Network pharmacology and molecular docking analysis reveal insights into the molecular mechanism of Gualou Qumai Wan in clear cell renal cell carcinoma

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Abstract

Background: To initially clarify the potential therapeutic targets and pharmacological mechanism regarding Gualou Qumai Wan (GQW), a kind of traditional Chinese medicine (TCM), in clear cell renal cell carcinoma (ccRCC) by virtue of the network pharmacology analysis and molecular docking analysis. Methods: The screening of bioactive components and targets of GQW was based on the Traditional Chinese Medicine System Pharmacology (TCMSP) and the UniProt platform served for standardizing their targets. Online Mendelian Inheritance in Man (OMIM), PharmGkb, TTD, DrugBank and GeneCards databases were searched to collect the disease targets of ccRCC. Cytoscape assisted in constructing herb-compound-target (H-C-T) networks. The STRING database was searched for constructing the target protein-protein interaction (PPI) networks, while the R programming language served for analyzing GO functional terms and the KEGG pathways related to potential targets. Analyses of core genes related to survival and tumor microenvironment (TME) were conducted respectively based on the GEPIA database and TIMER 2.0 database. Human Protein Atlas (HPA) and The Cancer Genome Atlas (TCGA) helped to obtain core genes’ protein expression as well as transcriptome expression level. Autodock Vina software validated the molecular docking regarding GQW components and pivotal targets. Results: The constructed H-C-T networks mainly had 33 compounds and 65 targets. A topological analysis of the PPI network identified that ESRI, AKTI, HIF1A, PTFG2, TP53 and VEGFA serve as core targets in the way GQW affects ccRCC. According to the GO and KEGG pathway enrichment analyses, the effects of GQW are mediated by genes related to hypoxia and oxidative stress as well as the Chemical carcinogenesis-receptor activation and PI3K-Akt signaling pathways. AKTI shows a close relation to the recruitment of various immune cells and can remarkably affect disease prognosis according to reports. Molecular docking and molecular dynamics simulations showed that diosgenin has higher affinity with core targets. Conclusion: The study makes a comprehensive explanation of the biological activity, potential targets, as well as molecular mechanism regarding GQW against ccRCC, which promisingly assists in revealing the action mechanism of TCM formulae in disease treatment and the respective and scientific basis.

Keywords: Gualou Qumai Wan; AKTI; PI3K-Akt signaling pathway; network pharmacology; diosgenin; clear cell renal cell carcinoma
Introduction

Renal cell carcinoma (RCC) is a malignancy common in urinary system, which ranks as the tenth most frequent tumor in the world [1]. RCC can be classified into renal papillary cell carcinoma, clear cell renal cell carcinoma (ccRCC), as well as chromophobe renal cell carcinoma [2-4]. ccRCC is the most common sub-form of RCC that takes up over 80% of renal cancers [5]. Surgical excision is commonly regarded as the primary therapeutic option for localized malignancies. However, more than 40% of patients have disease recurrence, predominantly during the initial 5 years following surgery [6]. Presently, the preferred therapy specific to advanced ccRCC is to use tyrosine kinase inhibitors targeting the vascular endothelial growth factor receptor [7]. Despite the certain inhibitory effect of anti-angiogenic drugs on tumor proliferation and their obvious positive impact on low risk ccRCC patients’ survival, their application can cause undesirable side-effects. In addition, such treatment exhibits an objective effectiveness rate < 30% and a median survival time < 12 months [8-10]. Furthermore, even patients effectively treated in the initial stage will undergo disease progression and show limited response to following treatment [11]. Hence, new treatment strategies and potential targets shall be developed urgently for treating ccRCC patients.

Gualou Qumai Wan (GQW) is a representative TCM herbal formula reported in Jingyiaovale (Synopsis of the Golden Chamber), and is mainly used for treating syndrome of kidny-yang deficiency. Kidney-yang-deficiency-syndrome is a neuroendocrine disease caused by the dysfunction of the adrenal-pituitary-target gland axis. Kidney-yang is considered as the root of yang-qi, and can warm the viscera, thereby being the impetus of human body’s functional activities. The concept of Yang Qi in Traditional Chinese Medicine (TCM) has many similarities with mitochondria in modern medicine. Kidney-yang weakness frequently occurs in overfatigue, chronic illness, aging, and deficient Yang constitution, etc. In the case that deficient Yang is incapable of warming the body, endogenous cold syndrome will occur, featuring pallor, cold limbs, and lassitude. The humpus is residence of the kidneys, and the waist-knee weakness and soreness and aversion to cold in the back and waist can be induced by kidney-yang insufficiency. The kidney controls the water metabolism and the qi transformative function. Deficient yang-qi will lead to abnormal transformative function of qi, causing urinary difficulty and edema. Wang Sanhu et al applied it to clear cell renal cell carcinoma, but the mechanism of GQW for ccRCC is still unclear. Network pharmacology combines pharmacology and pharmacodynamics, and puts more emphasis on disease-gene-target-drug integration, which well helps to explore the overall treating effect of drugs macroscopically and systematically. Hence, the network pharmacology approach was adopted here for revealing the mechanisms underlying GQW in ccRCC treatment and molecular docking was adopted for the reverse verification.

