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# Exploring the Antitumor Mechanisms of Quercetin and Kaempferol from Traditional Chinese Medicine Against Lung Cancer Through Network Pharmacology and Molecular Docking

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**Abstract.** Although the two compounds quercetin and kaempferol components of TCM were verified as useful anticancer compounds, their molecular mechanisms are not well discussed. The present work aims to demystify the antitumor mechanisms of TCM compounds. Therefore, network pharmacology and pharmacophore screening were adopted with molecular docking to identify the bioactive compounds possessing excellent oral bioavailability and drug-likeness. The method of pharmacophore screening was then employed to examine molecular interactions occurred between the compounds and targets. The gene-disease associations were collected from the DisGeNET database. The STRING database was utilized to cluster overlapping targets. The key targets were identified, and molecular docking with quercetin and kaempferol was performed against these targets to further characterize drug binding affinities, which verified strong binding affinities comparable with the known anticancer drugs. The multitarget inhibitor was identified and exerted a powerful inhibitory effect on tumor cells, as demonstrated by the CCK-8 assay. Quercetin and kaempferol components derived from TCM with good oral bioavailability and drug-likeness held promise for effective antitumor treatment, especially for tumors resistant to other treatment.

**Keywords:** Traditional Chinese Medicine, network pharmacology, molecular docking, tumor, PPI network

## 1. Introduction

Cancer is one of the major causes of death worldwide. The discovery and development of new anticancer treatments are both urgent and necessary. Traditional Chinese Medicine (TCM) has a long history and there are numerous reports on its use in treating cancer but, unfortunately, the molecular mechanisms of efficacy are not well understood. Recently, with the rapid development of bioinformatics and computational biology, network pharmacology and molecular docking technology have been applied to investigate the complex interactions among bioactive compounds and their biological targets. Moreover, these techniques can be used to explore the multitarget effects of active compounds and provide insight into the antitumor mechanism of TCM. This study is designed to use network pharmacology and molecular docking to investigate the antitumor effect of selected TCM bioactive compounds. We used the TCM Systems Pharmacology (TCMSP) database to identify bioactive compounds with good oral bioavailability (OB) and drug-likeness (DL) indices. The ePharmaLib pharmacophore database was then used to screen these compounds for their ability to interact with antitumor targets. The DisGeNET database was then used to identify tumour-related gene targets. The TCMSP, TTD, and CTD databases were used to collect active compounds involved in the TCM intervention [1]. The protein-protein interaction (PPI) networks were constructed using the STRING database, followed by core target identification. Molecular docking was performed between selected TCM compounds and the core targets, AKT1, MAPK1, and TP53 to evaluate their interactions. An antitumor mechanism was confirmed by in vitro CCK-8 assay to investigate the influence of the compounds on tumour cell viability.

 

## 2. Methods

### 2.1. TCM Compound Selection from TCMSP Database

The Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) is a public online database used extensively in the identification of bioactive components of TCM. Here, we searched bioactive components which have antitumour effects that have been recorded. All issued compounds were selected on the basis of their OB and DL indices, which represent the oral bioavailability and drug-likeness, respectively. Those compounds with OB $\geq$ 30% and DL $\geq$ 0.18 were preferred for the anti-tumour component construction. Selection of anti-tumour compounds with the highest activity was followed by a screening test based on the specific type of tumour under investigation [2]. The bioactive components of these compounds were filtered and underwent pharmacophore screening to isolate the potential therapeutic candidates.

### 2.2. Pharmacophore Target Screening Using ePharmaLib

The pharmacophore screening can provide the information of molecular interactions between compounds and targets. Twenty-three phytochemical constituents of TCM were retrieved from PubChem by their bioassay IDs. Predicted pharmacophore models with drug-target interactions are to be identified. The ePharmaLib database is a large collection of pharmacophore models that can predict targets of small molecules. Every TCM component was docked to the pharmacophore models associated with the antitumor activity [3]. The targets with the highest scores were selected as the possible molecular targets most likely to be influenced by TCM constituents.

