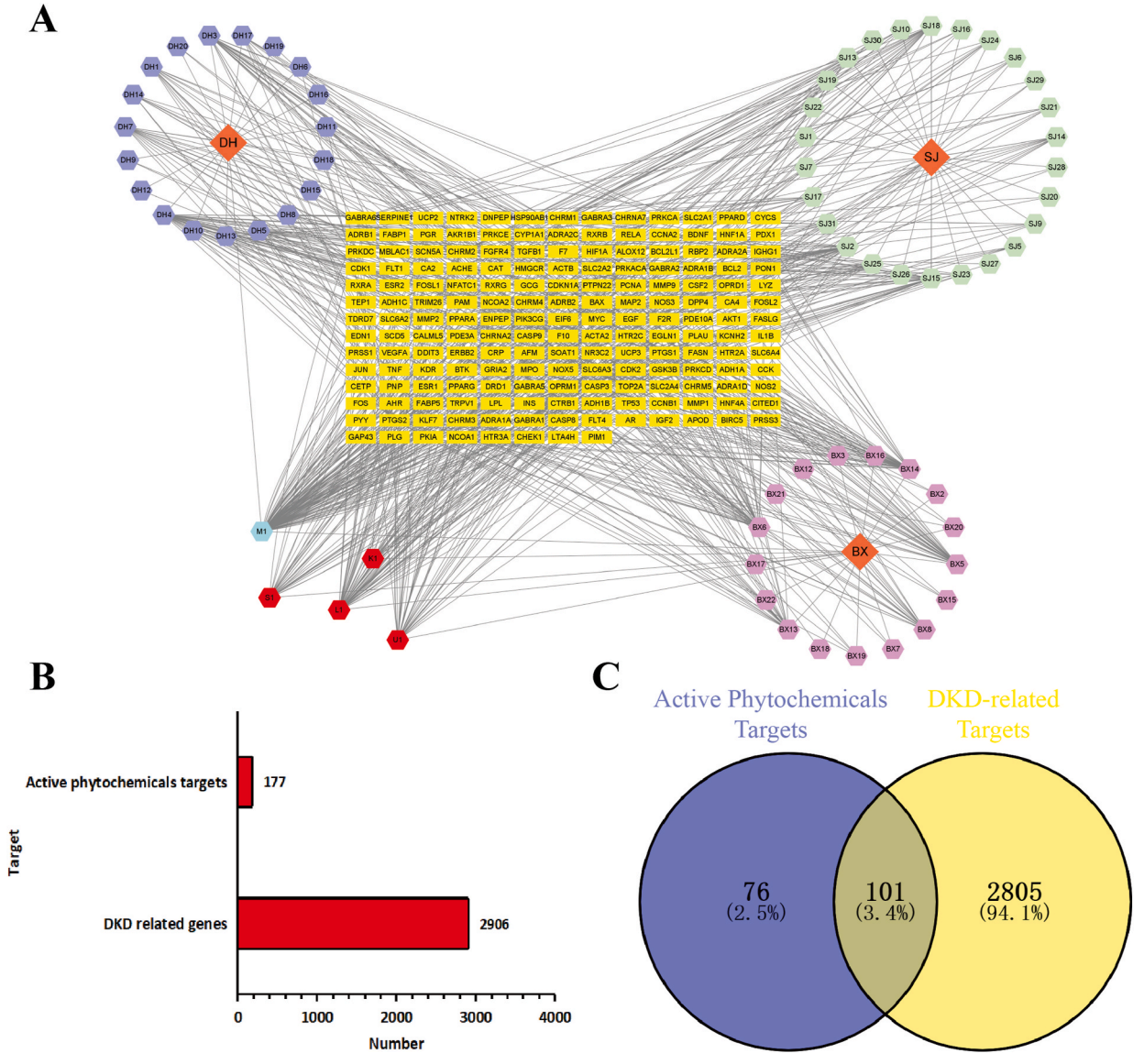


Fig. 2. Screening of disease and drug targets. A. DSBD components and targets network diagram. Orange diamond represents Chinese medicine, light purple hexagon represents the active ingredients of *rhubarb*, light green hexagon represents the active ingredients of *ginger*, the light pink hexagon represents the active ingredients of *pinellia*, the light blue hexagons represent *rhubarb*, *ginger*, and *pinellia* as common effective ingredients, the red hexagons represent *ginger* and *pinellia* as common effective ingredients, and the yellow rectangles represent the ingredients of interest. See Table 1 for the Chinese names of the active ingredients represented by the numbers in the figure and their sources. B. Number of targets of DSBD and DKD. C. Venn diagram of the intersection of DSBD targets and DKD targets.