Materials and methods

Composition of Gualou Qumai Wan
GQW is a decoction of 5 botanicals after water extraction, that includes Trichosanthes koriwii Maxim (Trichosanthis Radix), Smdax glabra Roxb (Portia), Dioscorea oppositifolia L (Dioscoreae Rhizoma), Cyperus rotundus L (Aconiti Lateralis Radix Praeparata), Dianthus chinensis L (Dianthi Herba), and they all possess medicinal values. The plant classification referred to the MPNS database (http://mpns.kew.org) and drug names referred to the 2020 edition of the Chinese Pharmacopoeia.

Database establishment
We identified the active GQW components in the TCMSP database and analytical platform (http://tcmspw.com/tcmsp). We then screened out more active compounds of which the OB and DL were higher, (thresholds: OB ≥ 30%; DL ≥ 0.18). The protein database UniProt (http://www.uniprot.org/uploadlists/) converted the obtained target proteins targeting each component in the TCMSP database to uniform gene names.

Putative target gene (PTG) identification for clear cell renal cell carcinoma
The DrugBank (https://www.drugbank.ca/), GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), PharmGkb (https://www.pharmgkb.org/), and TTD (http://db.idrblab.net/td/) databases were searched using the keyword “clear cell renal cell carcinoma” and the species “Homo sapiens.” The retrieved gene data were merged, and duplicate values were filtered for obtaining target data related to disease.

GQW and ccRCC target screening and H-C-T network construction
Active ingredient targets were mapped to disease targets by virtue of the R software “Venn” function package, thereby creating a Venn diagram. In the diagram, overlapping targets stood for core GQW targets for ccRCC therapy. Cytoscape (version 3.8.0) served for constructing a H-C-T network.

PPI network construction
We imported the target data shared by drugs and diseases into the STRING 12.0 database (https://string-db.org/) for constructing the PPI networks. We set interaction score ≥ 0.4 as the screening threshold and removed free proteins, which ensured the analysis robustness. Cytoscape assisted in the visualization of the network. CytoNCA calculated the topological parameters for identifying core proteins and central nodes in the network.

GO enrichment and KEGG pathway analysis
The GO enrichment and KEGG pathway analyses for core biological processes and essential signaling pathways were performed by the R software (version 4.2.2). The software plotted the first 10 items of GO analysis and 30 items of KEGG analysis as bubble plots. At last, key signaling pathways associated with ccRCC were generated using R software.

Core gene prognostic values and tumor-infiltrating immune cell analysis
The GEPIA2 database (http://geopia2.cancer-pku.cn/) also served for validating the gene expression information of the screened ccRCC-associated genes for KIRC in box plots and survival analysis. Also, each protein expression of the screened ccRCC-related genes with the same antibody were checked in KIRC tissues and normal kidney tissues by HPA portal (https://www.proteinatlas.org/). We adopted the TIMER (http://timer.comp-genomics.org/) for analyzing the core targets of GQW components, thereby exploring their relation to immune cell infiltration and to disease prognosis.

Verification of ingredient-target interaction via molecular docking
The 2D molecular structures possessed by the potential active ingredients in GQW could be observed in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) while the 3D structure shown by the core target proteins could be observed in the Protein Database Bank (https://www.rcsb.org/). After being imported into AutoDockTools, they were pre-treated in different ways, like being hydrogenated, dehydrated, etc. The receptor and ligand underwent molecular docking, together with the binding activity analysis. The lowest binding energy stood for the optimal docking conformation, and the energy < -5 kJ/mol exhibited spontaneous binding. Heatmaps were plotted to visualize these values in R. The PyMOL software served for visualizing the docking results.

Results

The primary active ingredients of GQW and PTGs specific to ccRCC

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GQW ingredients underwent target prediction by the TCMSp database and 64 compounds of which the OB and DL were high enough were identified by the screening standards OB ≥ 30% and DL ≥ 0.18. They had 11 compounds from Trichosanthes kirilowii Maxim, 15 compounds from Smilax glabra Roxb, 16 compounds from Dioscorea oppositifolia L, 21 compounds from Cyperus rotundus L, and 1 compound from Dianthus chinensis L.

The UniProt database served for searching potential targets information for active ingredients confirmed in the TCMSp database. After standardizing target gene names and removing duplicates, we obtained 173 target genes.

