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Network Pharmacology and Molecular Docking Strategy Reveal the Mechanism of *Tripterygium wilfordii* in Treating Castration-Resistant Prostate Cancer

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Abstract

Background: Castration-Resistant Prostate Cancer (CRPC) is an inevitable type of progression in Prostate Cancer (PCa) after Androgen Deprivation Therapy (ADT). *Tripterygium wilfordii* (TW), a traditional Chinese herbal medicine, is frequently used to treat cancer and other diseases. In order to identify the potential mechanism of TW in the treatment of CRPC, we jointly utilized network pharmacology and molecular docking to perform preliminary investigation and validation.

Methods: The active components and corresponding protein targets of TW were screened on TCMSP database, the targets of CRPC were obtained on GeneCards database and DisGeNET database. The intersectional targets of TW and CRPC were obtained on Venn software. Then, we used STRING database to construct a PPI network based on the intersectional targets, further performed GO and KEGG enrichment analyses on the intersectional targets. Finally, molecular docking was approved for the main active components and core targets. In addition, we also carried out immunohistochemical analysis and expression analysis of core targets, which were conducted to determine how TW affects CRPC and what are the most important components, potential targets, and signaling pathways.

Results: Our study reveals that kaempferol is the key component of TW treatment for CRPC. The findings of the network pharmacology indicated that TP53, AKT1 and TNF are the potential target genes for TW connected to anti-CRPC actions. Additionally, KEGG analysis revealed that p53 signaling pathway and PI3K-Akt signaling pathway may be the driver of the anti-CRPC impact of TW. The results of molecular docking showed a high affinity between major active components in TW and the key targets in CRPC.

Conclusion: This study reveals the potential mechanisms of TW in the treatment of CPRC through multiple targets and signaling pathways and is validated through molecular docking. TW exerts pharmacological effects in CRRC, p53 signaling pathway is of great value. Furthermore, the present study provided a reference for the wide application of TW in clinically managing CRPC.

Keywords: ***Tripterygium wilfordii*; Castration-Resistant Prostate Cancer; Network pharmacology; Molecular docking**

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Abbreviations

TW: *Tripterygium wilfordii*; CRPC: Castration-Resistant Prostate Cancer; PCa: Prostate Cancer; ADT: Androgen Deprivation Therapy; nmCRPC: non-metastatic Castration-Resistant Prostate Cancer; mCRPC: metastatic Castration-Resistant Prostate Cancer; OB: Oral Bioavailability; DL: Drug-Likeness

Introduction

Prostate Cancer (PCa) is the second most prevalent malignancy and the fifth leading cause of cancer death in males worldwide. In 2020, the global median rates of prostate cancer-related morbidity and mortality for men are 30.7/100,000 and 7.7/100,000 respectively [1,2]. The morbidity and mortality of PCa in China have been rising yearly, particularly for people over 60 years old,

albeit not being as high as in Europe and the United States [3,4]. There is a gap in the 5-year relative survival rate in PCa between China (66.4%) and developed countries (90.9%-99.5%). In addition to the different cancer spectrums between China and developed countries, late clinical diagnosis and irregular clinical diagnosis in advanced cases are the primary causes of this disparity [5]. Although Androgen Deprivation Therapy (ADT) has good efficacy for PCa patients, the majority of patients continue to convert from Hormone-Sensitive Prostate Cancer (HSPC) to Castration-Resistant Prostate Cancer (CRPC), which is divided into non-metastatic Castration-Resistant Prostate Cancer (nmCRPC) and advanced-stage metastatic Castration-Resistant Prostate Cancer (mCRPC) [6]. Currently, several new anti-androgen therapeutic agents which can prolong the MFS of nmCRPC have been recommended for the treatment of nmCRPC, such as darolutamide and enzalutamide. Though conditions of patients with initially diagnosed metastatic PCa can be improved to some extent, the majority of patients still develop mCRPC within two years [7,8]. Now, new endocrine therapies such as abiraterone and enzalutamide are recommended as first-line drugs for mCRPC, and previous studies have confirmed that these drugs benefit patients with mCRPC to a certain degree, especially patients who featured age ≥ 75 years, visceral metastases, pathological glandular cavity pattern or AR-expressing activity [9]. Chemotherapeutic agents such as docetaxel and platinum, targeted therapies such as the PARP inhibitor Olaparib, and immunotherapies like PD-1 and PD-L1 inhibitors are also available for the treatment of mCRPC, but the efficacy and safety of drugs remain controversial. The multidrug resistance exhibited by PCa is a major cause of cancer recurrence, metastasis, and treatment failure [10]. In the absence of effective treatment, the median survival of mCRPC is only 12 months. Therefore, exploring new drugs to optimize the treatment of patients with CRPC is urgent to prolong patient survival, delay disease progression and improve quality of life.

As a traditional Chinese medicine and one of the most researched herbal medicines among many herbal monomers, *Tripterygium wilfordii* (TW) has the efficacy of activating blood circulation, clearing heat and detoxifying, anti-rheumatoid and anti-cancer, especially in the treatment of various tumors such as breast cancer [11], lung cancer [12], cervical cancer [13] and leukemia [14], etc. Its main components, such as triptolide, can act on multiple aspects of tumors, including induction of apoptosis, blocking the cancer cell cycle, inhibiting cancer cell invasion and metastasis, inducing cancer cell autophagy. However, the molecular mechanism of the TW regimen for CRPC treatment has not been fully clarified. Network pharmacology is a comprehensive research method based on theories of systems biology and genomics. It can reflect the "component-target-disease" relationship, which provides guidance and treatment methods for drug discovery and selection in various diseases [15]. In this study, we used network pharmacology and molecular docking to investigate the mechanism of TW for the CRPC treatment and provide a basis for subsequent clinical treatment and experimental research. The flowchart of this study is shown in Figure 1.

Materials and Methods

Identification of bioactive components and corresponding targets of TW

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; http://old.tcmsp-e.com/tcmsp.php) was used to obtain the components of TW, and the Oral Bioavailability (OB) ≥ 30% and Drug-Likeness (DL) ≥ 0.18 were used as the preset criteria to obtain the main bioactive ingredients of TW. The corresponding targets of each bioactive component in TW were gained using TCMSP database, then we established the protein-target database of TW. Finally, the target proteins were standardized in the Universal Protein Resource (Uniprot; https://www.uniprot.org/), and the target information of "Homo sapiens" was reserved for further analysis.

