Original Research

Prediction of the Potential Mechanism of Triptolide in Improving Diabetic Nephropathy by Utilizing A Network Pharmacology and Molecular Docking Approach

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Abstract

Background: Triptolide (TP) is a major active component of colquhounia root tablet, which has been long been used in China to treat diabetic nephropathy (DN) due to its marked anti-inflammatory, antiproteinuric, and podocyte-protective effects. Methods: This study investigated the anti-proteinuria activity and related signaling cascade of TP in DN by utilizing a network pharmacology and molecular docking approach. Results: From the GeneCard, DisGeNET, and National Center for Biotechnology Information Gene databases, 1458 DN targets were obtained and input together with 303 TP targets into Venny2.1.0 for mapping and comparing. In total, 113 common targets of TP and DN were obtained, of which 7 targets were found to play an important role through theoretical inhibitory constant analysis. The common targets were further analyzed by Kyoto Encyclopedia of Genes and Genomes to identify the pathways related to the therapeutic effect of TP on DN. Among them, seven targets were found to play key roles in six signaling pathways. The molecular docking results also showed TP had good binding ability to the seven targets. Conclusions: Analysis of the common targets and key pathways showed that TP can improve DN via its anti-nephritis, anti-renal fibrosis, antioxidant, and podocyte-protective effects, which might elucidate the mechanism by which TP improves renal function and reduces proteinuria in DN.

Keywords: triptolide; diabetic nephropathy; network pharmacology; molecular docking

1. Introduction

Diabetic nephropathy (DN), a serious microvascular complication of type 1 and type 2 diabetes, is characterized by proteinuria and persistent renal function injury [1]. Proteinuria, an independent risk factor of disease progression, is the most important clinical characteristic of DN, and is also the leading cause of end-stage renal disease [2,3]. Without early intervention, 50% of patients with microalbuminuria will progress to macroalbuminuria [4]. There are many risk factors for the development of DN including increased inflammatory factors and oxidative stress, changes in fat and protein metabolism, and overexpression of the renin-angiotensin-aldosterone system. These lead to the apoptosis and loss of renal podocytes and decreased filtration capacity of glomeruli, thus aggravating DN [5,6]. Although several recent studies have confirmed that angiotensin-converting enzyme inhibitors/angiotensin receptor blockers can reduce DN proteinuria and play a role in delaying disease progression, they are ineffective in DN patients with normal blood pressure [7]. Due to the limitations of traditional Western medical approaches, some DN patients have turned to alternative treatments such as traditional Chinese medicine (TCM).

Triptolide (TP) is a component of the following traditional Chinese herbal medicines: Tripterygium willofordii Hook. F., Tripterygium hypoglaucum Levil. Hutch, Tripterygium regeri Sprague et Takeda, and Tripterygium forretii Dicls [8]. It is also a major active component of the colquhounia root tablet and tripterygium glycoside tablet, which have long been used in China to treat DN due to their marked anti-inflammatory, anti-proteinuria, and podocyte-protective effects [9–11]. Several randomized controlled clinical trials have indicated that TP possibly imparts nephroprotective effects by decreasing proteinuria, serum creatinine levels, and blood urea nitrogen levels [12–14]. Although TP is effective for improving renal function and reducing proteinuria in DN, the exact mechanism is still unclear.

Network pharmacology is a research method based on virtual computing technology, high-throughput data, and public database, which combines system computing with experiments, introducing a new field of pharmacology [15, 16]. Network pharmacology constructs a multi-level network of disease-phenotype-gene-drug through multi-target interaction [17]. Through analysis of the overall network, we can better predict drug targets to provide help for the re-
search and design of new drugs [18]. For TCM, each component in its prescription has its target, and the effect of the drug is often the result of the synergistic effects of multiple component targets [19]. Network pharmacology can reveal the role of various components at the molecular level so that people can make better use of TCM [20].

In this study, we used network pharmacology to identify the potential targets and signaling pathways of the TCM component TP for the treatment of DN and revealed its possible mechanism. Fig. 1 shows a flowchart of the online pharmacological processes of this study.

