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Exploring the Mechanisms of Action of Quercetin for the Treatment of Cervical High-Risk-Human Papilloma Virus Using Network Pharmacology and Molecular Docking

Shanyun Wang[1,2] Huisi Hong[3] Yiming Yuan[4] Yinhao Yin[5] Jianfeng Zeng[5] Jing Xiao[6]

[1] Department of Yuelai Clinic, Zhongshan Hospital of Traditional Chinese Medicine, Zhongshan, Guangdong, China
[2] The Second Clinical School, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, China
[3] Department of Science and Education, Zhongshan Hospital of Traditional Chinese Medicine, Zhongshan, Guangdong, China
[4] Department of Clinical Pharmacy, Zhongshan Hospital of Traditional Chinese Medicine, Zhongshan, Guangdong, China
[5] Department of Andrology, Zhongshan Hospital of Traditional Chinese Medicine, Zhongshan, Guangdong, China
[6] Department of Gynecology, the Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, China

Address for correspondence Jianfeng Zeng, Associate Chief Physician, Department of Andrology, Zhongshan Hospital of Traditional Chinese Medicine, No. 3 Kangxin Road, Xiqu Street, Zhongshan, Guangdong, China (e-mail: 317528928@qq.com).

Jing Xiao, PhD, Professor, Department of Gynecology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, 111 Dade Road, Yuexiu District, Guangzhou, Guangdong, China (e-mail: xiaojingson_2004@126.com).

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Abstract

Objective Our objective was to investigate the therapeutic mechanism of quercetin in the management of cervical HR-HPV through the integration of network pharmacology and molecular docking techniques.

Methods The GeneCard database was utilized to analyze and identify potential therapeutic targets in HR-HPV. Subsequently, a protein–protein interaction (PPI) network was constructed by employing the String database. The visualization and construction of PPI networks were accomplished using Cytoscape. The R language was utilized to conduct Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses.

Results A total of 154 active constituents of quercetin were identified through screening. Additionally, 139 target genes associated with the effects of quercetin on cervical HR-HPV were predicted. PPI analyses revealed that threonine kinase Akt1, mitogen-activated protein kinase1, human IL-6 protein, signal transducer and activator of transcription 3, and epidermal growth factor receptor (EGFR) may serve as potential targets for quercetin in the treatment of cervical HR-HPV. Furthermore, GO and KEGG analyses demonstrated that quercetin is involved in various functional pathways, biological processes, molecular categories, including the Th17 signaling pathway, tumor necrotizing factor (TNF) signaling pathway, EGFR signaling pathway, PI3K-Akt signaling pathway, among others.

Conclusion Quercetin exhibits multifaceted characteristics, targeting multiple components, pathways, and targets, in the therapeutic intervention of HR-HPV, primarily by modulating inflammatory responses, oxidation reactions, and apoptotic processes.

Keywords
- HR-HPV
- quercetin
- network pharmacology
- molecular docking

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Introduction

Human papilloma virus (HPV) is a group of DNA viruses that infect humans, with over 200 subtypes identified. It can be categorized as high-risk or low-risk based on its potential to cause cancer. High-risk HPV (HR-HPV) is linked to various cancers.[1–3] Cervical cancer is the fourth most common cancer in women worldwide, caused primarily by persistent infection with HR-HPV.[4–7] Preventing cervical cancer relies on stopping persistent HR-HPV infection. Qingdu Fang, a TCM compound, has been proven effective in treating HR-HPV infection. Through network pharmacology and mass spectrometry, it was found that quercetin is the main component of Qingdu Fang. Quercetin, a well-studied natural flavonoid, has various pharmacological benefits such as antioxidant, anticancer, antiviral, antimicrobial, and anti-inflammatory propertiest.[8,9] However, there is limited research on quercetin for treating cervical HR-HPV infection. This study used network pharmacology and molecular docking to study how quercetin targets HR-HPV, aiming to identify therapy targets and understand the molecular mechanism and signaling pathways involved in treating HR-HPV.