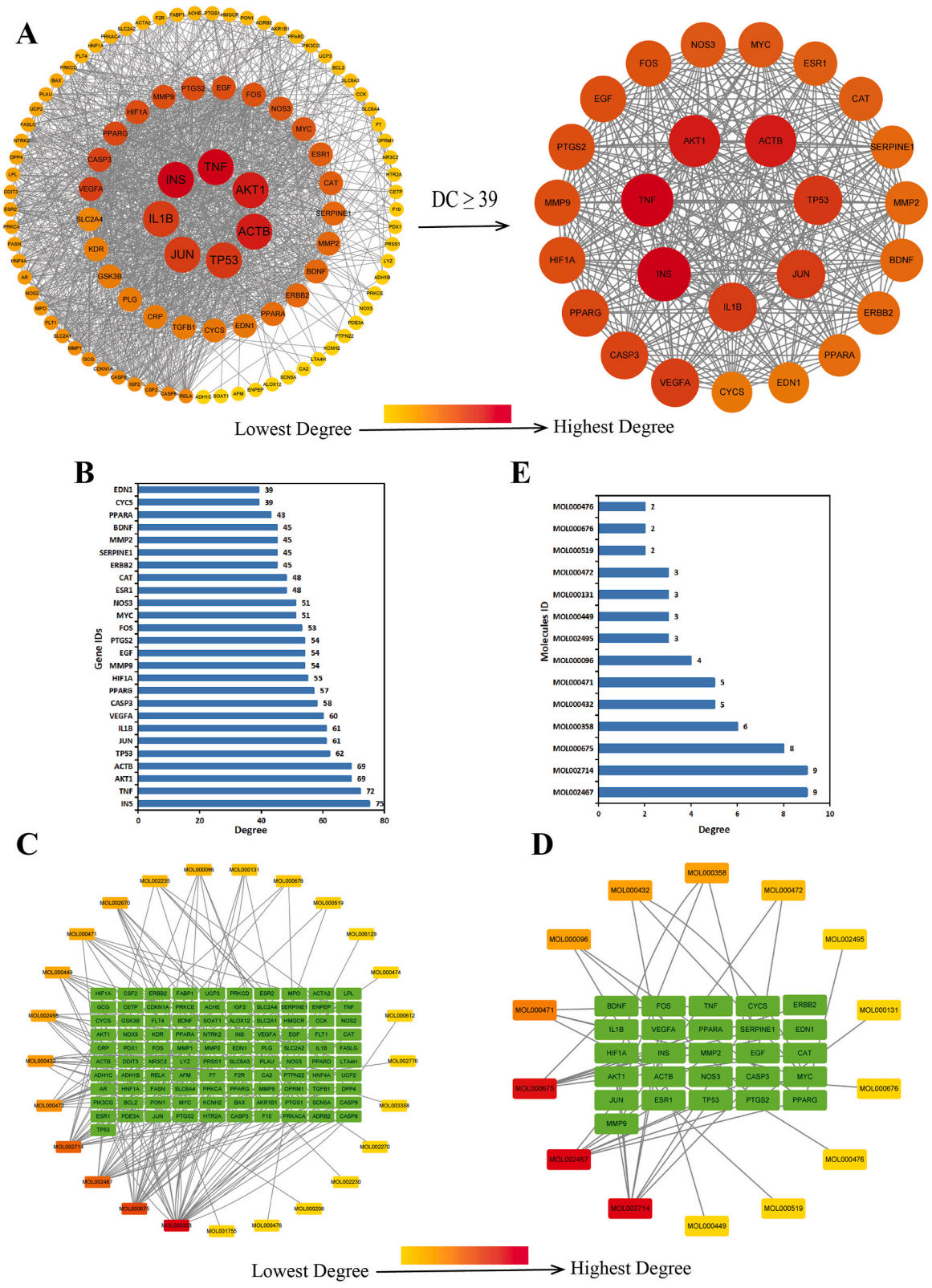


Fig. 3. Initial screening of the core targets. A. PPI network diagrams sorted by degree value, core targets were initially screened out according to DC $\geq$ 39. B. Degree of the core targets. C. Intersection targets and network diagram of active constituents. D. Core targets and active components network diagram. E. Degree of active ingredients.

nlm.nih.gov/) and the PDB (https://www.rcsb.org/), respectively. After being converted to file format by Open Babel 3.1.1, then visualized by AutoDock Vina for docking, and finally PyMol for visualization and output.

3. Results

3.1. Active components and targets of the DSBD

A total of 33 effective components of *Rhubarb* were obtained in the TCMSP, corresponding to 199 targets. There were 39 effective components of *Rhizomes*, corresponding to 294 targets. And there were 30 effective components of *Pinellia*, corresponding to 282 targets. After removing duplicate targets, 177 targets were obtained. We used Cytoscape 3.9.1 software to create a network diagram of "drugs-ingredients-targets" (Fig. 2A).

3.2. Targets of DKD

The DKD targets obtained from GeneCards, OMIM, DrugBank, TTD, and DisGeNET databases were de-weighted to obtain 2906 disease targets. Visual output of DKD targets and the number of DSBD targets in 2.1 (Fig. 2B).

3.3. Intersection targets of DSBD and DKD

177 DSBD targets and 2906 DKD targets were mapped to each other using the online tool Venny 2.1.0, and 101 intersection targets were generated (Fig. 2C).

3.4. Construction of the PPI network

Using the intersection targets of the resulting DSBD and DKD, we were able to obtain the PPI network diagram across the STRING. The number of nodes is 101 and the number of edges is 1353, and nodes represent proteins. Downloading the TSV file from the PPI network diagram and analyzing the network features using the CytoNCA plug-in in the Cytoscape 3.9.1 software package. According to

Table 1
Active ingredients and sources represented by numbers in Fig. 2A.

ID	Active Ingredients	Chinese Name	ID	Active Ingredients	Chinese Name
DH1	MOL000096	大黄	SJ21	MOL002153	生姜
DH3	MOL000471	大黄	SJ22	MOL002186	生姜
DH4	MOL000472	大黄	SJ23	MOL002335	生姜
DH5	MOL000476	大黄	SJ24	MOL002573	生姜
DH6	MOL002230	大黄	SJ25	MOL003358	生姜
DH7	MOL002235	大黄	SJ26	MOL003480	生姜
DH8	MOL002240	大黄	SJ27	MOL003575	生姜
DH9	MOL002243	大黄	SJ28	MOL003978	生姜
DH10	MOL002244	大黄	SJ29	MOL005743	生姜
DH11	MOL002258	大黄	SJ30	MOL005964	生姜
DH12	MOL002259	大黄	SJ31	MOL010380	生姜
DH13	MOL002262	大黄	BX2	MOL001755	半夏
DH14	MOL002268	大黄	BX3	MOL001818	半夏
DH15	MOL002270	大黄	BX5	MOL002670	半夏
DH16	MOL002280	大黄	BX6	MOL002714	半夏
DH17	MOL002281	大黄	BX7	MOL002776	半夏
DH18	MOL002284	大黄	BX8	MOL000357	半夏
DH19	MOL002288	大黄	BX12	MOL005030	半夏
DH30	MOL002297	大黄	BX13	MOL000519	半夏
SJ1	MOL001566	生姜	BX14	MOL000675	半夏
SJ2	MOL002467	生姜	BX15	MOL006936	半夏
SJ5	MOL006129	生姜	BX16	MOL006944	半夏
SJ6	MOL000066	生姜	BX17	MOL006951	半夏
SJ7	MOL000025	生姜	BX18	MOL006956	半夏
SJ9	MOL000208	生姜	BX19	MOL006957	半夏
SJ10	MOL000266	生姜	BX20	MOL006958	半夏
SJ13	MOL000474	生姜	BX21	MOL003578	半夏
SJ14	MOL000612	生姜	BX22	MOL006967	半夏
SJ15	MOL000676	生姜	M1	MOL000358	大黄、生姜、半夏
SJ16	MOL000935	生姜	K1	MOL002495	生姜、半夏
SJ17	MOL000938	生姜	S1	MOL000131	生姜、半夏
SJ18	MOL001179	生姜	U1	MOL000432	生姜、半夏
SJ19	MOL001390	生姜	L1	MOL000449	生姜、半夏
SJ20	MOL001771	生姜			

the topological analysis results, nodes with a degree greater than or equal to 39 were discovered, and a preliminary PPI network of the DKD core targets treated by DSBD was obtained. Its targets are *VEGFA*, *HIF1A*, *MMP9*, *JUN*, *TP53*, *EGF*, *CASP3*, *TNF*, *PTGS2*, *INS*, *AKT1*, *FOS*, *ACTB*, *IL1B*, *SERPINE1*, *MMP2*, *CAT*, *MYC*, *CYCS*, *NOS3*, *PPARG*, *BDNF*, *ESR1*, *ERBB2*, *PPARA*, and *EDN1* (Fig. 3A). The shade of the color in the figure changes positively with the degree value. The degree values of 26 core targets were visualized (Fig. 3B).

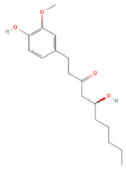
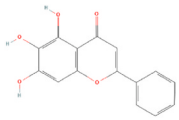
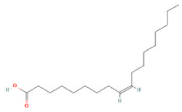
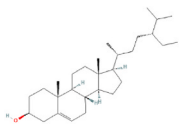
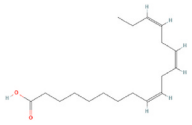
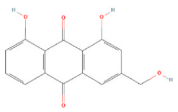
3.5. Compositions-targets network construction

PPI selected targets and selected core targets are reconstructed with active components of DSBD respectively. After being analyzed by Cytoscape 3.9.1, the interaction network between active components of DSBD and 101 common targets were obtained (Fig. 3C), as was the network relationship between active components of DSBD and 26 core targets (Fig. 3D). The shade of the color in the figure changes positively with the degree value, and the degree value of active components was visualized (Fig. 3E). See Table 2 for the top 6 effective components of degree value and their related information.