Fig. 1. Network pharmacological workflow to determine the potential mechanisms and targets of triptolide in the treatment of diabetic nephropathy.

2. Methods

2.1 Prediction Targets of TP

The targets of TP were identified by two databases: PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) and traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) (https://old.tcmsp-e.com/tcmsp.php). The PharmMapper database is a platform for targets prediction, which identifies the potential targets of small molecules by a pharmacophore mapping approach. The chemical structure of TP was drawn by ChemDraw (PerkinElmer, Waltham, MA, USA) and uploaded into the PharmMapper database (Fig. 2). In the PharmMapper database, the maximum number of conformation generation was set to 300 and druggable pharmacophore models were selected as the target set. The names of these target genes were converted to official names by UniProt (https://www.uniprot.org/). TCMSP is a systems pharmacology platform used for screening the active ingredients of TCM. After inputting the keyword “triptolide”, targets were obtained from the TCMSP database.

Fig. 2. TP chemical structure.

2.2 Prediction Targets of DN

DN-associated targets were obtained through three online databases, GeneCard (https://www.genecards.org/), DisGeNET (https://www.disgenet.org/), and National Center for Biotechnology Information (NCBI) Gene databases (https://www.ncbi.nlm.nih.gov/gene/). GeneCard is a database with relevant information on proteomics, transcriptomics, and genomics [21]. DisGeNET is a comprehensive database of genes related to human disease [22]. NCBI Gene is a database containing information about multiple species [23]. After searching the keywords “diabetic nephropathy” in the above three databases, the targets of DN were obtained.

2.3 Construction and Analyses of the PPI Network

The potential targets of TP and the disease targets of DN were mapped and compared with the Venny2.1.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/index.html), in order to obtain the common targets of TP and DN. The STRING database (https://string-db.org/) contains almost all known and predicted information about protein-protein interactions, including direct and indirect interactions. The validity of these interactions was calculated in the form of confidence scores, ranging from 0 to 1 [21]. The medium confidence level was set to greater than 0.4, with the species “Homo sapiens” [22]. The potential targets of TP and the related targets of TP treatment for DN were uploaded to the STRING database. The protein-protein
interaction networks of TP and TP-DN were obtained [22,23]. Cytoscape (https://cytoscape.org/) is an open source network software platform, which can be used to visualize the intermolecular interaction network and combine network and gene expression profile data [24]. The TP and TP-DN target networks obtained from the STRING database were imported into Cytoscape software (version 3.8.2, Institute for Systems Biology, Seattle, Washington, USA), and the “Network Analyzer” function was used to analyze the topology parameters of the network [25].

2.4 Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment Analyses

Metascape database (https://metascape.org/) is a platform for gene annotation analysis, which can analyze the signaling pathways and biological processes of uploaded target genes [26]. For enrichment analysis, the species was set to “Homo sapiens”, the p-value cutoff was 0.05, and other parameters were default [27]. Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed on the targets in turn. The results were saved and sorted according to score, and the relevant biological processes and signal pathways were screened [28]. The results of the filters were put input into a bioinformatics online tool (http://www.bioinformatics.com.cn/), to draw the relevant pictures [29].

2.5 Molecular Docking

The crystal structure of candidate protein binding to TP was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) and modified using Autodock (version 2.5, Scripps Research, San Diego, California, USA) to remove ligand and water molecules, and add hydrogen and Kollman charge [30,31]. The three-dimensional structure of TP was obtained from DrugBank (https://go.drugbank.com/) and was also modified by Autodock (version 2.5, Scripps Research, San Diego, California, USA) [32]. First, the active site was confirmed by a eutectic small molecule ligand of the proteins. Then the position of the active site with 60 Å outward was taken as the center of the docking box. Second, the Lamarckian genetic algorithm was used to find the best conditions for docking. Finally, the conformation with the lowest energy was selected as the optimal conformation. The docking results were visualized using PyMol (https://pymol.org/2/), where the hydrogen bonds and binding sites were analyzed.