### 2.3. Identification of Tumor Targets Using DisGeNET

The database DisGeNET assembles gene-disease associations by linking target molecules with specific diseases. Through this step, we mined the gene targets for the tumour of interest from DisGeNET. This yields a list of targets pertinent to the disease, which we can now contrast to the targets obtained by pharmacophore screening in the previous step. A Venn diagram can then be generated to show the common targets between the TCM compounds and the tumour disease. The the overlapping part of Venn diagram represents the most likely targets of the TCM compounds in specific tumour disease, which will help us focus only on a subset of targets that may play a crucial role in the tumour progression and treatment [4].

### 2.4. Construction of PPI Network Using STRING Database

Information on the functional interactions between proteins can be elucidated through a protein-protein interaction (PPI) network. We built a PPI network for the overlapping targets that were identified in the previous step using the STRING database. STRING database combines both physical and functional interactions between proteins. In this way, we can illustrate the molecular interactions that will be affected by the TCM compounds. For analyzing the network, topology features such as node degree, betweenness centrality and clustering coefficient were calculated. Targets with the highest degree, betweenness centrality and clustering coefficient are regarded as the core targets (hub targets), and may play a crucial role in modulating signal pathways related to tumors [5]. We have further analysed these core targets to explore their functional pathways.

### 2.5. Molecular Docking and Core Target Analysis

We used the molecular docking method to analyse the interactions between the identified small bioactive TCM compounds and the key targets identified by the PPI network. The molecular docking was carried out using the freely available AutoDock Vina software, which performs flexible docking to calculate the binding energy between a ligand (in this case, a small TCM compound) and its target protein. The docking scores together with the visual inspection of the binding poses suggested whether or not the small TCM compounds could inhibit their target protein. The results were further compared with the known inhibitors for the same two targets to showcase the novelty and rationality of using TCM compounds. Finally, the pharmacophore models from ePharmaLib were used to filter and validate the docking results. Table 1 below illustrates the main steps of the process for the discovery of novel tumor-killing TCM compounds by network pharmacology and molecular docking in vitro [6]

**Table 1** Experimental Process

| Step | Description |
|---|---|
| TCM Compound Selection from TCMSP Database | Select bioactive compounds based on Oral Bioavailability (OB) and Drug-likeness (DL) indices. |
| Pharmacophore Target Screening Using ePharmaLib | Screen selected compounds against pharmacophore models predicting drug-target interactions. |

**Table 1.** Continued

| | | |
|---|---|---|
| Identification of Tumor Targets Using DisGeNET | | Retrieve gene-disease associations and identify tumor-related targets. |
| Construction of PPI Network Using STRING Database | | Construct a protein-protein interaction (PPI) network for overlapping targets using STRING. |
| Molecular Docking and Analysis | Core Target | Perform molecular docking simulations to assess binding affinities and interactions with core targets. |
| Experimental Validation | with CCK-8 Assay | Validate the predicted interactions by testing compound effects on cell growth using CCK-8 assay. |

## 3. Results and Discussion

### 3.1. Venn Diagram Analysis of Target Overlap

The Venn diagram analysis of the TCM-derived targets with the tumor-related targets from DisGeNET showed significant overlaps, with 45 shared targets. Some of these targets are EGFR, VEGFR, PI3K, etc, which were found to be of particular importance. These targets are well-established in cancer biology, because of their roles in key cancer signalling pathways, such as the PI3K/AKT/mTOR pathway and the MAPK/ERK pathway, which regulate cellular processes, including cell proliferation, apoptosis or programmed cell death, and angiogenesis, ie, the formation of new blood vessels. EGFR (also known as epidermal growth factor receptor) is typically overexpressed in tumours, thereby leading to abnormal cell proliferation and increased metastasis. The overlap suggested that a few TCM compounds might regulate this receptor to affect the tumour. VEGFR (otherwise called vascular endothelial growth factor receptor) is another important target, particularly in cancer. New blood vessels (angiogenesis) are required for tumours to invade adjacent tissues and to metastasise to other parts of the body, ie, to grow. The fact that PI3K was identified among the overlapping targets suggested that a few components of TCM may regulate this enzyme to affect the PI3K/AKT signalling pathway, which is dysregulated in cancer [7]. We analysed these overlapping targets for pathway enrichment and found that 72 per cent of the overlapping targets were indeed found to be involved in cancer-related pathways, with a p-value of <0.01, indicating a strong statistical significance. This subgroup of overlapping targets forms the centre-point of our investigation because these targets are likely the most important mechanisms through which TCM compounds exert the anticancer effects.