3.6. Analysis of GEO chip differences

According to Part 1.5, the GSE142153 data set file was downloaded, and after screening, a total of 1094 differential genes was obtained after removing duplicate items, among which were 555 up-regulated genes and 539 down-regulated genes. Venn diagrams of DEGs and DSBD targets were performed to obtain 1 up-regulated gene and 12 down-regulated genes (Fig. 4A). And DEGs were visualized in a histogram (Fig. 4B). A venn diagram was made among the DEGs and the 26 core targets obtained in 2.3 (Fig. 4C), and finally 6 core targets were obtained, which were *IL1B*, *MMP9*, *EGF*, *VEGFA*, *HIF1A*, and *PTGS2*. The six targets were mapped differently

Table 2
The top 6 effective components of degree value and their related information.

Mol ID	Phytochemical Name	Structure	OB	DL	Degree
MOL002467	6-gingerol		35.64	0.16	9
MOL002714	baicalein		33.52	0.21	9
MOL000675	oleic acid		33.13	0.14	8
MOL000358	beta-sitosterol		36.91	0.75	6
MOL000432	linolenic acid		45.01	0.15	5
MOL000471	aloe-emodin		83.38	0.24	5

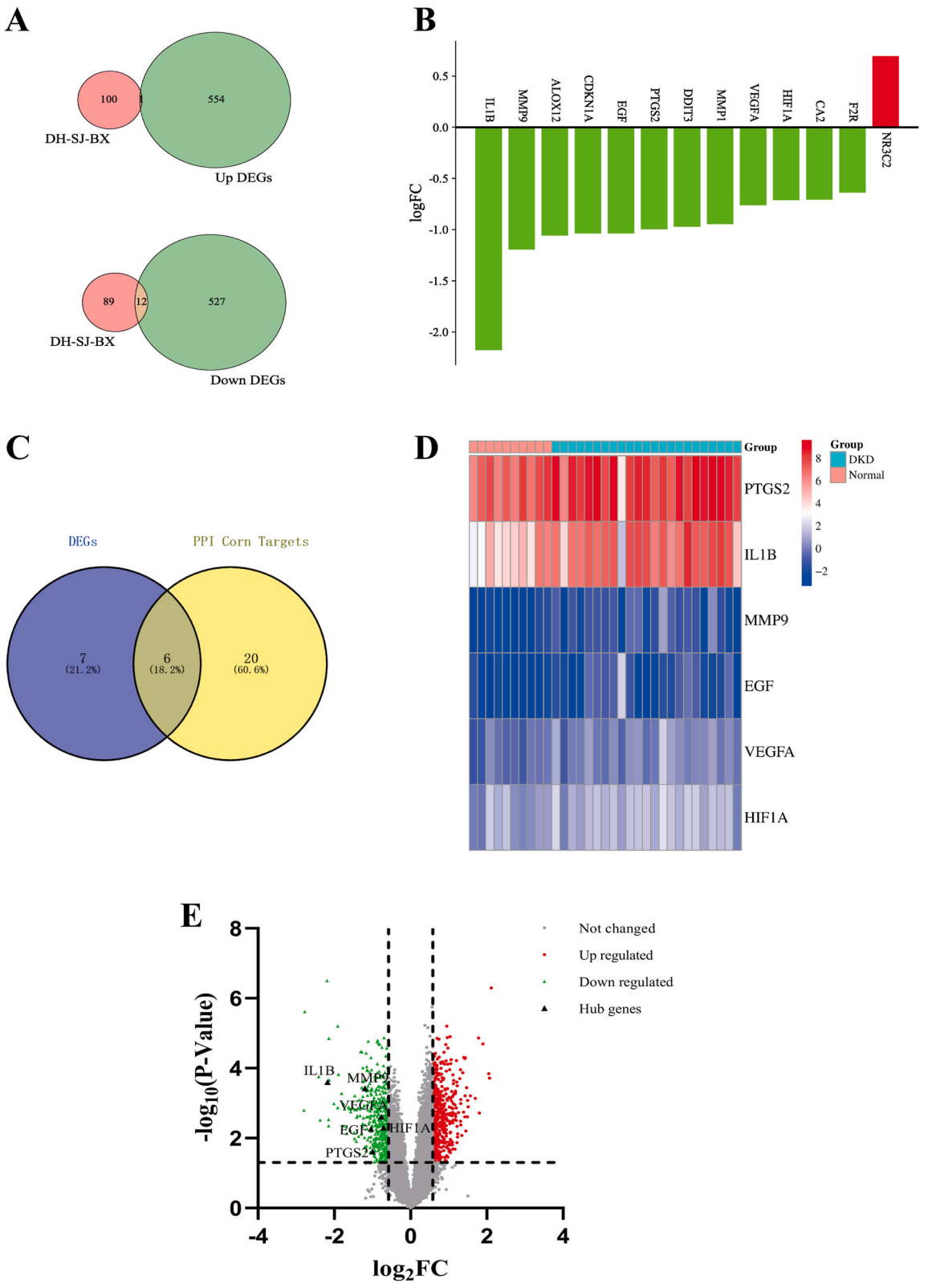


Fig. 4. Screening of DEGs and further screening of core targets. A. DSBD and an up-down-regulated genes are intersected in venn diagrams. B. Up-down-regulated gene histogram. C. Venn diagram of the intersection of DEGs and PPI core targets. D. Heatmap of the core targets. E. Volcano plot of the DEGs.