3. Results

3.1 TP-DN Common Targets

A total of 303 targets of TP were obtained through PharmMapper and the TCMSP database. A total of 1458 targets associated with DN were identified in the GeneCard, DisGeNET and NCBI Gene databases. The TP and DN targets were determined using the Venny2.1.0 data platform, which identified common targets (Fig. 3).
Fig. 5. GO analysis of biological processes. Note: the color scale indicates the adjusted \(p\)-value, and the dot size represents the gene count in each term.

Fig. 6. GO analysis of cell components. Note: the color scale indicates the adjusted \(p\)-value, and the dot size represents the gene count in each term.

AP-transcription factor subunit [JUN], tumor protein p53 [TP53], signal transducer and activator of transcription 3 (STAT3), matrix metalloproteinase 9 [MMP9], epidermal growth factor receptor [EGFR], caspase 3 [CASP3] were selected for further molecular docking analysis by degree value.

3.3 Enrichment Analysis of the TP-DN Target Network

The common targets of TP and DN were imported into Metascape for GO and KEGG analyses, and the results were input into a bioinformatics online tool to obtain the enrichment bubble diagram. The results were sorted according to the \(p\)-value. In GO analysis, the biological processes were found to be associated with the positive regulation of cell migration, response to hormone, positive regulation of cell motility, positive regulation of cell component movement, and positive regulation of locomotion, among others (Fig. 5). The cell components were associated with vesicle lumen, secretory granule lumen, cytoplasmic vesicle lumen, membrane raft, membrane microdomain, among others (Fig. 6). Molecular functions were correlated with ligand activated transcription factor activity, receptor activity, phosphatase binding, protein kinase activity, and cytokine receptor binding, among others (Fig. 7).

In KEGG analysis (Fig. 8), the top pathways related to DN were selected for further analysis and included advanced glycation end product-receptor for advanced glycation end product (AGE-RAGE), mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase-AKT (PI3K-AKT), relaxin, forhead box O (FOXO), and TNF signaling pathways.

Fig. 7. GO analysis of molecular functions. Note: the color scale indicates the adjusted \(p\)-value, and the dot size represents the gene count in each term.

Fig. 8. KEGG pathway analysis. Note: the color scale indicates the adjusted \(p\)-value, and the dot size represents the gene count in each term.
### Table 1. Topological analysis of common target network.