Collection of relevant targets of TW in the treatment of CRPC

By using the DisGeNET database (https://www.disgenet.org/) and GeneCards database (https://www.genecards.org/), the keyword "castration resistant prostate cancer" was used to retrieve the disease-related targets. Intersections of TW targets and CRPC targets were regarded as the targets of TW action in the treatment of CRPC. The common targets between the components and CRPC were obtained by using the Venny 2.1.0 tool (https://bioinfogp.cnb.csic.es/tools/venny/).

Construction of the Protein-Protein Interaction (PPI) network

We used the Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING database; https://string-db.org/) to analyze the interaction between common targets. In our study, the species was limited to "*Homo sapiens*", and the minimum required interaction score was set to be medium confidence (0.400). Then we obtained the PPI networks from the STRING database, we used Cytoscape 3.7.2 software to perform network topology analysis for obtaining the degree of connectivity between intersectional targets and mapping the network of potential target interactions based on the Degree value. Finally, we used MCC methods in the "cytoHubba" plugin of Cytoscape 3.7.2 software to identify the top 10 target genes as the hub targets.

Immunohistochemical analysis and survival analysis of core targets

We used The Human Protein Atlas database (https://www.proteinatlas.org/) to obtain the images of hub targets, then we used the GEPIA database (https://gepia.cancer-pku.cn/) to compare the expression of hub target genes in the prostate tissues of PCa patients and normal people, and also to explore the impact of core genes on survival prognosis of PCa patients.

GO and KEGG pathways enrichment analysis

We used R Studio software to perform functional enrichment analysis of the intersectional targets, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Then we used the "clusterProfiler" and "ggplot2" packages in R studio software to visualize the enriched pathways. The statistical significance threshold of enrichment analysis was $P<0.05$. The top 20 enriched terms were shown on a chord plot.

Construction of the component-target-signaling pathway network

We used Cytoscape 3.7.2 software to construct the "component-target-signaling pathway" network to clarify the relationship between bioactive compounds in TW, potential targets and main signaling pathways. Then, we analyzed the network with "Analyze network" plugin in Cytoscape 3.7.2 software to explore the main components and targets as well as demonstrate the relationship between the active components, targets, and major signaling pathways. Finally, the

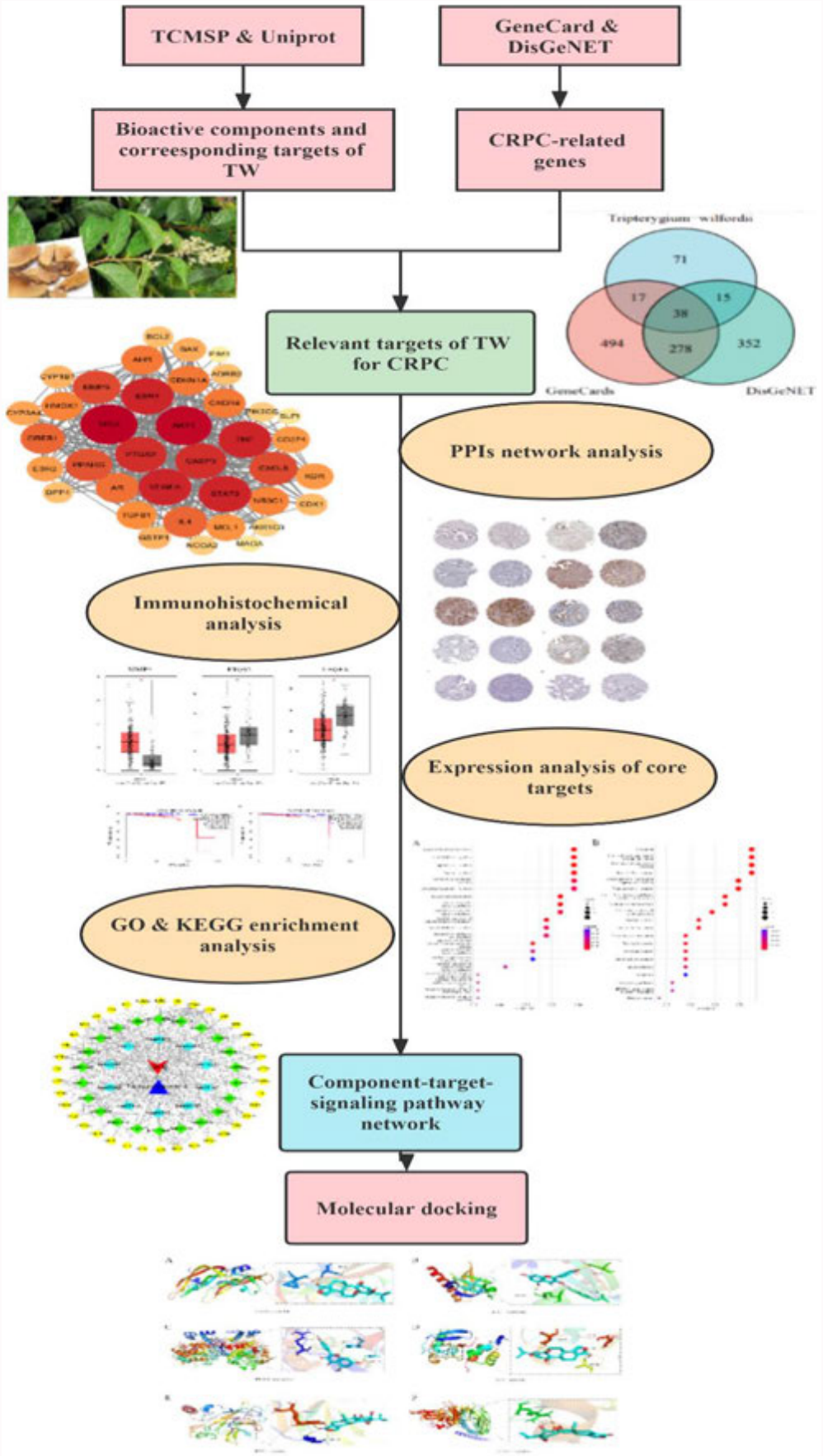

Figure 1: The flowchart of this study.

network was visualized using Cytoscape 3.7.2 software.

Molecular docking simulation

We selected the main active components and hub targets in the "component-target-signaling pathway" network for molecular docking. We used PubChem (https://pubchem.ncbi.nlm.nih.gov/) to download the 3D structures of the main active components. The structures of the hub target protein were obtained from the PDB database (http://www.rcsb.org/). The target proteins were de-watered, hydrogenated, and charge calculated by using AutoDockTools 1.5.6 software, then run AutoDock Vina using "autogrid" and "autodock" plugins for molecular docking, and the Lamarckian Genetic Algorithm (LGA) was used in the docking process. Binding Energy (BE) (Kj/mol) was used to assess the binding activity between small molecules and proteins. Generally, BE ≤ -5.0 Kcal/mol is considered to have good binding properties. Finally, PyMOL 2.4.0 software was used to visualize the results.