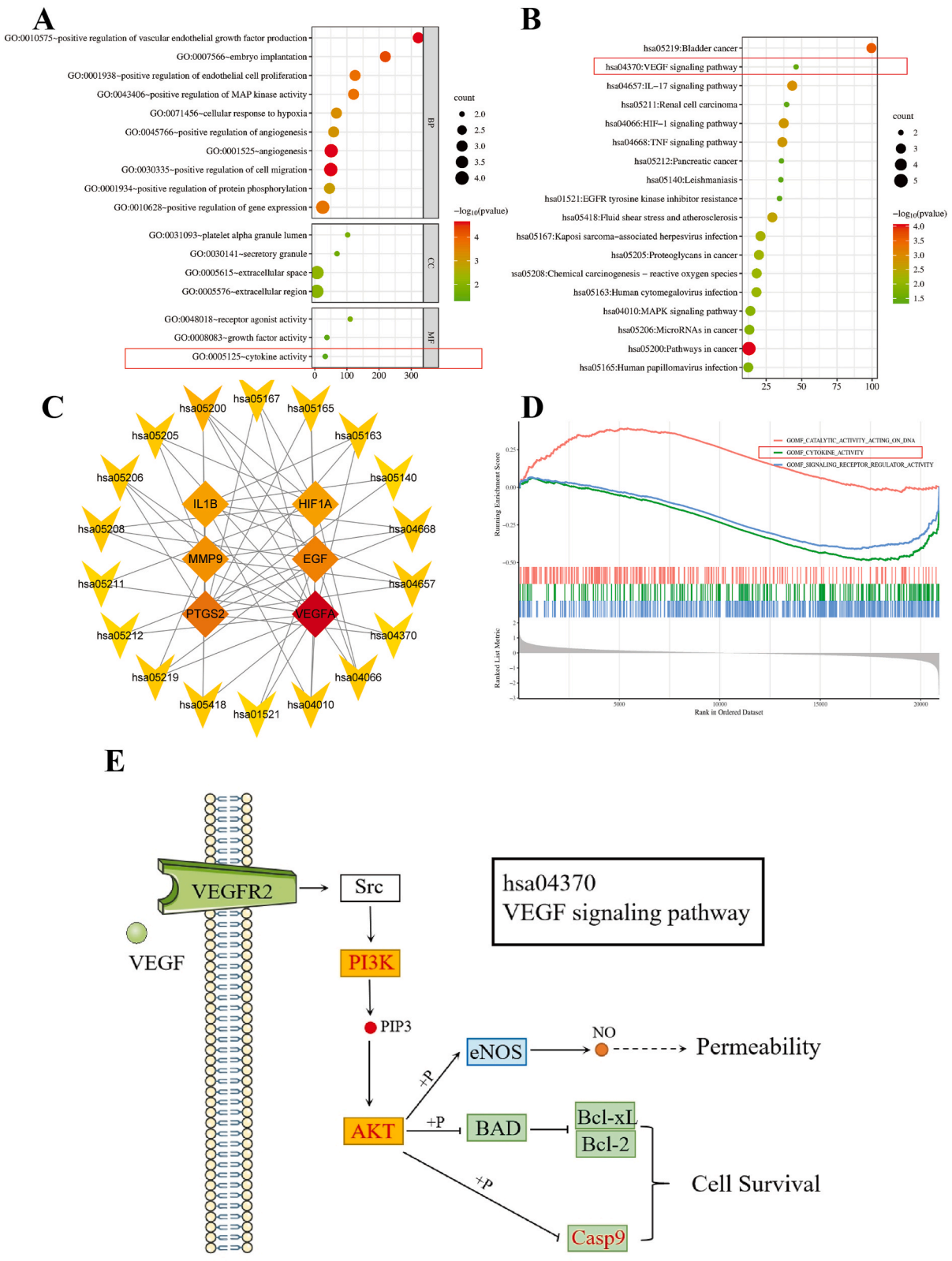


Fig. 5. Enrichment analysis and pathway selection. A. GO enrichment analysis. B. KEGG enrichment analysis. C. KEGG pathway network diagram with six core targets. The V nodes represents the pathways, and the diamond represents the core targets. D. GSEA-GOMF enrichment analysis. E. VEGF/PI3K/AKT signaling pathway diagram.

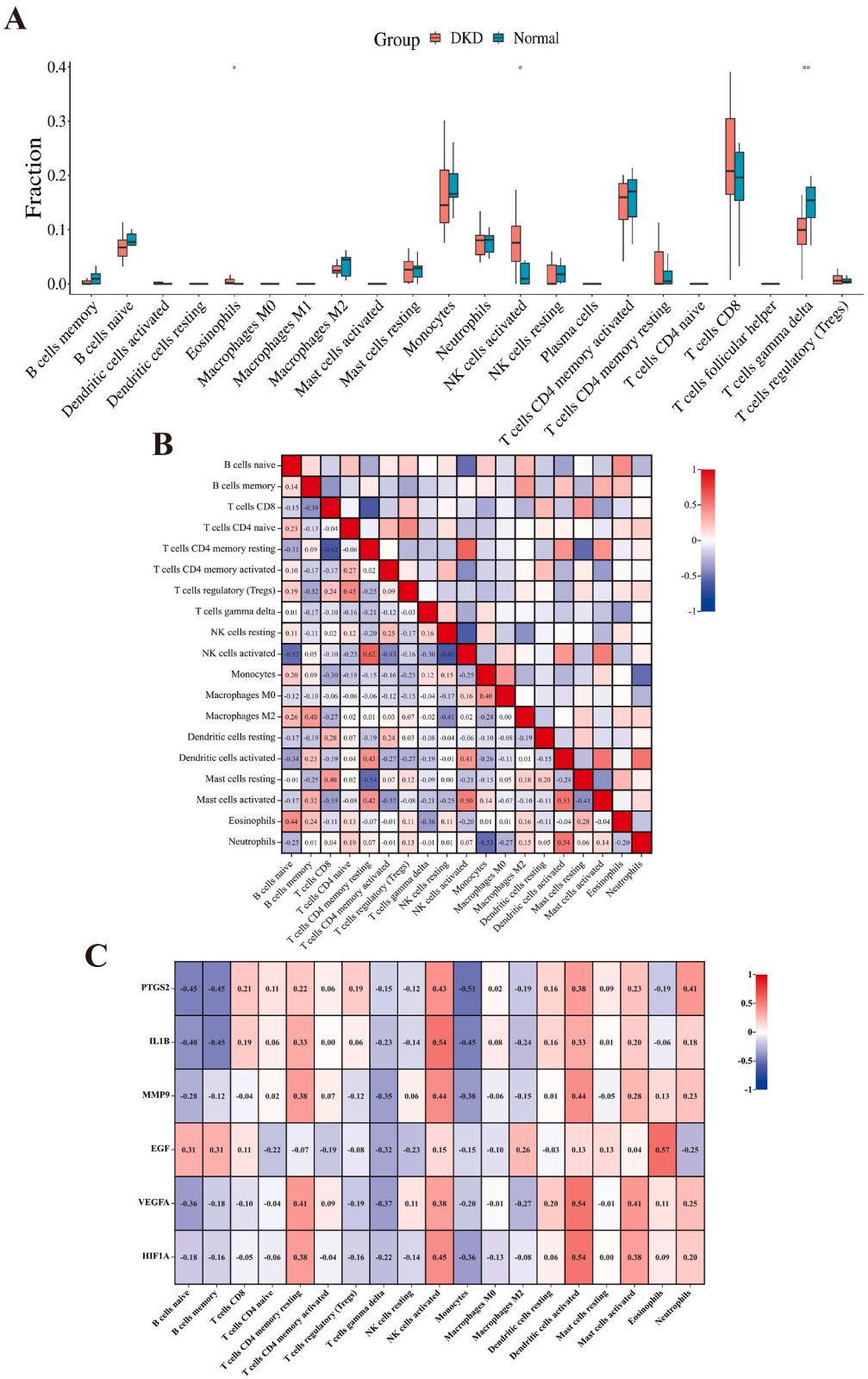


Fig. 6. Immunoinfiltration analysis. A. Immunoinfiltration analysis. B. Immune cell correlation analysis. C. Correlation analysis between core genes and immune cells.