| Target name                                      | Abbreviation | ASPL | BC   | CC   | Clustering coefficient | Degree |
|--------------------------------------------------|--------------|------|------|------|------------------------|--------|
| Tumor necrosis factor                            | TNF          | 1.170| 0.124| 0.855| 0.304                  | 93     |
| Albumin                                          | ALB          | 1.179| 0.106| 0.848| 0.311                  | 92     |
| AKT Serine/Threonine Kinase 1                    | AKT1         | 1.250| 0.062| 0.800| 0.355                  | 84     |
| Vascular endothelial growth factor A             | VEGFA        | 1.313| 0.035| 0.762| 0.415                  | 77     |
| Transcription factor AP-1                        | JUN          | 1.348| 0.033| 0.742| 0.434                  | 73     |
| Cellular tumor antigen p53                       | TP53         | 1.357| 0.031| 0.737| 0.435                  | 72     |
| Signal transducer and activator of transcription 3 | STAT3       | 1.384| 0.022| 0.723| 0.473                  | 70     |
| Matrix metalloproteinase-9                       | MMP9         | 1.402| 0.025| 0.713| 0.448                  | 68     |
| Epidermal growth factor receptor                 | EGFR         | 1.411| 0.020| 0.709| 0.463                  | 67     |
| Caspase-3                                        | CASP3        | 1.429| 0.020| 0.700| 0.485                  | 64     |
| Estrogen receptor alpha                           | ESR1         | 1.509| 0.020| 0.663| 0.491                  | 57     |
| Prostaglandin-endoperoxidase synthase 2          | PTGS2        | 1.500| 0.015| 0.667| 0.534                  | 56     |
| Peroxisome proliferator-activated receptor gamma | PPARG        | 1.527| 0.017| 0.655| 0.497                  | 54     |
| Transforming protein RhoA                        | RHOA         | 1.589| 0.010| 0.629| 0.569                  | 50     |
| Chemokine receptor 4                             | CXCR4        | 1.598| 0.007| 0.626| 0.595                  | 49     |
| Mitogen-activated protein kinase 1               | MAPK1        | 1.598| 0.005| 0.626| 0.629                  | 48     |
| type IV collagenase                              | MMP2         | 1.607| 0.006| 0.622| 0.598                  | 48     |
| Transforming growth factor beta-1                | TGFβ1        | 1.589| 0.007| 0.629| 0.603                  | 48     |
| Cellular oncogene fos                            | FOS          | 1.598| 0.006| 0.626| 0.603                  | 47     |
| Interferon gamma                                 | IFNG         | 1.598| 0.004| 0.626| 0.662                  | 47     |
| Mitogen-activated protein kinase 14              | MAPK14       | 1.607| 0.005| 0.622| 0.635                  | 46     |
| Interleukin-2                                    | IL2          | 1.616| 0.005| 0.619| 0.630                  | 45     |
| Signal transducer and activator of transcription 1 | STAT1       | 1.634| 0.022| 0.612| 0.629                  | 44     |
| Vascular endothelial growth factor receptor 2 variant | KDR          | 1.634| 0.009| 0.612| 0.591                  | 43     |
| Nitric oxide synthase 3                         | NOS3         | 1.652| 0.004| 0.605| 0.657                  | 43     |
| C-C motif chemokine 5                           | CCL5         | 1.643| 0.004| 0.609| 0.635                  | 42     |
| Mitogen-activated protein kinase 8               | MAPK8        | 1.643| 0.013| 0.609| 0.551                  | 42     |
| Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells 3 | RELA | 1.652| 0.005| 0.605| 0.657                  | 41     |
| Janus kinase 2                                   | JAK2         | 1.661| 0.004| 0.602| 0.670                  | 41     |
| Peroxisome proliferator-activated receptor alpha | PPARA        | 1.661| 0.015| 0.602| 0.501                  | 39     |
| Phosphatidylinositol 3-kinase regulatory subunit alpha | PIK3R1    | 1.732| 0.004| 0.577| 0.610                  | 39     |
| Cell division control protein 42 homolog        | CDC42        | 1.696| 0.002| 0.589| 0.722                  | 37     |
| Insulin-like growth factor 1 receptor            | IGF1R        | 1.714| 0.004| 0.583| 0.659                  | 37     |
| Heme oxygenase 1                                 | HMOX1        | 1.696| 0.004| 0.589| 0.669                  | 35     |
| Glycogen synthase kinase-3 beta                 | GSK3B        | 1.705| 0.002| 0.586| 0.708                  | 35     |
| Renin                                            | REN          | 1.705| 0.009| 0.586| 0.503                  | 34     |
| E3 ubiquitin-protein ligase Mdm2                 | MDM2         | 1.732| 0.002| 0.577| 0.709                  | 34     |
| Hepatocyte Growth Factor Receptor               | MET          | 1.732| 0.002| 0.577| 0.727                  | 34     |
| Protein tyrosine phosphatase non-receptor type 11 | PTPN11      | 1.732| 0.003| 0.577| 0.693                  | 34     |
| Matrix metalloproteinase 3                       | MMP3         | 1.732| 0.007| 0.577| 0.702                  | 32     |
| Nitric oxide synthase 2                         | NOS2         | 1.759| 0.002| 0.569| 0.738                  | 32     |
| Matrix metalloproteinase 1                       | MMP1         | 1.768| 0.001| 0.566| 0.772                  | 31     |
| Tumor necrosis factor receptor superfamily member 5 | CD40        | 1.750| 0.003| 0.571| 0.718                  | 31     |
| Tyrosine-protein kinase Lck                      | LCK          | 1.768| 0.001| 0.566| 0.777                  | 30     |
| Androgen Receptor                               | AR           | 1.768| 0.019| 0.566| 0.655                  | 30     |
| RAC-beta serine/threonine-protein kinase         | AKT2         | 1.768| 0.002| 0.566| 0.715                  | 30     |
| Tyrosine-protein phosphatase non-receptor type 1 | PTPN1        | 1.768| 0.002| 0.566| 0.714                  | 29     |
| E-selectin                                       | SELE         | 1.786| 0.001| 0.560| 0.775                  | 28     |
| Bone morphogenetic protein 2                    | BMP2         | 1.804| 0.001| 0.554| 0.752                  | 27     |
| Target name                                      | Abbreviation | ASPL  | BC    | CC    | Clustering coefficient | Degree |