The GeneCards database was searched for to identify the 16599 ccRCC-related genes, and the OMIM database, PharmGkb database, TTD database, and DrugBank database were searched for to identify 46, 374, 12, and 43 ccRCC-related genes, respectively. After combining above 5 sets of genes as well as removing the duplicates, we obtained 16734 ccRCC-related genes to be further analyzed (Figure 1A).

**GQW and ccRCC target gene screening**

For confirming the underlying gene targets of GQW that impacted ccRCC, we compared the group of 16734 early selected ccRCC-related genes with the group of 173 target genes of the GQW components, confirming 65 common potential target genes (Figure 1B).

Cytoscape software served for visualizing the H-C-T relationships as well as ranking the GQW components in descending order by degree. The top 6 core compounds of GQW against ccRCC were stigmasterol, kadsurenone, hancinone C, diosgenin, hederagenin and AIDS180907. A "H-C-T" network has 91 nodes and 171 edges, that includes 33 active ingredients and 65 targets (Figure 1C).

![Figure 1](https://example.com/figure1.png)

Figure 1 Construction of the disease target genes set. (A) Venn diagram of disease targets and drug targets. (B) and herb-compound-target network. (C) Orange represents dioscorea oppositifolia L, blue represents aconitii lateralis radix praepatata, red represents smilax glabra roxb, and green represents trichosanthes kirilowii maxim.
Construction of PPI network and screening of core targets

A String database was adopted for constructing a PPI network that had 616 edges and 62 nodes, thereby obtaining key proteins of GQ in ccRCC treatment (Figure 2A). Later, we set the thresholds of BC, CC, DC, EC, LAC, and NC in two steps as per the topological parameters in CytoNCA, and filtered out 6 key proteins, namely ESR1, AKT1, HIF1A, PTGS2, TPS3 and VEGFA, which were strongly linked to ccRCC (Figure 2B).

GO and genome encyclopedia enrichment analysis

In GO enrichment analysis, BP primarily contained pathways associated with response to xenobiotic stimulus, to decreased oxygen levels, and to hypoxia and others. CCs were related to synaptic membrane and postsynaptic membrane. MFs were related to G protein-coupled amine receptor activity, and nuclear receptor activity, etc. (Figure 3A). KEGG analysis identified pathways were Neuroactive ligand-receptor interaction, the PI3K-Akt, chemical carcinogenesis-receptor activation, and Calcium signaling pathway (Figure 3B). Thereinto, PI3K-Akt signaling pathway remarkably impacted the effects of ccRCC (Figure 3C).

Figure 2 Network diagram of the PPI network and core clusters. (A) PPI network. (B) It's key target network.

Figure 3 Enrichment analysis. (A) GO enrichment analysis. (B) KEGG enrichment analysis. (C) The PI3K-AKT signaling pathway.
Prognostic values and immune infiltration
The study focused on analyzing the differential expressions of proteins in cancer as well as para-carcinoma tissues, for validating whether these identified proteins can be therapeutic targets. According to the IHC results in the HPA database, only AKT1 showed higher expression in cancer tissue compared to normal renal tissues (Figure 4A–4B). The TCGA database expanded the validation sample size and assisted in obtaining the gene expression data at the transcriptome level. According to the box plots of the differential expression analysis (Figure 4C), normal tissues exhibited obviously overexpressed AKT1, relative to cancer tissues. Hence, our study confirmed AKT1 as a representative gene and paid attention to ccRCC for exploring their relationship. A significant correlation (P ≤ 0.05) existed between the AKT1 expression and kidney clear cell carcinoma (KIRC) patients’ survival (Figure 4D).

For more deeply exploring possible immune mechanisms, our study adopted the TIMER database to investigate the association among gene expression, tumor tissue purity, and immune infiltrating cell abundance. Tumor tissue purity refers to the ratio of cancer cells to the entire tumor sample and can help to effectively select genes associated with deconvolving immune cells. As shown in Figure 5E, AKT1 expression level failed to show an obvious relation to the tumor tissue purity, while could regulate the TME to impact the disease prognosis. AKT1 expression presented a positive relation to the infiltration of CD4+ T cells, B cells, macrophages and neutrophils (Figure 4E–4H).

Molecular docking analysis
For evaluating the affinity possessed by candidate biologically active compounds specific to associated targets, we conducted molecular docking analysis to simulate the way small ligand molecules interact with receptor protein macromolecules. Autodock Vina v.1.5.6. assisted in obtaining the binding patterns and interaction between 6 ingredients and 6 proteins. Besides, we calculated the binding energy between the two counterparts specific to each interaction, for predicting their affinity. Docking results demonstrated the 3D interactions between the abovementioned two types of molecules in PyMOL software. The heatmap showed the lowest docking binding energy of the PTGS2 and AIDS180907 (~ 9.7 kcal/mol), revealing their higher stability relative to other combinations (Figure 5). Figure 6 gives the detailed local structures of molecular docking. The key active ingredients of GQW achieves a good binding with key target genes, namely ESR1 (diosgenin) (Figure 6A), AKT1 (diosgenin) (Figure 6B), HIF1A (diosgenin) (Figure 6C), PTGS2 (AIDS180907) (Figure 6D), TP53 (diosgenin) (Figure 6E), and VEGFA (diosgenin) (Figure 6F). In other words, a potent binding activity could have a significant effect on the treatment of ccRCC. The docking results indicate that GQW contributes to the evident anti-inflammatory and anti-tumor effects.