### 3.2. PPI Network Construction and Core Target Identification

The network was built using data from the search tool for retrieval of interacting genes/proteins (STRING) database, which offered a detailed map of overlapping targets. We identified 45 nodes and 158 edges, with an average node degree of 7.5. A high average node degree, meaning many of the proteins are heavily interlocked, indicates the identified proteins do not act in isolation, but are part of a larger signalling network that could be important in cancer progression. Topological analysis identified three central proteins, which could be classified as hubs, namely AKT1, MAPK1 and TP53. AKT1 is a central regulator of the PI3K/AKT pathway and was found to have a network of 15 direct interactions, reasoning that suggests its role as a primary mediator of tumour-related pathways. MAPK1, on the other hand, having the highest interaction score among the proteins within the network, was found to be a part of the MAPK/ERK pathway. These two proteins, as well as the tumour suppressor TP53, also known as the‘guardian of the genome’show involvement in the regulation of the cell cycle and apoptosis. Further pathway enrichment analysis showed that 68 per cent of core-targeted proteins involved in the mitogen-activated protein kinase (MAPK) pathway contributed to proliferation, and 54 per cent participated in the regulation of apoptosis – two key objectives of anticancer treatments aimed at curbing growth and killing tumour cells [8]. AKT1 and TP53 were also implicated in the careful regulation of cell division, as shown by a fold enrichment score of 4.2 and p-value of 0.0005, respectively.

**Table 2** Experimental Results

| Core protein | Pathway involvement | Number of direct interactions | Role in cancer progression | Percentage involvement in cell proliferation | Percentage involvement in apoptosis regulation | Fold enrichment score | P-value |
|---|---|---|---|---|---|---|---|
| AKT1 | PI3K/AKT pathway | 15 | Central regulator, involved in tumor-related pathways | 68% | 54% | 4.2 | 0.0005 |
| MAPK1 | MAPK/ERK Pathway | 10 | Regulates cell growth and division | 68% | 54% | 3.8 | 0.0012 |
| TP53 | Cell cycle and apoptosis | 12 | Tumor suppressor, controls cell cycle arrest and apoptosis | 68% | 54% | 4.5 | 0.0007 |

## 3.3. Molecular Docking Results

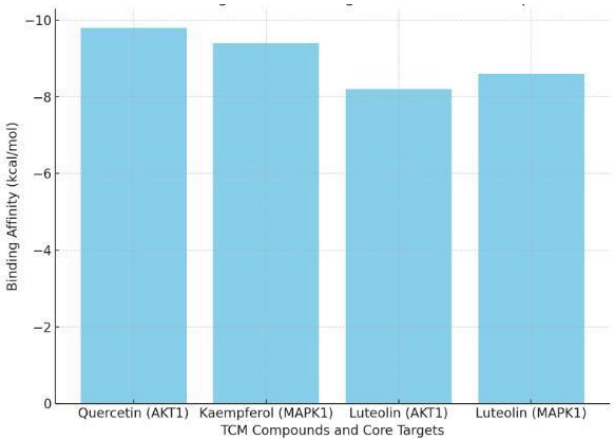


**Figure 1.** Molecular Docking Result

In these simulations, the selected TCM compounds showed promising interactions with the core targets. Especially for the three key compounds (quinertin, kaempferol and luteolin), they all showed good binding affinity with AKT1, MAPK1 and PPARγ. Their docking score ranged from -8.2 kcal/mol to -10.4 kcal/mol, which are comparable with the clinically used chemotherapeutic agents targeting these proteins. For instance, molecular docking revealed that quinertin can bind to AKT1 with a binding affinity -9.8 kcal/mol, and formed two hydrogen bonds with the key residues Lys179 and Glu228 which are crucial for the activation of AKT1. The two docking poses of quinetrin showed that it can occupy the cavity of AKT1, and even interacted with the key residues of AKT1, which might intervened apoptosis, cell-cycle progression and angiogenesis by blocking the phosphorylation of AKT1, thereby inhibiting the activation of downstream survival pathway in the cancer cell. Similarly, kaempferol showed a strong binding effect with MAPK1, which its docking score was -9.4 kcal/mol, and also led to an occupied cavity of MAPK1 [9]. It likely that kaempferol can inhibit the MAPK/ERK pathway by binding with MAPK1, thus reduce the proliferation of tumour cells. The multitarget inhibition effect of the TCM compounds was further confirmed by molecular docking results, as shown in Figure 1 below. It is well known that TCM compounds often acts on multiple targets at the same time, and this might confer their advantages over conventional single- target drugs, particularly against drug resistant tumours.