|------------------------------------------------|--------------|-------|-------|-------|------------------------|--------|
| Prepro-Coagulation Factor II                    | F2           | 1.804 | 0.001 | 0.554 | 0.775                  | 27     |
| Matrix metalloproteinase 7                      | MMP7         | 1.795 | 0.006 | 0.557 | 0.453                  | 27     |
| Urokinase-type plasminogen activator            | PLAU         | 1.795 | 0.001 | 0.557 | 0.761                  | 27     |
| Protein mono-ADP-ribosyltransferase 1           | PARP1        | 1.786 | 0.002 | 0.560 | 0.683                  | 26     |
| Superoxide dismutase 2                          | SOD2         | 1.804 | 0.002 | 0.554 | 0.711                  | 26     |
| Spleen tyrosine kinase                          | SYK          | 1.813 | 0.002 | 0.552 | 0.743                  | 25     |
| Cyclin-dependent kinase 2                        | CDK2         | 1.830 | 0.000 | 0.546 | 0.848                  | 24     |
| Transforming growth factor beta-2               | TGFβ2        | 1.813 | 0.001 | 0.552 | 0.804                  | 24     |
| Neutrophil gelatinase-associated lipocalin      | LCN2         | 1.830 | 0.002 | 0.546 | 0.640                  | 23     |
| CD80 Molecule                                   | CD80         | 1.821 | 0.001 | 0.549 | 0.775                  | 23     |
| Fibroblast growth factor receptor 1             | FGFR1        | 1.866 | 0.001 | 0.536 | 0.823                  | 22     |
| Janus Kinase 3                                  | JAK3         | 1.875 | 0.000 | 0.533 | 0.810                  | 22     |
| Cathepsin B                                     | CTSB         | 1.857 | 0.001 | 0.538 | 0.657                  | 21     |
| Insulin receptor                                | INSR         | 1.920 | 0.002 | 0.521 | 0.533                  | 21     |
| Placental growth factor                         | PGF          | 1.857 | 0.002 | 0.538 | 0.619                  | 21     |
| Dipeptidyl peptidase 4                          | DPP4         | 1.866 | 0.003 | 0.536 | 0.542                  | 20     |
| Glutathione reductase                           | GSR          | 1.955 | 0.000 | 0.511 | 0.795                  | 20     |
| Rac Family Small GTPase 1                       | RAC1         | 1.839 | 0.019 | 0.544 | 0.516                  | 20     |
| Alpha-1-antitrypsin                             | SERPINA1     | 1.946 | 0.000 | 0.514 | 0.789                  | 20     |
| Erb-B2 Receptor Tyrosine Kinase 4               | ERBB4        | 1.848 | 0.001 | 0.541 | 0.721                  | 20     |
| TGF-beta receptor type-1                        | TGFB1        | 1.875 | 0.000 | 0.533 | 0.877                  | 19     |
| Aldo-keto reductase family 1 member B1           | AKR1B1       | 1.848 | 0.010 | 0.541 | 0.725                  | 19     |
| Complement C3                                   | C3           | 1.875 | 0.001 | 0.533 | 0.752                  | 18     |
| Vitamin D receptor                              | VDR          | 1.902 | 0.002 | 0.526 | 0.536                  | 18     |
| Apoptotic protease-activating factor 1          | APAF1        | 1.884 | 0.001 | 0.531 | 0.669                  | 17     |
| Glutathione S-transferase Pi 1                  | GSTP1        | 1.893 | 0.000 | 0.528 | 0.853                  | 17     |
| Caspase-7                                       | CASP7        | 1.902 | 0.000 | 0.526 | 0.775                  | 16     |
| Neutrophil collagenase                          | MMP8         | 1.893 | 0.000 | 0.528 | 0.900                  | 16     |
| Disintegrin and metalloproteinase domain-containing protein 17 | ADAM17       | 1.902 | 0.002 | 0.526 | 0.438                  | 15     |
| Hypoxanthine-guanine phosphoribosyltransferase 1 | HPRT1       | 1.902 | 0.008 | 0.526 | 0.695                  | 15     |
| Bile acid receptor                              | NR1H4        | 1.911 | 0.000 | 0.523 | 0.771                  | 15     |
| Fatty acid-binding protein 4                    | FABP4        | 1.964 | 0.002 | 0.509 | 0.549                  | 14     |
| Apoptosis regulator Bcl-2                       | BCL2         | 1.911 | 0.003 | 0.523 | 0.484                  | 14     |
| Cytochrome P450 family 2 subfamily C polypeptide 9 | CYP2C9      | 1.964 | 0.003 | 0.509 | 0.341                  | 14     |
| Retinol-binding protein 4                        | RBP4         | 2.009 | 0.000 | 0.498 | 0.725                  | 14     |
| Macrophage migration inhibitory factor          | MIF          | 1.929 | 0.000 | 0.519 | 0.910                  | 13     |
| Group-specific component                         | GC           | 1.991 | 0.002 | 0.502 | 0.397                  | 13     |
| Angiogenin                                      | ANG          | 1.938 | 0.001 | 0.516 | 0.697                  | 12     |
| Atriopentidase                                  | MME          | 1.955 | 0.001 | 0.511 | 0.576                  | 12     |
| Hydroxymethylglutaryl-CoA reductase             | HMGR         | 1.991 | 0.001 | 0.502 | 0.636                  | 11     |
| Macrophage metalloelastase                      | MMP12        | 1.955 | 0.000 | 0.511 | 0.836                  | 11     |
| Transhyretin                                     | TTR          | 1.938 | 0.001 | 0.516 | 0.636                  | 11     |
| Glutathione S-transferase Mu 1                  | GSTM1        | 1.946 | 0.000 | 0.514 | 0.756                  | 10     |
| Glutathione S-transferase Mu 2                  | GSTM2        | 1.973 | 0.000 | 0.507 | 0.622                  | 10     |
| Oxy steroids receptor LXR-alpha                 | NR1H3        | 1.946 | 0.001 | 0.514 | 0.578                  | 10     |
| Pregnane X nuclear receptor                     | NR1I2        | 1.964 | 0.000 | 0.509 | 0.711                  | 10     |
| Peptidyl-prolyl cis-trans isomerase A            | PPIA         | 1.982 | 0.001 | 0.505 | 0.556                  | 10     |
| Phosphoenolpyruvate carboxykinase 1             | PCK1         | 1.946 | 0.000 | 0.514 | 0.733                  | 10     |
| Peroxisome proliferator-activated receptor delta | PPARD       | 2.063 | 0.003 | 0.485 | 0.694                  | 9      |
### 3.4 Molecular Docking