Results

Identification of bioactive components and corresponding targets of TW

We obtained 144 components of TW through the TCMSP database. After filtering by preset criteria (OB ≥ 30% and DL ≥ 0.18), a total of 51 active components were retrieved, the detailed information regarding these compounds is shown in Table S1. Then, we also predicted 141 potential targets which were identified for 51 bioactive components in TW through the TCMSP database. Afterward, potential targets were extracted and converted to gene names using Universal Protein Resource.

Acquisition of intersectional targets of TW and CRPC

We obtained 683 relevant targets of CRPC from the DisGeNET database and 827 from the GeneCards database. Then we used Venny 2.1.0 to obtain the intersection between the corresponding target of the bioactive components in TW and the targets of CRPC. Finally, we obtained 38 targets for the treatment of CRPC with TW (Figure 2).

PPIs network analysis of overlapping targets

To further investigate the relationship between the 38 overlapping genes, we constructed the PPIs network by using the STRING database. Then the network was analyzed and visualized by Cytoscape 3.7.2, showing a total of 38 nodes and 322 edges. The result was shown in Figure 3. After that, we used "cytoHubba" plugin to screen the top 10 hub targets, which were TP53, AKT1, TNF, VEGFA, STAT3, PTGS2, CXCL8, CASP3, MMP9 and ESR1, indicating the importance of these genes to the mechanism of TW action to CRPC.

Immunohistochemical analysis and expression analysis of core targets

The Human Protein Atlas database showed the expressions of the hub targets in prostate tissues of PCa patients and normal people were different (Figure 4). Through the analysis using the GEPIA database, by setting the standard of |Log2FC|=1 and p-value <0.01, the results showed that MMP9 expressed higher than normal prostate tissues (P<0.01), PTGS2 and VEGFA expressed lower than normal prostate tissues (P<0.01) (Figure 5). By setting the standard that: 1) group cutoff =50%, 2) p-value <0.05, the results of the Kaplan–Meier survival curves showed that the high-risk group had a lower overall survival rate than the low-risk group in the TP53 and VEGFA gene groups (Figure 6).

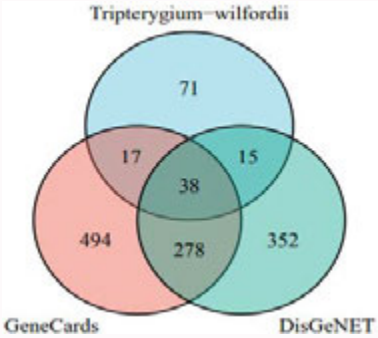

Figure 2: Venn diagram. 38 overlapping targets were obtained from three databases (TCMSP, GeneCards, and DisGeNET). These targets were identified as the targets of TW in the treatment of CRPC.

Table 1: The binding energy of compounds and core targets.

Docking molecular combination	Binding Energy (Kcal/mol)
triptolide-VEGFA	-8.4
triptolide-TP53	-7.4
triptolide-STAT3	-7.9
triptolide-TNF	-8.5
kaempferol-AKT1	-6
kaempferol-PTGS2	-9

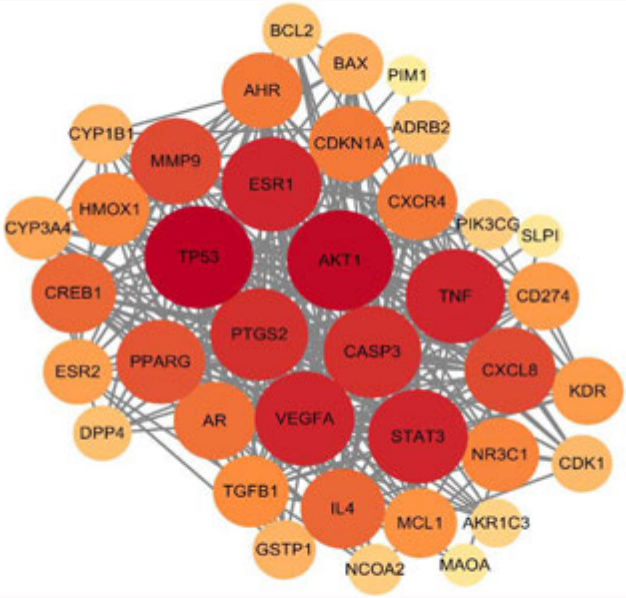

Figure 3: The PPI network of target proteins of Tripterygium wilfordii in the treatment of CRPC. The node size from large to small and node color from deep to light represents the descending order of the degree value.

GO and KEGG pathways enrichment analysis

We performed the GO enrichment analysis of 38 overlapping targets using the package "clusterProfiler" in the R. The GO enrichment analysis showed that the biological processes were mainly enriched in the pathways of cell migration, apoptosis, oxidative stress response, proliferation of immune cells, regulation of cell cycle, and autophagy regulation. The results of the KEGG pathway enrichment analysis showed that the treatment of CRPC with TW was mainly associated with AGE-RAGE signaling pathway in diabetic complications, chemotactic-receptor activation, proteoglycans in cancer, microRNA

Table S1: 51 bioactive components in *Tripterygium wilfordii*.