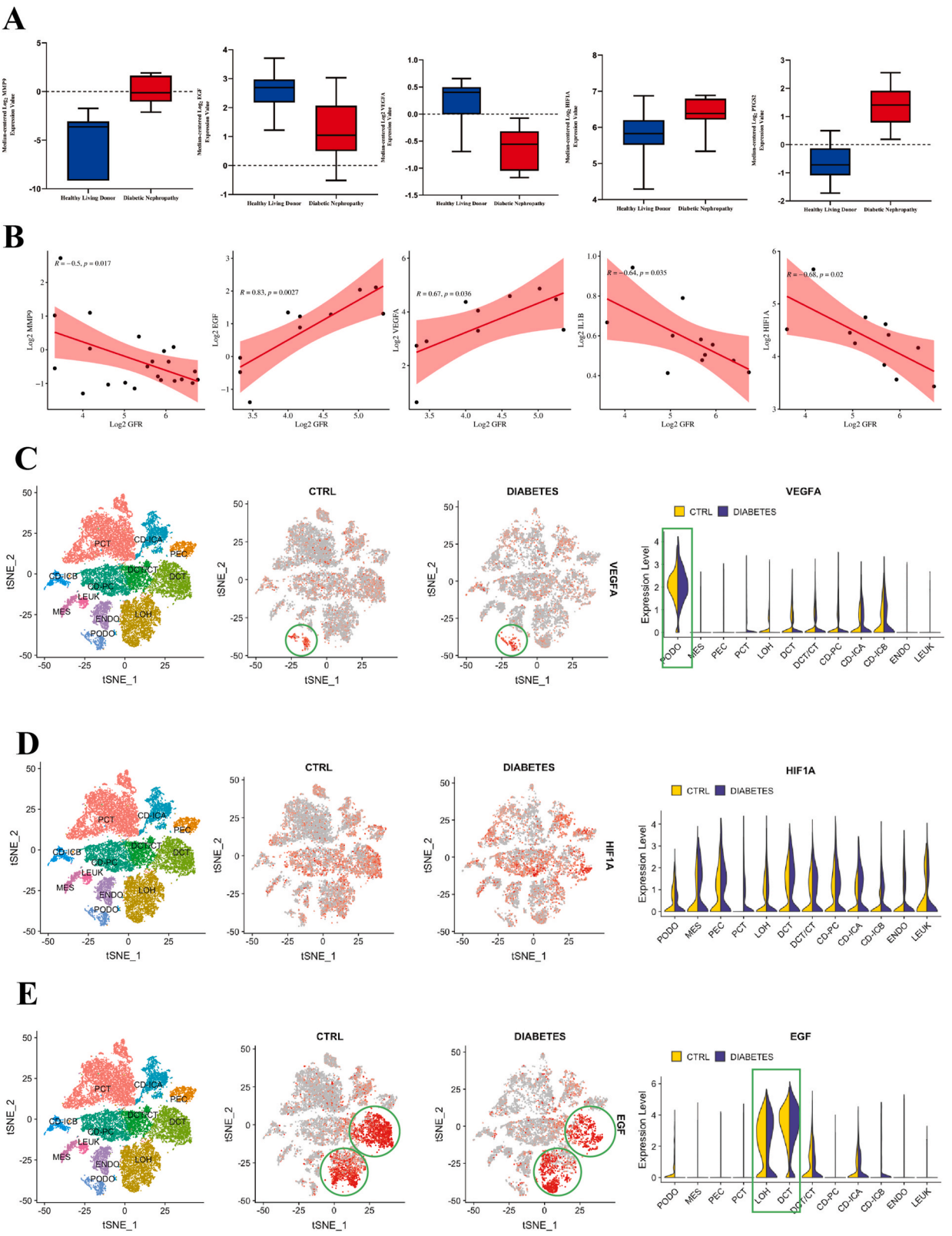


Fig. 7. Clinical correlation and single-cell sequencing analysis. A. Disease correlation analysis of core targets. B. Correlation analysis between core targets and eGFR. C-E. Single-cell sequencing analysis of core targets.

(Fig. 4D), in which red meant high expression, white meant intermediate expression, and blue meant low expression. A volcano plot of DEGs was also made (Fig. 4E), in which the green, red and grey dots represented unregulated genes in the normal group, DKD group and genes with no difference between the two groups, severally. The core targets were labeled with black triangles.

3.7. GO and KEGG enrichment analysis

After GO enrichment analysis and filtering, we obtained a total of 52 enrichment results, 45 of which corresponded to BP. It mainly involves angiogenesis, positive regulation of cell migration, embryo implantation, etc. CC has 4 pieces: extracellular space, extracellular region, platelet alpha granule lumen, and secretory granule. There are 3 MF involved in the cytokine activity, growth factor activity and receptor agonist activity. The first 10 enrichment results of the BP were selected and visualized with CC and MF (Fig. 5A). After KEGG enrichment analysis and screening, 18 enrichment results were obtained. It involves Pathways in cancer, Bladder cancer, Leishmaniasis, etc. Indicating that these pathways may be important pathways for the DSBD treatment of DKD. 18 enrichment results were visualized (Fig. 5B). We used Cytoscape 3.9.1 software to construct the "pathway-target" network diagram (Fig. 5C) and analyzed the CytoNCA plug-in. The shade of the color in the figure changes positively with the degree value.

3.8. GSEA enrichment analysis

GSEA-GOMF enrichment shows that, in terms of MF, the data set is significantly enriched in catalytic activity acting on DNA, cytokine activity and signaling receptor regulation activity (Fig. 5D). Therefore, we finally selected the VEGF/PI3K/AKT signaling pathway for study, and the signaling pathway diagram is shown in Fig. 5E.

3.9. Analysis of immune infiltration

The visualization showed that three kinds of immune cells in DKD had significant changes. Two of them were up-regulated, and one was down-regulated (Fig. 6A). This indicates that there are different infiltration modes of immune cells in DKD. A correlation analysis was performed to evaluate whether different immune cells interact with each other. We found a lot of immune cells that was positively or negatively correlated. The synergistic effect of NK cells activated and T cells CD4 memory resting was the strongest ($r = 0.62$). T cells CD4 memory resting and T cells CD8 were the most competitive ($r = -0.62$) (Fig. 6B). Later, in order to understand the correlation between core targets and immune cells, the Pearson correlation coefficient between core targets and immune cells was calculated (Fig. 6C). All six core targets had a moderate-to-high correlation with immune cells ($r > 0.4$), particularly eosinophils and EGF ($r = 0.57$).

3.10. Analysis of the correlation between core targets and diseases and clinical indicators

In order to verify the correlation between the expression levels of the core targets and the clinical indicators of DKD, the online tool Nephroseq V5 was used to analyze the core targets (Fig. 7A). We found that, with the exception of the level of *IL1B* expression in DKD patients that was not recorded in the Nephroseq V5 database, the expression level of *MMP9*, *HIF1A*, and *PTGS2* in DKD patients was up-regulated compared with that in healthy living donors, and the expression level of *EGF* and *VEGFA* was down-regulated. At the same time, correlations between six core genes and the eGFR of the renal function index were performed in the Nephroseq V5 database (Fig. 7B). In addition to *PTGS2*, which was not recorded in the database, three targets were negatively correlated with eGFR level (*IL1B*, *MMP9*, and *HIF1A*), and two targets were positively correlated (*EGF* and *VEGFA*).