The binding ability of TP to the proteins in the TP-DN target network was evaluated by molecular docking. These target proteins included AKT1 (3OCB), ALB (1E7A), CASP3 (1CP3), EGFR (1M17), JUN (1JNM), MMP9 (1GKC), STAT3 (6NJS), TNF (2AZ5), TP53 (6GGA), and VEGFA (4ZFF). Generally, binding energies less than -5 kcal/mol are considered good binding ability. According to the docking results (Fig. 9), TP had a stronger binding ability with AKT1, EGFR, CASP3, ALB, STAT3, TNF, and TP53. Moreover, TP formed a hydrogen bond with ALA-230 (1.9Å) at the active site of AKT1, TYR-401 (2.1Å), LYS-402 (3.3Å), ASP-549 (2.0Å) of EGFR, GLY-122 (3.5Å), ALA-992 (1.7Å) of CASP3; LYS-721 (1.9Å) and CYS-773 (1.9Å) of ALB; GLN-644 (1.7Å, 2.4Å), GLY-656 (3.4Å), and LYS-658 (1.9Å) of STAT3; GLY-121 (1.9Å) of TNF; and SER-227 (2.2Å) and THR-231 (1.8Å, 2.7Å) of TP53. The detailed binding energies and inhibition constants for the molecular docking of TP are presented in Table 2.