MOL ID	Molecule Name	OB (%)	DL
MOL000296	hederagenin	36.91	0.75
MOL003182	(+)-Medioresinol di-O-beta-D-glucopyranoside_qt	60.69	0.62
MOL003184	81827-74-9	45.42	0.53
MOL003185	(1R,4aR,10aS)-5-hydroxy-1-(hydroxymethyl)-7-isopropyl-8-methoxy-1,4a-dimethyl-4,9,10,10a-tetrahydro-3H-phenanthren-2-one	48.84	0.38
MOL003187	triptolide	51.29	0.68
MOL003188	Tripchlorolide	78.72	0.72
MOL003189	WILFORLIDE A	35.66	0.72
MOL003192	Triptonide	67.66	0.7
MOL003196	Tryptophenolide	48.5	0.44
MOL003198	5 alpha-Benzoyl-4 alpha-hydroxy-1 beta,8 alpha-dinicotinoyl-dihydro-agarofuran	35.26	0.72
MOL003199	5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin	61.85	0.54
MOL003206	Canin	77.41	0.33
MOL003208	Celafurine	72.94	0.44
MOL003209	Celallocinnine	83.47	0.59
MOL003210	Celapanine	30.18	0.82
MOL003211	Celaxanthin	47.37	0.58
MOL003217	Isoxanthohumol	56.81	0.39
MOL003222	Salazinic acid	36.34	0.76
MOL003224	Tripdiotolnide	56.4	0.67
MOL003225	Hypodiolide A	76.13	0.49
MOL003229	Triptinin B	34.73	0.32
MOL003231	Triptoditerpenic acid B	40.02	0.36
MOL003232	Triptofordin B1	39.55	0.84
MOL003233	Triptofordin B2	107.71	0.76
MOL003234	Triptofordin C2	30.16	0.76
MOL003235	Triptofordin D1	32	0.75
MOL003236	Triptofordin D2	30.38	0.69
MOL003238	Triptofordin F1	33.91	0.6
MOL003239	Triptofordin F2	33.62	0.67
MOL003241	Triptofordin F4	31.37	0.67
MOL003242	Triptofordinine A2	30.78	0.47
MOL003244	Triptonide	68.45	0.68
MOL003245	Triptonoditerpenic acid	42.56	0.39
MOL003248	Triptonoterpene	48.57	0.28
MOL003266	21-Hydroxy-30-norhopan-22-one	34.11	0.77
MOL003267	Wilformine	46.32	0.2
MOL003278	salaspermic acid	32.19	0.63
MOL003279	99694-86-7	75.23	0.66
MOL003280	TRIPTONOLIDE	49.51	0.49
MOL000358	beta-sitosterol	36.91	0.75
MOL000211	Mairin	55.38	0.78
MOL000422	kaempferol	41.88	0.24
MOL000449	Stigmasterol	43.83	0.76
MOL002058	40957-99-1	57.2	0.62
MOL003283	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	66.51	0.39
MOL004443	Zhebeiresinol	58.72	0.19
MOL005828	nobiletin	61.67	0.52
MOL007415	[(2S)-2-[[(2S)-2-(benzoylamino)-3- phenylpropanoyl] amino]-3-phenylpropyl] acetate	58.02	0.52
MOL007535	(5S,8S,9S,10R,13R,14S,17R)-17-[(1R,4R)-4-ethyl-1,5-dimethylhexyl]-10,13-dimethyl-2,4,5,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,6-dione	33.12	0.79
MOL009386	3,3'-bis-(3,4-dihydro-4-hydroxy-6-methoxy)-2H-1-benzopyran	52.11	0.54
MOL011169	Peroxyergosterol	44.39	0.82

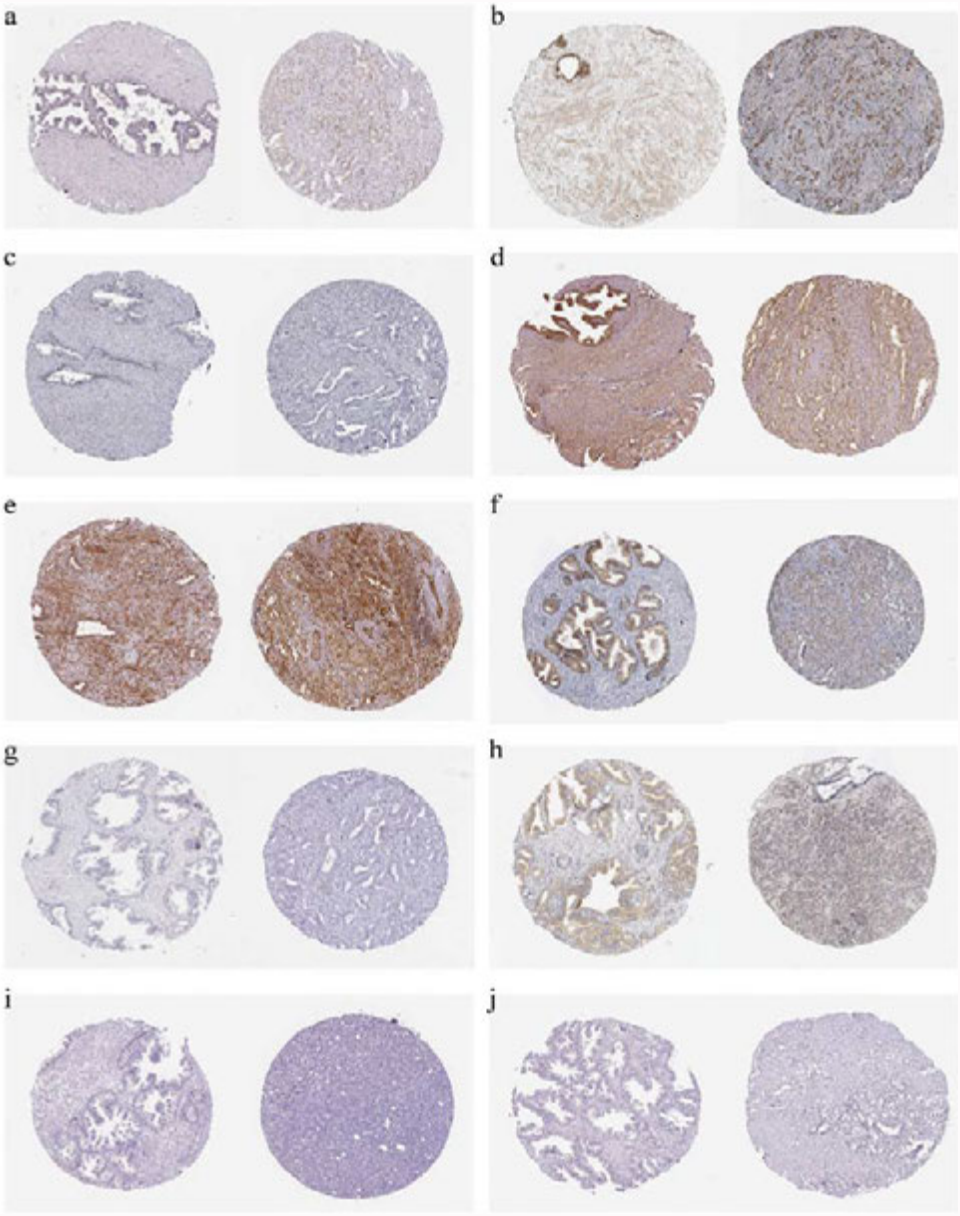

Figure 4: Immunohistochemistry. The expression of 10 key targets in prostate cancer tissues (right side picture) and normal people tissues (left side picture) (a) TP53 (220 um Antibody CAB039238). (b) AKT1 (220 um Antibody CAB003765). (c) TNF (220 um Antibody CAB048769). (d) VEGFA (220 um Antibody CAB069907). (e) STAT3 (220 um Antibody HPA001761). (f) PTGS2 (220 um Antibody CAB000113).

in cancer, endocrine resistance, p53 signaling pathway, estrogen signaling pathway, PI3K-Akt signaling pathway, IL-17 signaling pathway, JAK-STAT signaling pathway, HIF-1 signaling pathway, and TNF signaling pathway. The GO/KEGG enrichment analysis were displayed in Figure 7. The above biological processes were closely related to the development of CRPC, suggesting that TW can treat CRPC through a multi-targets and multi-pathways approach.