Figure 4 Prognostic value and immune infiltration of hub genes. (A–B) Validation of the expression of AKT1 at the protein level using the HPA database. (C) Validation of the expression of AKT1 at the transcriptome level using the TCGA database. (D) Association of AKT1 with the prognosis of differential expression. (E–H) Correlation of AKT1 expression with immune cells.

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Discussion

According to the experience of physicians, such as Wang Sanhu and Wu Xiongzhi, GQW is a common formula for warming yang to excrete water and is widely used in the treatment of ccRCC. With a spicy flavour and hot nature, Aconiti Lateralis Radix Praepatata is the major herb for warming the kidney-yang to achieve the activation of the transformative function of qi [12]. Dioscoreae Rhizoma and Trichosanthis Radix are used to nourish lung yin, while these two herbs can strengthen the lung's purging and descending "from top to bottom" to give strength to the kidneys, which can "consolidate" the kidney qi to ensure that the tumor does not spread, and at the same time can fill up the essence of yang qi in the Human Body [13–15]. The inclusion of Poria in the formula is effective in eliminating excess moisture and

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clearing turbidity, hence alleviating the strain on the kidneys [16]. The core difference of this formula lies in the use of Dianthis Herba, bitter flavor and cold property, which has the functions of clearing away heat, resolving the turbidity, dissipating stasis and resolving swelling, promoting blood circulation and eliminating the toxin, effectively reducing the stagnated heat and turbidity within the tumor and inhibiting the growth of the tumor, and it is the eye-catching medicine of this formula [17].

Cancer comprises various diseases, and cancer cells exhibit uncontrolled proliferation ability and are capable of invading and metastasizing to other body parts [18]. Lesions within the same tumor possibly possess unique genomic changes, biological actions, and local microenvironments, which possibly make various treatment responses. Even tumor cells in different regions of the same lesion possibly carry various somatic mutations [19]. In recent years, with the discovery of artemisinin and the clarification of mechanism of arsenic trioxide which the main active components of realgar-Indigo naturalis formula, TCM has received increasingly attention [20]. TCM could ameliorate the progression of cancer via multicomponent, multitarget, and multipath ways mode, different from a single drug involving few targets [21]. Herbal formulas are not cobbled together from drugs of the same nature, and there are strict principles for prescribing them. When multiple medicines are used together to ensure that their strengths are fully utilized while limiting their weaknesses, they will show superiority over single medicines in the treatment of diseases. Pharmacodynamic material basis and effective mechanism are the two main issues in deciphering the mechanism of action of herbal medicines in treating diseases [22]. The development and application of network pharmacology has promoted the safety, efficacy, and mechanism research of traditional Chinese medicine, thereby enhancing its credibility and popularity [23].

In our study, network pharmacology, molecular docking and validation by TCGA database and HPA database were used for revealing the biologically active compound as well as molecular mechanisms of GQW for ccRCC treatment. We selected 33 biologically active compounds and 65 protein targets from public databases. A topological analysis of the PPI network identified that ER1, AKT1, HIF1A, PTGS2, TP53 and VEGFA serve as core targets in the action of GQW on ccRCC. According to the KEGG results, the PI3K-AKT signaling pathway, of which the gene count enrichment is the highest relative to other cancer-related signal pathways, critically impacts the way GQW goes against ccRCC. Subsequently, molecular docking and database validation assisted in further verifying the molecular mechanism of diosgenin that is proved as the most significant biologically active compound of GQW in ccRCC treatment. According to molecular docking results, diosgenin could be capable of interacting with AKTI through three bonds, hence, AKTI serves as a pivotal action target impacting the way diosgenin resists ccRCC. On that account, it is hypothesized that GQW impacts the immunomicroenvironment, thereby improving tumor patients’ prognosis and such proposal can be evidenced in the GEPIA2 and TIMER databases [24].

Conclusions

In conclusion, we revealed the multi-component, multi-target and multi-pathway mechanisms of GQW by network pharmacological analysis. The core targets of GQW against ccRCC may be ER1, AKTI, HIF1A, PTGS2, TP53, and VEGFA, and the underlying mechanisms may be related to the regulation of the chemical attenuator receptor activation and the PI3K-Akt signaling pathway and so on.

References


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