3.11. Single-cell sequencing analysis of core targets

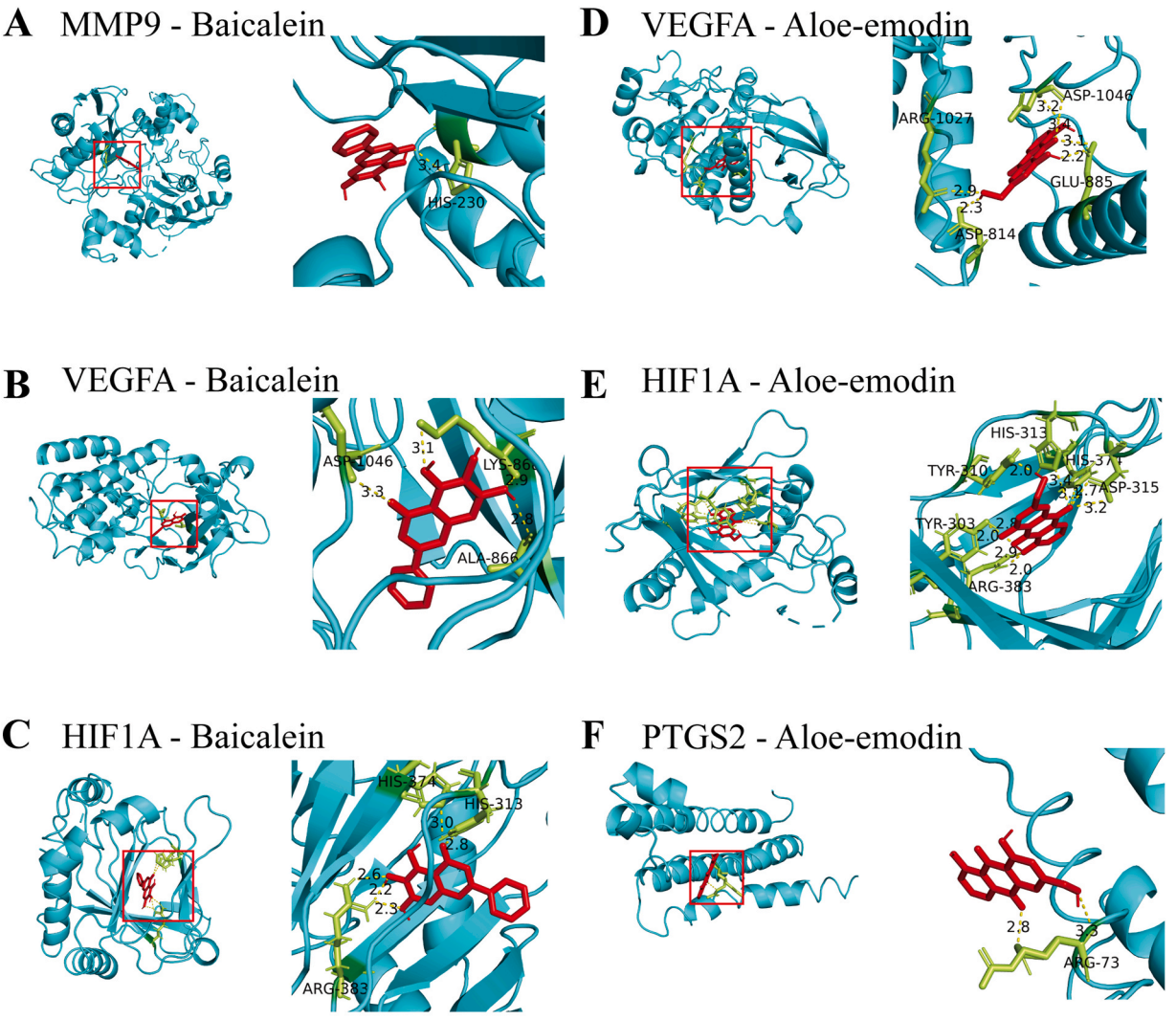
Single-cell sequencing analysis can understand genes expression in a single cell. According to the results (Fig. 7C–E), *VEGFA* is highly expressed in podocyte, *HIF1A* is widely distributed in cells, and *EGF* is highly expressed in the Henle loop (LOH) and distal convoluted tubule (DCT), moreover, the expression of *EGF* was different between the normal group and the diabetic group.

3.12. Molecular docking

In order to further analyze the feasibility of treating DKD with DSBD, the top 6 active ingredients, 6-gingerol, baicalein, oleic acid, β-sitosterol, linolenic acid, and aloe emodin, were interlinked with the core targets *IL1B*, *MMP9*, *EGF*, *VEGFA*, *HIF1A*, and *PTGS2*. The PDB ID of IL1B is 5bow, that of MMP9 is 5ue4, that of EGF is 1zt3, that of VEGFA is 3vnt, that of HIF1A is 4bqy, and that of PTGS2 is 4al0. After docking with AutoDock Vina, the binding activity was evaluated according to binding energy. The greater the negative binding energy, the more stable the conformation and the stronger the spontaneous binding ability. The lowest six binding energies to visual docking with PyMol (Fig. 8A–F), and the consequences of molecular binding activity to heat map drawing (Fig. 8G). Baicalein-VEGFA showed the smallest binding energy, which was -9.2 kcal mol^{-1}.

4. Discussion

The purpose of this study was to predict the mechanism of action of DSBD in the treatment of DKD at the molecular level through



Molecule ID	Molecule Name	Binding Affinity(kcal/mol)					
		IL1B	MMP9	EGF	VEGFA	HIF1A	PTGS2
MOL002467	6-gingerol	-5.6	-5.7	-4.8	-7.1	-6.1	-6.6
MOL002714	baicalein	-7.5	-8.9	-6.0	-9.2	-8.3	-7.5
MOL000675	oleic acid	-5.1	-4.7	-3.8	-6.6	-5.5	-5.4
MOL000358	beta-sitosterol	-7.5	-7.1	-7.5	-8.7	-7.1	-8.2
MOL000432	linolenic acid	-5.7	-6.2	-4.5	-8.7	-5.4	-6.2
MOL000471	aloe-emodin	-7.7	-7.2	-6.4	-7.8	-8.7	-8.1

Fig. 8. Molecular docking. A. MMP9 - Baicalein B. VEGFA - Baicalein C. HIF1A - Baicalein D. VEGFA - Aloe-emodin E. HIF1A - Aloe-emodin F. PIGS2 - Aloe-emodin G. Heatmap of the molecular docking binding energy.

cyberpharmacology. We found that DSBD inhibited proliferation of renal tubule epithelial cells via VEGF/PI3K/AKT signaling pathway. The graphic summary of the mechanism of action of DSBD in treating DKD is shown in Fig. 9.