Construction of the component-target-signaling pathway network

The relationships between active components, intersectional targets, and major signaling pathways in the mechanism of TW action for CRPC were shown in Figure 8. From the network, we found that active components of TW acted on 38 intersectional targets through multiple signaling pathways for the treatment of CRPC. The active components with network neutrality values ≥ 10 were kaempferol, triptolide, and nobiletin, suggesting these components were the key components of TW for the treatment in CRPC. The targets with network neutrality values ≥ 10 were PTGS2, NCOA2, ADRB2, AKT1, BCL2, TP53, CASP3 and STAT3, suggesting that these targets were the core targets of TW for the treatment of CRPC. The top pathways in the network neutrality values were MicroRNAs in cancer, Chemical carcinogenesis-receptor activation, and Proteoglycans in cancer, suggesting that these pathways were the core pathways of TW for the treatment of CRPC.

Molecular docking results

To further validate potential targets in CRPC, the molecular docking study was performed on the main components (kaempferol and triptolide) with the core target proteins (TP53, AKT1, PTGS2, TNF, VEGFA, and STAT3). The Binding Energy (BE) were predicted by docking analysis successfully, they were all negative and less than

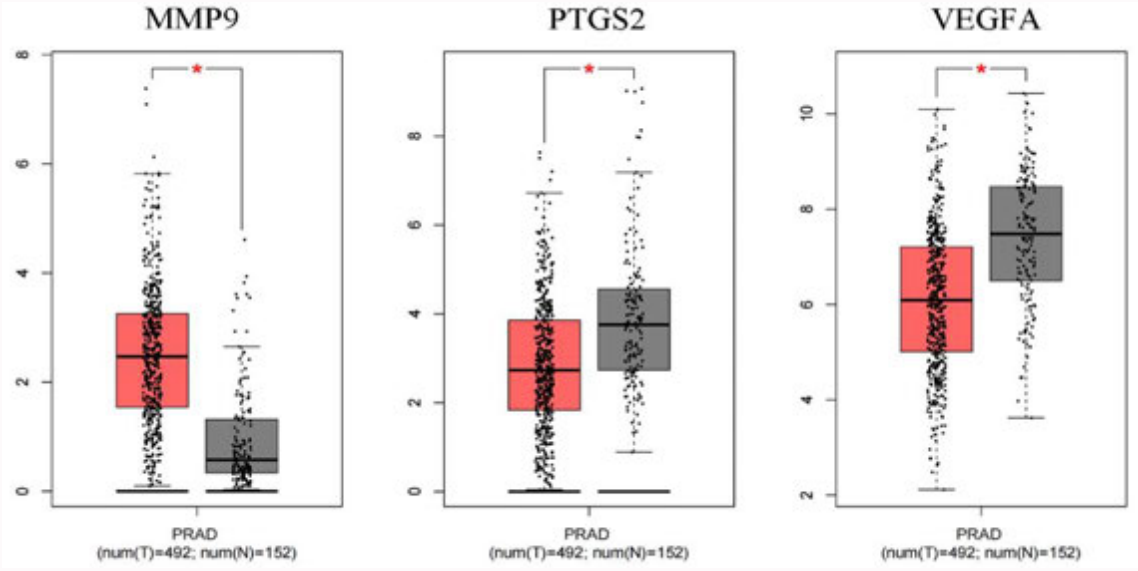

Figure 5: The related gene expression of three targets in prostate cancer (red, n=492) and normal people (grey, n=152).

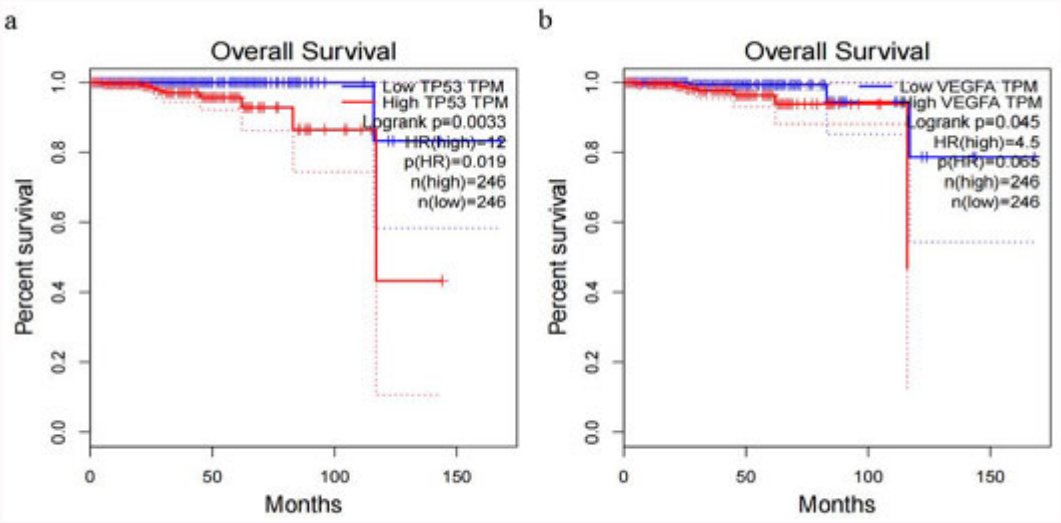

Figure 6: The Kaplan–Meier survival curves of two genes target. (a) TP53, (b) VEGFA.

-5, between the main components and target proteins. The lowest binding energy between molecules and gene proteins was shown in Table 1, and the smaller the binding energy between molecules, the better their docking properties were indicated. The results showed that kaempferol and PTGS2 had the best binding properties (Figure 9).

Discussion

Prostate cancer is one of the most common malignancies of the male urological system [5]. Perhaps 30% of initially diagnosed prostate cancer patients are metastatic Hormone-Sensitive Prostate Cancer (mHSPC), and most patients with early prostate cancer cases can still develop Castration-Resistant Prostate Cancer (CRPC) after Androgen Deprivation Therapy (ADT). Currently, there are androgen therapy, chemotherapy, radionuclide therapy, PARP inhibitors, and immunotherapy for the treatment of CRPC patients, but the efficacy and safety of these therapeutic measures remain unclear and have little impact on delaying disease progression in patients with CRPC. Therefore, we should innovate and explore new therapeutic approaches or combination therapy strategies to improve the prognosis of patients. We also should control the toxic effects of drugs and reduce adverse drug reactions as much as possible to improve the quality of life and prolong the survival time of patients.