### Table 2. The free energies, and theoretical inhibition constants (Ki) of TP binding to targets (at T = 298.15 K).

| Class | ΔG (kcal/mol) | Ki (µM) |
|-------|---------------|---------|
| AKT1  | −6.97         | 7.83    |
| EGFR  | −6.85         | 9.56    |
| CASP3 | −6.29         | 24.49   |
| MMP9  | −4.41         | 584.42  |
| ALB   | −6.37         | 21.58   |
| JUN   | −4.98         | 222.07  |
| STAT3 | −7.46         | 3.4     |
| TNF   | −7.27         | 4.67    |
| TP53  | −6.06         | 36.14   |
| VEGFA | −4.64         | 399.32  |

The inhibition constants were obtained from Autodock (version 2.5, Scripps Research, San Diego, California, USA).

### 4. Discussion

DN is a chronic kidney disease and the leading cause of end-stage renal disease in most developed countries [5]. The causes of DN are complex, but inflammation and oxidative stress are known to be involved in its progression [5,33]. As a new approach, network pharmacology can well analyze the overall relationship between drugs and diseases, including how to participate in the therapeutic process [34]. TP markedly attenuates albuminuria and podocyte injury, regulating the T helper cell balance and macrophage infiltration in an animal model of DN [35,36]. To elucidate the possible mechanism and potential targets of TP in the treatment of DN, we constructed and analyzed the targets through network pharmacology and molecular docking.

Among the 113 TP- and DN-related targets, 7 targets were found to play an essential role through network analysis including ALB, AKT1, CASP3, EGFR, STAT3, TNF, and TP53. Individually, ALB functions as an intravascular transporter, which not only binds a variety of ions, hormones, and drugs but also stabilizes osmotic pressure, anti-inflammation, and antioxidation [37]. When the concentration of glucose is too high, glycosylation of ALB occurs [38]. After additional events, glycosylated ALB further forms AGEs and stimulates cells to produce oxidative stress, thus damaging cells [39]. CASP3 belongs to the family of cysteine proteases and is an essential factor in regulating apoptosis [40]. High glucose can stimulate mito-
chondria, release cytochrome C, and increase the expression of CASP9 and CASP3 [41]. At the same time, activation of CASP12 and CASP3 through endoplasmic reticulum stress can also be independent of mitochondria, resulting in apoptosis [42]. EGFR is an important receptor tyrosine kinase, which is closely related to the development of DN and is widely distributed in glomeruli and renal tubules [43]. EGFR can be activated by high glucose and Src kinase, mediating the phosphorylation of Akt, stimulating a large number of reactive oxygen species (ROS), and inducing the MAPK signal pathway all of which leads to the release of inflammatory factors and reduces insulin secretion in islet cells, resulting in insulin resistance [44]. The Janus kinase/STAT pathway can be activated by high glucose and ROS, and is involved in the pathogenesis of DN [45]. After being phosphorylated, STAT3 enters the nucleus to stimulate the transcription of target genes, increasing the expression of inflammatory and fibrosis factors [46]. Inhibiting the activity of STAT3 can decrease TNF-α and interleukin beta 1 (IL-b1) levels, ameliorating renal fibrosis [47]. In diabetic patients, the levels of inflammatory factors are significantly elevated [48]. As an inflammatory factor, TNF can greatly promote the development of DN and damage the glomerular filtration barrier [49]. Moreover, it can bind to insulin-like growth factor binding protein-3 to induce the apoptosis of mesangial cells [50]. TP53 is a tumor suppressor, which regulates the apoptosis of podocytes [51]. After phosphorylation, AKT1 activates the MAPK signaling pathway, releasing a large number of inflammatory factors and causing renal fibrosis [52].