It is possible that 6-gingerol, baicalin, oleic acid, β-sitosterol, linolenic acid and aloe emodin is the representative active ingredients of DSBD in the treatment of DKD. Studies have shown that [26], 6-gingerol can improve fasting glucose, lipids, serum creatinine, and blood urea levels in DM rats induced by streptozotocin (STZ) and reduce the inflammatory response of DM rats by lowering levels of inflammatory markers, leading to a kidney protective effect. In parallel, 6-gingerol can obviously inhibit the level of NF-κB expression in renal tubular epithelial cells induced by the aldehyde acetone, reducing levels of reactive oxygen species (ROS), and increasing the activity of superoxide dismutase (SOD) to combat renal tubular epithelial cell injury [27]. Baicalin can lower the levels of serum urea and urinary albumin (U-ALB), two indicators of renal function, and regulate the expressions of AMPKα, hs-CRP, and FcγR to delay the progression of DKD and protect the kidney [28]. Leyi Ma et al. [29] showed that baicalin prevents DKD by activating Nrf2 and inhibiting MAPK signaling pathway to reduce oxidative stress and inflammation. In the presence of the inflammatory cytokine TNF-α, oleic acid was shown to have the capacity to increase insulin secretion, which resulted in reversal of inflammation-characterized symptoms in DM mice [30]. In the case of DKD, β-sitosterol exerts its effects primarily through inhibition of the IKKβ/NF-κB and JNK signaling pathways, as well as inhibition of TNF-α and IL-6 synthesis [31]. β-sitosterol stimulates adipocyte glucose uptake by suppressing the expression of AKT and PI3K [32]. α-linolenic acid and γ-linolenic acid are isomers of linolenic acid. α-linolenic acid can lower blood urea nitrogen (BUN) levels while increasing glomerular filtration rate (GFR), implying that the protective effect of α-linolenic acid on the kidney is primarily due to a reduction in microalbuminuria [33]. γ-linolenic acid can suppress the expression of MCP-1 and ICAM-1 to achieve anti-inflammatory and anti-fibrosis effects [34]. A study on the improvement of DKD by aloe emodin showed that aloe emodin could reduce the expression levels of IL-1β, IL-7, ratio of urinary albumin to urinary creatinine (ACR), and blood urea to protect the kidney injury of DKD rats and could regulate the downstream signaling pathway of IRF4 to inhibit the injury of podocyocytes [6]. The corn targets screened in this study were *IL1B*, *MMP9*, *EGF*, *VEGFA*, *HIF1A*, and *PTGS2*. IL1B is a proinflammatory cytokine, it has the ability to directly inhibit the insulin signaling pathway by restraining tyrosine phosphorylation of IRS-1 and decreasing IRS-1 expression [35]. MMP9 is a good marker for DKD large vascular disease complications and can help with renal diseases like albuminuria and tubulointerstitial fibrosis [36]. EGF can activate EGFR, and the combination of the two can produce biological activity, which involved in embryonic development, tissue regeneration and ion transport [37]. Furthermore, EGF can help to maintain renal function and protect the kidneys, and that a lack of EGF can lead to kidney diseases [38]. EGF produced by Henle loop

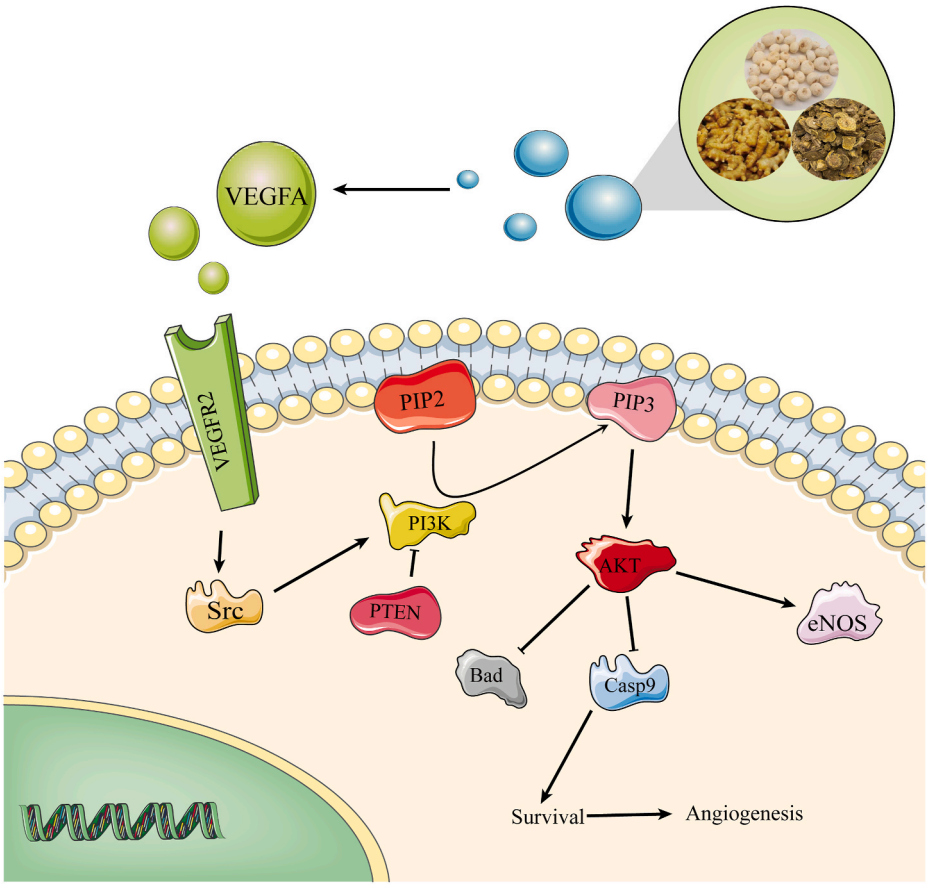


Fig. 9. Graphic summary.

and distal convoluted tubules gradually decreased with renal tubule injury in the late DN period [39], which was consistent with our single-cell sequencing results. VEGF is a key regulatory factor in angiogenesis and cancer, diabetes, and other diseases, with VEGFA playing a key role in angiogenesis regulation by prompting new blood vessel formation and increased vascular permeability [40]. In the study of Nan Hee Kim et al. [41], it was found that VEGF levels in the urine of DKD patients were synchronized with the progression of the disease and also closely correlated with urinary albumin excretion. The excretion of soluble VEGF receptor (sFLT-1) was also higher in DKD patients than in the control group. Studies have shown that abnormal regulation of VEGF/sFLT-1 can delay the progression of DKD and avoid harmful stimulation of the kidney. The results of single cell sequencing in this study showed that VEGFA showed high expression in renal podiocytes, and the expression in DN group was higher than that in normal group, which was consistent with the results of Chun-Liang Lin et al. [42]. Oxidative stress is closely related to DM. During its occurrence, ROS levels rise in the body, which will lead to body damage. Xiaowei Zheng et al. [43] found that promoting HIF-1 helps to maintain a normal level of ROS and prevent the occurrence of DKD. HIF1A, a subunit of HIF-1, is an important regulator of hypoxia. Studies have shown that HIF1A is associated with the complications of DM, and the loss of HIF1A can also affect the function of β cells. Simultaneously, an increase in HIF1A in the kidney increases the degree of renal fibrosis [44]. PTGS2, also known as COX2, is involved in pathophysiological processes such as tumors, inflammation, and kidney disease. The expression of PTGS2 in the kidney of DM patients is noticeably up-regulated, and the use of PTGS2 inhibitors can normalize the levels of glomerulosclerosis and albuminuria in DM patients. The above evidence proves that PTGS2 has adverse effects on the kidney and is also a relevant target for DKD treatment [45]. In this study, we used in vitro cellular assays to validate the network's pharmacological predictions, and we will follow up with animal studies and clinical trials to see if DSBD is effective in the treatment of DKD.