Network pharmacology is an emerging discipline that combines bioinformatics, pharmacology, computer science, and other multidisciplinary theories to explore the network relationship between "compound-gene-disease" [16]. In our study, we explored the mechanism of action of TW for the treatment of CRPC, screened and identified the therapeutic targets using network pharmacology, then analyzed the mechanism of action and related signaling pathways using GO and KEGG enrichment analysis, and finally validated the binding energy between main components and key target proteins using molecular docking. Ultimately, we screened out the top 10 key targets, which include TP53, AKT1, TNF, VEGFA, STAT3, PTGS2, CXCL8, CASP3, MMP9, and ESR1.

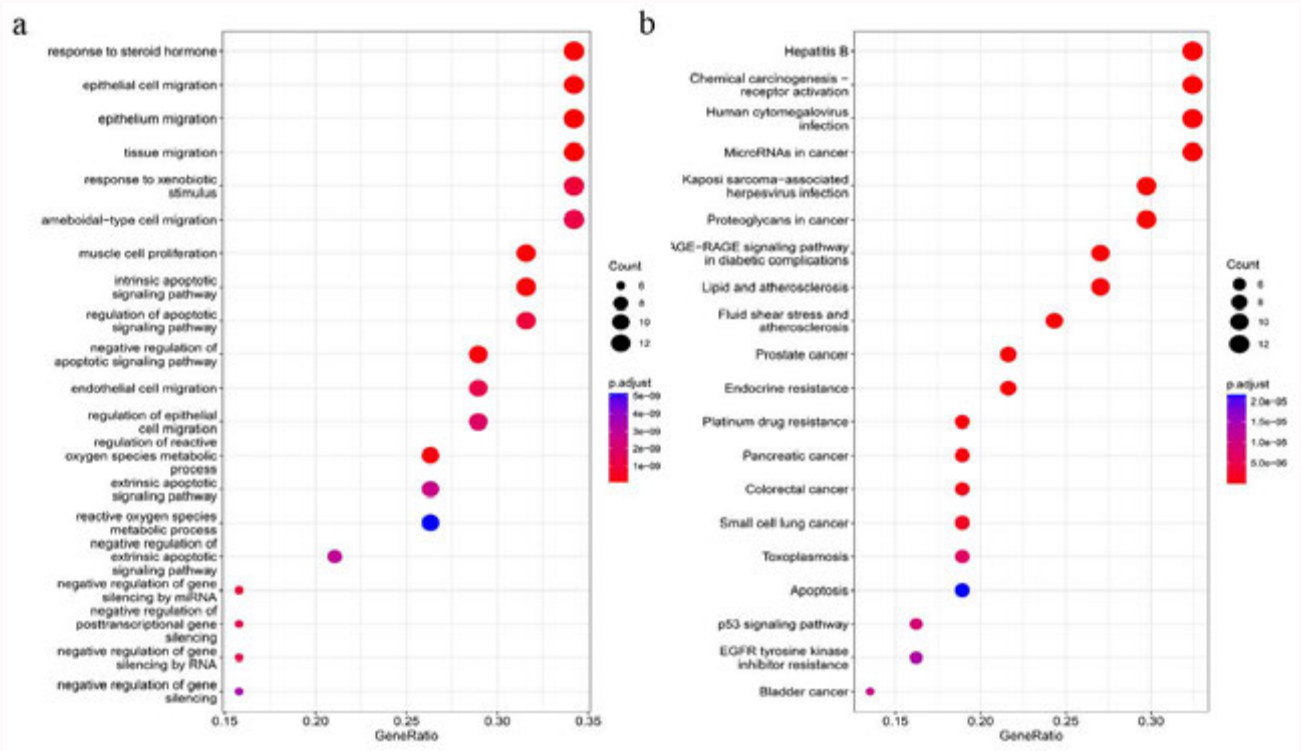

Figure 7: GO and KEGG enrichment analyses. (a) The top 20 items of GO functional enrichment analyses of 38 overlapping targets. (b) The top 20 items of KEGG enrichment analyses of 38 overlapping targets.

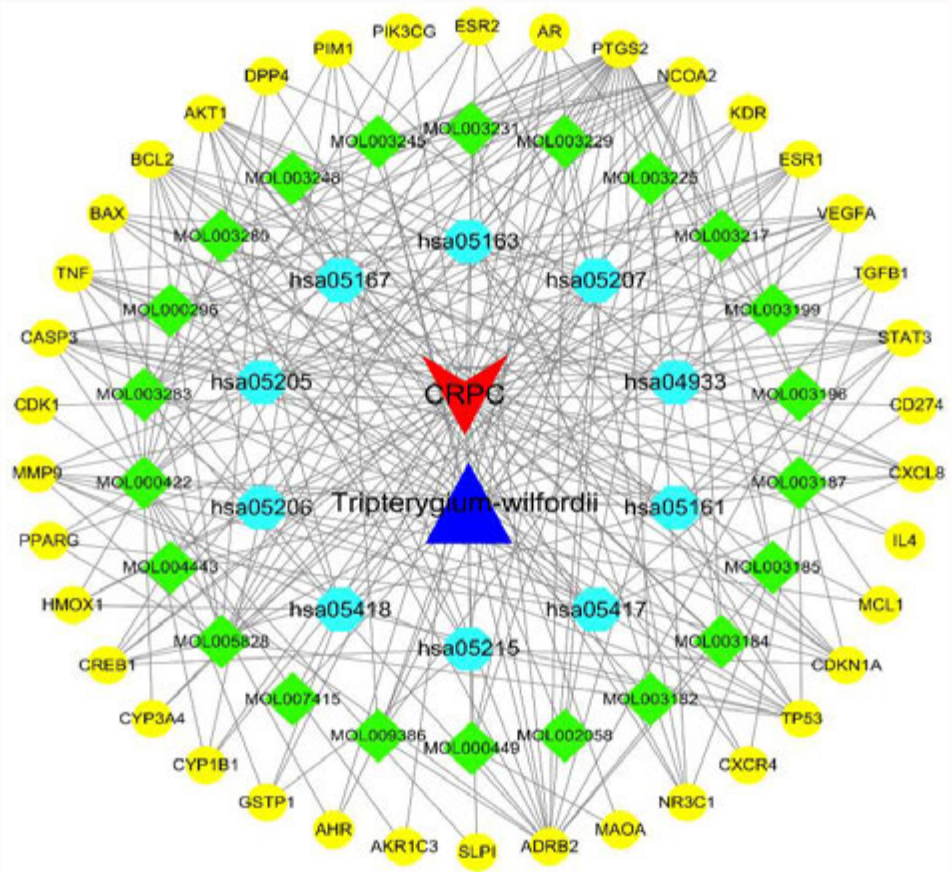

Figure 8: The component-target-signaling pathway network. (Blue circles represent the top 10 KEGG signaling pathways, the green diamond represents the main components in TW, and the yellow circles represent the 38 overlapping genes proteins).