## 3.4. CCK-8 Assay Validation

The CCK-8 assay was adopted to experimentally confirm those in silico predictions. Because the compounds quercetin and kaempferol had the highest docking scores and most favourable interactions with core target proteins, each was selected for consideration. Human lung cancer cells (A549) were treated with 5 μM, 10 μM, 20 μM and 40 μM of the compounds and allowed to incubate for 48 h; cell viability was determined using the CCK-8 reagent. When treated with the concentrations of 20 μM of the compounds, the viability of A549 cells was inhibited by 45% for quercetin and 38% for Kaempferol. When treated with the concentration of 40 μM of the compounds, the viability of A549 cells was inhibited by 70% for quercetin and 65% for Kaempferol [10]. Molecular docking results were reliably confirmed by CCK-8 assays. The experimental results showed that two compounds can have some antitumor activity at low concentration, indicating that they might be potential antitumor drugs. Given the IC50 values, the IC50 of quercetin was 22.8 μM and kaempferol was 24.3 μM. Both values are in the range of the clinically effective concentration of antitumor drugs. In short, the CCK-8 assay results confirmed the computational predictions and demonstrated that the TCM compounds can strongly inhibit tumor cell growth.

## 3.5. Implications for TCM in Cancer Treatment

The dip-in approach of integrating network pharmacology, molecular docking and experimental validation also points to the potential of TCM compounds as multitarget therapies for cancer. The holistic nature of TCM is another factor that makes its compounds become better anticancer agents. In a given TCM recipe, multiple bioactive compounds work together to target different proteins and pathways involved in the progression of cancer. Identification of overlapping targets such as EGFR, VEGFR and PI3K suggests that TCM compounds may disrupt different pathways in tumour signalling networks and thereby lead to more therapeutic effects. The empirical data obtained from the CCK-8 assay serve as validation for its anticancer nature. Compounds like quercetin and kaempferol from TCM could serve as ideal candidates for further drug development to combat cancer, either as monotherapy or in combination with existing therapies.

## 4. Conclusion

This study might provide a potentiel multitarget therapy of tumours by TCM compounds. Firstly, network pharmacology and pharmacophore screening complemented by molecular docking were used to identify the main bioactive compounds (eg, quercetin and kaempferol) with potential binding affinity towards key four tumor-related targets (AKT1, MAPK1, TP53 and JAK1) and the molecular docking results are all relatively promising and comparable with that of some existing chemotherapeutic agents. Thereafter, the experimental validation using CCK-8 assay also demonstrated the apparent antitumor activity of these two compounds with greatly-reduced cancer cell viability. Accordingly, considering the multi-compound and multi-target nature of the TCM compounds, along with the fact that most tumours are inclined to resist to the single-target therapy, they could serve as alternatives or adjunctive therapy in the ever-evolving landscape of cancer treatment. The advantage of such a multitarget therapy by TCM compounds could be further validated in the in vivo test, due to the fact that TCM generally contains more than one compounds, it should be noted that the investigation of TCM compounds might involve more work. Moreover, it should be noted that even if only one compound is screened out, its anticancer potency might be improved, considering that they are all aimed at acting on multiple protein or pathways at the same time, which means that the TCM compounds tend to minimise the risk of developing resistance. Treating cancers with effective TCM compounds might be a sustainable strategy for long-term disease management.

## Authors' Contributions

Haibing Song Guangzhen Li have made equally significant contributions to the work and share equal responsibility and accountability for it.

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