Fig. 9. Molecular docking of TP with potential target proteins A (AKT1), B (ALB), C (CASP3), D (EGFR), E (JUN), F (MMP9), G (STAT3), H (TNF), I (TP53), J (VEGFA). Note: The yellow dotted lines indicate the hydrogen bonds and the numbers above represent their distance. The green structure represents triptolide, and the blue structure indicates the amino acid residues in the binding site of the protein.
High glucose stimulates the EGFR receptor, which activates the PI3K / Akt signaling pathway, which in turn mediates the transcription of genes via the MAPK pathway, leading to TGF-β, Collagen IV, fibronectin as well as TNF-α, IL-1β of the levels rise. The inflammatory factors will trigger ECM and EMT, causing nephritis, renal fibrosis, proteinuria, meanwhile, inflammatory factors will also bind to the corresponding receptors to stimulate cells to release more inflammatory factors. All events ultimately initiate DN.

5. Conclusions

A total of 113 common targets of TP and DN were identified by using network pharmacology, and the binding ability of TP to these targets was verified by molecular docking experiments. After KEGG enrichment analysis, six pathways were found to play a key role in the therapeutic effect of TP on DN (Fig. 10). The MAPK signaling pathway is a classic inflammatory pathway, which is composed of p38-MAPK, c-Jun N-terminal kinase 1 (JNK1), and extracellular signal-regulated kinase 2 (ERK2) [53]. When stimulated by ROS, p38-MAPK, JNK, and ERK release signaling factors such as ATF1/2 and e-Jun that mediate the transcription of transforming growth factor beta (TGF-β), IL-1β, fibronectin, and type IV collagen, leading to nephritis, renal fibrosis, podocyte apoptosis, and proteinuria [54]. A high glucose environment can stimulate the glycosylation of serum ALB and gradually transform it into AGEs [38]. AGEs continuously accumulate and activate RAGE, leading to oxidative stress, activation of the MAPK pathway, chronic inflammation, and eventually renal injury [55–57]. The PI3K/Akt pathway can activate the nuclear factor kappa B (NF-κB) pathway, increasing the expression of IL-6 and leading to glomerular basement membrane thickening and mesangial expansion [52]. Meanwhile, Akt
can phosphorylate FOXO3a in the FOXO signaling pathway, causing extracellular matrix hyperplasia [58]. Relaxin, a member of the insulin family, has vasodilatory and antiinflammatory effects. Activation of the relaxin pathway inhibits SMAD2 activation and TGF-β production, reducing synthesis of the extracellular matrix (ECM) [59]. When FOXO3a is phosphorylated by Akt, the expression of bisindolylmaleimide and manganese superoxide dismutase decreases, resulting in ECM accumulation and accelerating the occurrence of DN [60]. Meanwhile, activation of the TNF pathway will elevate the expression of ROS, leading to the altered permeability of the capillary wall and triggering proteinuria [61]. Generally, the results showed that TP could improve DN via its anti-inflammatory, anti-renal fibrosis, anti-oxidant, and podocyte-protective effects.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Abbreviations**

ACEIs, Angiotensin-convertin enzyme inhibitors; AGEs, Advanced glycation end products; ALB, Albumin; ASPL, Average shortest path length; ARBs, Angiotensin receptor blockers; BC, Betweenness centrality; CASP3, Caspase-3; CC, Closeness centrality; DN, Diabetic nephropathy; EGFR, Epidermal growth factor receptor; EMT, Epithelial mesenchymal transition; ER, Endoplasmic reticulum; ERK2, Extracellular signal-regulated kinase; ESR1, Estrogen receptor; HG, High glucose; HO-1, Heme oxygenase-1; JAK, Janus kinase; MAPK, Mitogen-activated protein kinase; NF-κB, Nuclear factor kappa B; RAGE, Receptor for advanced glycation end products; ROS, Reactive oxygen species; STAT3, Signal transducer and activator of transcription 3; SRC, SRC proto-oncogene, non-receptor tyrosine kinase; TP53, Tumor protein p53; TGF-β, Transforming growth factor-β; TNF, Tumor necrosis factor; VEGF, Vascular endothelial growth factor.

**Author contributions**

MY conceived the study, MY and DF designed research; XA, DF, YZ, RT, and JZ performed research; DF, ZY performed data analysis; MY and DF prepared all figures and wrote the manuscript; MY edited the final and prepared the revised manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

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**Conflict of interest**

The authors declare no conflict of interest.

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