Tumor Protein p53 (TP53) is a key Tumor Suppressor Gene (TSG). Mutations in this gene are associated with a variety of human cancers. TP53 plays a pivotal role in genomic stability, cell cycle arrest, DNA repair, apoptosis, and other important cellular functions and signaling pathways [17]. TP53 mutation, reported in 6.9% of PCa, is one of the most common alternations, affecting 50% of metastatic PCa cases [18]. Many studies have demonstrated that TP53 status has prognostic clinical significance in Castration-Resistant Prostate Cancer (CRPC), and its low expressions may predict a shorter survival time for CRPC [19]. Tumor Necrosis Factor (TNF), as a mediator of the inflammatory process, few studies have examined associations between TNF polymorphisms and prostate cancer risk [20]. But TNF may interact with other inflammatory genes to play a role in prostate cancer development, such as VEGFA, AKT, MiR-130b, etc. [21]. AKT1, which is a proto-oncogene, is frequently activated in the tumor, due to mutation or activation of the P13K, or inactivation of PTEN [22]. The activation of AKT1 can regulate multiple cellular functions, including the proliferation, survival, metabolism, motility, and angiogenesis of malignant cells [23]. Overexpression of AKT1 has been associated with the development of various tumors, including prostate cancer, gastric cancer, and ovarian cancer. Genetic variants and dysregulation of gene expression of PI3K pathway components were detected in prostate cancer and 100% of metastatic prostate cancer patients. It was also reported that AKT levels may be a predictor of biochemical recurrence in patients undergoing radical prostatectomy [24,25]. Vascular Endothelial Growth Factor A (VEGFA) is a member of the PDGF/VEGF growth factor family, which can induce the proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis. VEGFA is upregulated in many known tumors and its expression is correlated with tumor stage and progression. Related research demonstrated that it is a strong negative association between VEGF and Prostate Specific Antigen (PSA) levels in PC patients [26]. Evidence also demonstrated the critical role of VEGF in tumor angiogenesis, and also suggested that VEGF has many properties of potential therapeutic targets whose inhibition would have specific anticancer effects [27]. The protein encoded by the Signal Transducer and Activator of Transcription 3 (STAT3) is activated through phosphorylation in response to various cytokines and growth factors including IL5, IL6, BMP2, and other gene proteins. In cancer cells, aberrant IL6 expression and intrinsic activation of STAT3 are closely associated with enhanced cancer cell proliferation. Overactivation of STAT3 plays a key role in malignant tumorigenesis, tumor progression, and metastatic dissemination. There were relevant reports indicating that IL-6 and STAT3 have a clear role in the progression of a substantial number of PCa patients [28]. STAT3 and STAT5A may be potential therapeutic targets in CRPC [29]. Prostaglandin-endoperoxide Synthase 2 (PTGS2), which has been shown to be expressed in most solid tumor types, such as colorectal cancer [30], pulmonary tumor [31], and prostate cancer [32], has been suggested as a target for chemoprevention, owing to its role in the development and progression of prostate cancer [33]. Furthermore, by inflammatory signaling through PTGS2 expression and ErbB family receptors/AKT activation, the progression from