Renal macrophages are a group of intrinsic macrophages in renal tissues, which play an important role in maintaining renal homeostasis, anti-infection and tissue repair [46]. Renal macrophages mainly include resident macrophages and infiltrating macrophages [47]. In kidney injury caused by DKD, renal function decline is often accompanied by infiltration of macrophages [48], which shows the close relationship between immune cells and DKD. Therefore, studying the relationship between different immune cells and DKD may provide new opportunities for the diagnosis and treatment of DKD. In this study, the expression of Eosinophils and NK cell activated was up-regulated and the expression of T cells gamma delta was down-regulated through immunoinfiltration analysis. Studies have shown that interstitial eosinophile aggregates are found in DKD patients, and blood eosinophils are the strongest predictors of interstitial eosinophile aggregates and are strongly associated with eGFR decline [49]. In a study of tuberculosis co-morbidity with diabetes, T cells gamma delta expressed cytokines and cytotoxicity less frequently in the co-morbidity group than in the non-diabetic group, which is consistent with our prediction [50]. As for NK cells, the existing studies on their expression in DM are contradictory. In Jeannig Berrou et al. 's study, the level of NK cells in T2DM patients was reduced, which also explains why T2DM patients are more prone to infection and cancer [51]. In Paweł Piątkiewicz 's study, NK cells increased in number but decreased in activity, which may be the reason for the increased risk of cancer [52].

A research conducted by Tingting Zhao et al. suggested that Yishen Huashi Granules inhibited the degree of fibrosis in the kidney and maintained the integrity of the filter barrier via the PI3K/AKT/mTOR signaling pathway, thus retarding the progression of DKD [14]. After PI3K is activated, it catalyzes the generation of PIP3, a second messenger, from PIP2, followed by activation of AKT upon binding to AKT and PDK1. The activated AKT can act directly on mTOR and activate mTOR and its downstream pathways. Among these, mTOR is a key signaling regulator that is activated to regulate cell proliferation, metabolism, and apoptosis [53]. Yaling Hu et al. [54] indicated that the levels of HIF-1α, JAK-2, and STAT3 in DKD rats were higher than those in the control group after intervention with Yishen Capsule, suggesting that Yishen Capsule improves renal injury by interfering with the HIF-1α and JAK2/STAT3 signaling pathways. The biological processes such as immunity, proliferation, and apoptosis are contained within the action of the JAK/STAT signaling pathway. Specific binding of cytokines to receptors causes receptor dimerization, which subsequently brings receptor-coupled JAK kinases in close proximity to each other, with activation of each other's tyrosine residues by phosphorylation. The activated JAK kinase phosphorylate the complex residues on the receptor, thereby recruiting STAT and phosphorylating them via JAK kinase. The phosphorylated STAT molecules are released from the receptor and rely on their respective SH2 structural domains to form homodimer that then translocates to the target gene promoter and regulate the gene expression [55]. Zhao Liang et al. [56] investigated the mechanism of action of the Yishen Tongluo Formula in the treatment of DKD and found that it mainly inhibits inflammation as well as renal fibrosis by suppressing the expression of inflammatory factors such as TGF-β1 and IL-6, which is mainly achieved through AGE-RAGE and PI3K/Akt signaling pathways. The AGEs bind to the receptors for RAGE and release pro-inflammatory cytokines, which later acts together to combat oxidative stress, suppress inflammatory responses, delay renal fibrosis, and maintain the integrity of the glomerular filtration membrane through activation of downstream signaling pathways such as p38MAPK, NF-κB, and so forth [57]. And in this study, we chose the VEGF/PI3K/AKT signaling pathway as the object of study. VEGF usually refers to VEGFA, and VEGF receptors include VEGFR1 and VEGFR2, of which VEGFR2 is an important transducer receptor for vascular neogenesis and mitosis. After VEGFA binds to VEGFR2, the Src protein is activated, which stimulates the PI3K/Akt signaling pathway. P-AKT can improve vascular endothelial cell survival by inhibiting BAD and CASP9 activity, and it can also improve vascular permeability by activating eNOs to produce NO [58].

Considering the limitations of network pharmacological analysis, we will follow up with in vitro cell experiments, animal experiments, and clinical observations to verify the scientific validity of cell experiments, and there is much more we can do. Since there are quite a lot of components in herbal compounding, we need to figure out which component exerts the therapeutic effect on the disease. As a result, we can use metabolomics to measure the active ingredients of raw herbal medicines in blood after DSBD gavage in DKD rats and then analyze the top-ranked active ingredients to see if they have the effect of delaying DM, such as improving DKD, lowering glucose, and improving renal blood circulation. In addition, exosomes are specifically secreted extracellular vesicles that are naturally present in body fluids and have related functions involving apoptosis, inflammation, and oxidative stress. Studies have

demonstrated that in a high glucose environment, exosomes are involved in podocyte protection, inhibition of podocyte migration, injury, and renal fibrosis, suggesting that exosomes play an important biological role in DKD [59]. Therefore, we can extract exosomes from blood or body fluids after gavage in rats and measure exosome levels to demonstrate the therapeutic effect of DSBD on DKD. Finally, we can also use proteomics, transcriptomics, and other histological methods to elucidate the mechanism of action of DSBD in the treatment of DKD at a deeper level.

5. Conclusions

The aim of this study was to use network pharmacology to investigate the molecular mechanism of DSBD in the treatment of DKD. Ultimately, the core *IL1B*, *MMP9*, *EGF*, *VEGFA*, *HIF1A*, and *PTGS2* targets were obtained by screening for DSBD active components, targets of DKD disease, and DEGs. The core active ingredients are 6-gingerol, baicalein, oleic acid, beta-sitosterol, linolenic acid and aloe-emodin. Enrichment analysis of GO and KEGG has shown that DSDB is predominantly through angiogenesis, positive regulation of cell migration and positive regulation of gene expression, positive regulation of vascular endothelial growth factor production and other biological processes, as well as related pathways such as VEGF signaling pathway and IL-17 signaling pathway to play a therapeutic role. In conclusion, clinical correlation analysis, single-cell sequencing analysis, and molecular docking demonstrated that *VEGFA* could be a key target gene in DSBD treatment of DKD. There was, however, no experimental verification involved in the present study, but it did provide the direction for the research that follows.

Ethics statement

Informed consent was not required for this study because all data were derived from a database.

Consent for publication

Not applicable.

Availability of data and materials

All the data can be obtained from the open source platform provided in the article.

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Data availability

The data in this study are contained in publicly available repositories, including TCMSP, UniProt, Gene Cards, OMIM, DrugBank, TTD, DisGeNET, GEO, CIBERSORT X, Nephroseq V5.

CRediT authorship contribution statement

Duojie Xu: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Ling Yuan:** Conceptualization. **Mengying Che:** Methodology, Data curation. **Wenjing Liu:** Software, Formal analysis, Data curation. **Xiangyang Li:** Methodology, Data curation. **Yifan Yang:** Supervision, Software, Resources. **Kaili Wang:** Visualization, Software. **Yi Nan:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

DSBD	Dahuang-Shengjiang-Banxia Decoction
DKD	Diabetic kidney disease
DM	Diabetes mellitus

ESRD	End-stage kidney disease
TCM	Traditional Chinese medicine
OB	Oral availability
DL	Drug-likeness
DEGs	Differentially expressed genes
GO	Gene ontology
BP	Biological process
MF	Molecular function
CC	Cellular composition
KEGG	The Kyoto gene and genome
GSEA	Gene set enrichment analysis
eGFR	Estimated glomerular filtration rate
STZ	Streptozotocin
ROS	Reactive oxygen species
SOD	Superoxide dismutase
U-ALB	Urinary albumin
BUN	Blood urea nitrogen
GFR	Glomerular filtration rate
ACR	Ratio of urinary albumin to urinary creatinine

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