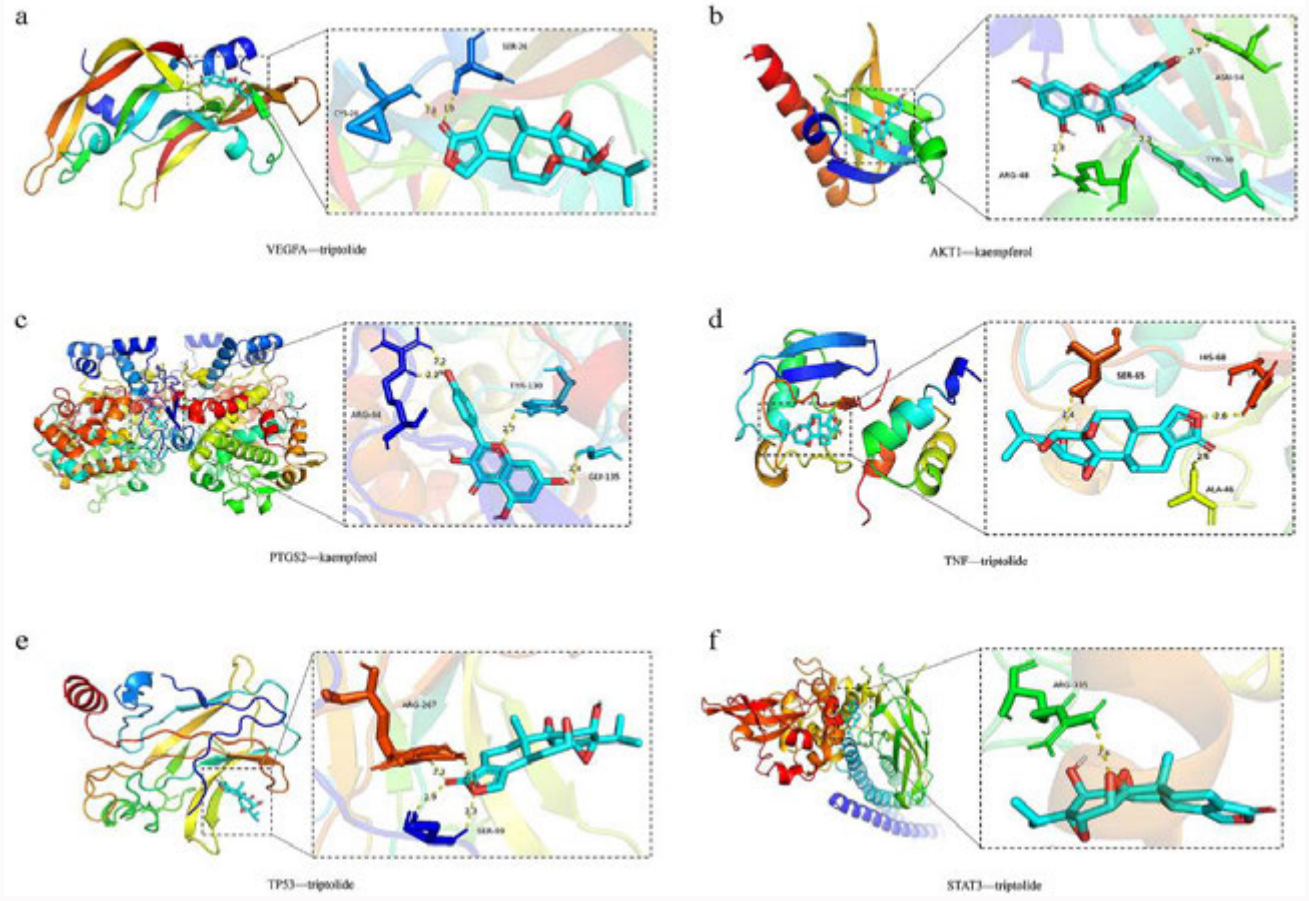

Figure 9: Combined model diagram of core targets molecular docking. (a) VEGFA-triptolide; (b) AKT1- kaempferol; (c) PTGS2- kaempferol; (d) TNF- triptolide; (e) TP53- triptolide; (f) STAT3- triptolide.

androgen-sensitive PCa to CRPC is promoted [34]. C-X-C motif chemokine Ligand 8 (CXCL8), its encoded protein is commonly referred to as Interleukin-8 (IL-8). The increasing of IL8 secretion by PCa cells is associated with malignant biological behaviors of PCa cells. Poor clinicopathological features including high Gleason score and advanced pathological stage of PCa are associated with an increased mRNA expression of IL8 [35]. A combination of serum IL8 levels and f/tPSA ratios may provide a substantial improvement in predicting PCa patients' outcomes [36]. Moreover, elevated IL-8 in men with metastatic prostate cancer starting Androgen-Deprivation Therapy (ADT) is associated with a shorter time to castration resistance and overall survival [37]. Caspase 3 (CASP3) is a major mediator of apoptosis activated during radiotherapy or immunotherapy. It is often used as a marker for the effectiveness of cancer treatments. However, recent studies have suggested that CASP3 may promote tumor growth in a variety of ways, such as through the paracrine signaling pathway and providing a pro-angiogenic microenvironment [38,39]. Another recent study suggested that CASP3 may affect the survival time in patients after ADT for prostate cancer, so the analysis of CASP3 may help identify patients at higher risk of adverse prognosis in PCa patients [40]. Matrix Metallopeptidase 9 (MMP9), a recent study indicated that the expression level in cancerous tissues was significantly higher than the adjacent normal tissues in PCa. Furthermore, higher levels of MMP9 expression are strongly related to the Gleason score and age of PCa patients [41]. In addition, it has been reported that MMP9 protein is closely associated with mCRPC and the downregulation of MMP9 protein levels also can reduce the invasion and metastasis of CRPC cells [42]. It also has been demonstrated that the AKT signaling pathway also can affect the expression of MMP9 protein, leading to CRPC progression [43]. Estrogen Receptor 1 (ESR 1) is implicated in prostate cancer susceptibility by stimulating aberrant prostate growth, controlling prostate cell growth, and programming prostate cell death [44]. Recently, several ESR1 gene polymorphisms have been identified as candidates for prostate cancer susceptibility and among these, ESR1 PvuII (rs2234693 C>T) and XbaI (rs9340799 A>G) polymorphisms were suggested to possess significant associations with the development of prostate cancer. Both PvuII and XbaI can affect ESR1 transcription activity and possibly contribute to the elevated risk of prostate cancer [45]. but the exact effects of ESR1 gene mutations on prostate epithelial cells are still debated despite the fact that estrogen is already used in treating prostate cancer due to its growth-inhibitory effects.

In order to further explore the biological functions of TW for CRPC treatment, we performed GO enrichment analysis and KEGG pathway enrichment analysis on 38 intersectional targets, which showed that these targets are involved in multiple biological pathways to inhibit the proliferation and metastasis of prostate cancer cells, inhibit prostate cancer angiogenesis and promote apoptosis of prostate cancer cells. GO enrichment analysis showed that the biological processes of the overlapping targets were mainly enriched in the pathways of cell migration, apoptosis, oxidative stress response, the proliferation of immune cells, regulation of cell cycle, and autophagy regulation. The results of the KEGG pathway enrichment analysis showed that the treatment of CRPC with TW was mainly associated with AGE-RAGE signaling pathway in diabetic complications, chemotactic-receptor activation, proteoglycans in cancer, microRNA in cancer, endocrine resistance, p53 signaling pathway, estrogen signaling pathway, PI3K-Akt signaling pathway, IL-17 signaling pathway, JAK-STAT signaling pathway, HIF-1 signaling pathway, and TNF signaling pathway. The PI3K/AKT pathway is the most commonly aberrantly activated signaling pathway in tumor cells, and aberrant activation of the PI3K-Akt pathway is associated with prostate cancer denervation resistance, disease progression, and poor prognosis [18,46]. It has been reported that activation of the PI3K/AKT signaling pathway by AGE binding to RAGE may promote prostate cancer cell proliferation by regulating retinoblastoma protein. In addition, it has also been shown that PI3K/AKT pathway can contribute to prostate cancer development and progression through interacting with other cell signaling pathways, such as AR and the RAS/RAF/MEK signaling pathways. As reported by the study, the amplification of AR signaling, mutations in the ligand-binding domain of AR, and induction of AR splice variants have been shown to promote the development of CRPC [47]. Therefore, the development of PI3K/AKT pathway inhibitors in CRPC is extremely important.

In summary, this study initially investigated the key chemical components, action targets, and signaling pathways of TW for the treatment of CRPC, so as to exploit the effective therapeutic effects of TW. The related key targets can also be used as potential molecular markers for the diagnosis and treatment of CRPC. The molecular docking technique also verified the good binding between the key components and the key targets. This study laid a certain theoretical foundation for the treatment of CRPC by TW and also provided some ideas for the subsequent experimental design and research.

Conclusion

This present study revealed the potential mechanisms of *Tripterygium wilfordii* in treating castration-resistant prostate cancer through multiple targets and signaling pathways, the molecular docking technology also validated the high affinity between the main bioactive components in *Tripterygium wilfordii* and the core targets in CRPC. Furthermore, the present study provided a reference for the wide application of TW in clinically managing CRPC.

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