Materials and Methods

Collection of Quercetin Targets

In this study, the target genes associated with the active ingredients of quercetin were obtained through the utilization of the database of Traditional Chinese Medicines Systems Pharmacology Platform (TCMSP, https://tcmsp-e.com/). The potential active compounds of Ginseng were screened based on their oral bioavailability (OB) being equal to or greater than 0.3 and drug-likeness (DL) index being equal to or greater than 0.18. In this investigation, it was found that the OB of quercetin was 46.43%, which exceeds the threshold of 30%, and the DL of quercetin was 0.28, which is greater than the required value of 0.18. Consequently, it can be inferred that quercetin possesses certain potential activities. The TCMSP database was then utilized to query the targets of the active components of quercetin. The standardization of gene naming was achieved through the utilization of the databases of Human Gene DataBase (Gencards, http://www.genecards.org) and Universal Protein (Uniprot, http://www.uniprot.org/uniprot). Disease targets were extracted from these databases, consolidated, and any duplicate targets were eliminated to obtain a set of HR-HPV disease-related targets.

Acquisition and Collection of Disease Targets

In order to obtain a comprehensive understanding of the relationship and mechanisms of action between HR-HPV and quercetin at the protein level, the keyword "HR-HPV" or "high risk human papillomavirus" was utilized to search Genecards. Additionally, the target genes of quercetin were uploaded to the Venny 2.1.0 website (http://bioinfogp.cnb.csic.es/tools/venny) to determine the intersection of genes between the active targets and disease targets.

Construction and Analysis of the Protein–Protein Interaction Network

The gene data at the intersection were imported into the String database (https://string-db.org/). The confidence score threshold was set at a value greater than 0.9, and outlier targets were excluded. The analysis was limited to the "Homo sapiens" species to obtain the interaction relationships. Subsequently, the Tab Separated Values (TSV) file containing the protein–protein interaction (PPI) network was imported into Cytoscape 3.7.2 for visualization. Core targets were identified based on a screening condition of count $\geq 3\times$.

Biological Pathway Analysis

The obtained targets of quercetin were imported into the Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/). The data types were set to "gene list," the species type was set to "Homo sapiens", and the background was set to the species. Functional annotation was performed using Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The screening qualifier was set to $p < 0.05$, false discovery rate (FDR) < 0.05, and disease associations were studied to identify biologically significant processes. The analysis focused on the biological pathways and mechanisms of action of quercetin as a treatment for cervical HR-HPV.

Targets-Pathway Network Analysis

The initial selection of the first 20 KEGG pathways was determined by considering the top 25 targets according to the results obtained from PPI analysis. Subsequently, a histogram was generated to depict the network diagram of the "target–pathway" relationship.

Key Active Compounds and the Targets Verification

In order to evaluate the veracity of the association between the target and the compound, compounds exhibiting the highest numbers of pertinent critical genes, along with their shared core genes, were chosen for molecular docking. The crystal structures were obtained from the Protein Data Bank (http://www.rcsb.org/pdb/), while the two-dimensional (2D) structures were acquired from the Pubchem Compound database

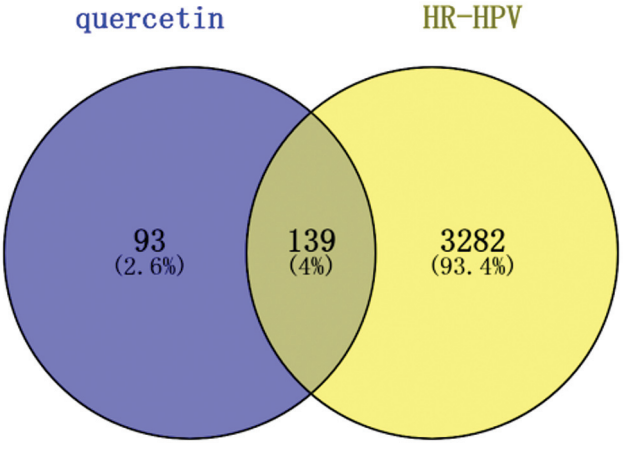

Fig. 1 Veeny of quercetin and HR-HPV targets intersection.

(https://pubchem.ncbi.nlm.nih.gov/) in structured data file format. Molecular docking via CB-Dock was employed to forecast the binding activities of proteins to compounds. The measurement of credibility perceptions between compounds and targets was conducted through docking affinity, whereby a docking affinity value less than −7.0 kcal/mol indicates a stronger binding between the two groups. The Ligplot software (Version 2.2) was utilized to display the optimal dock group.

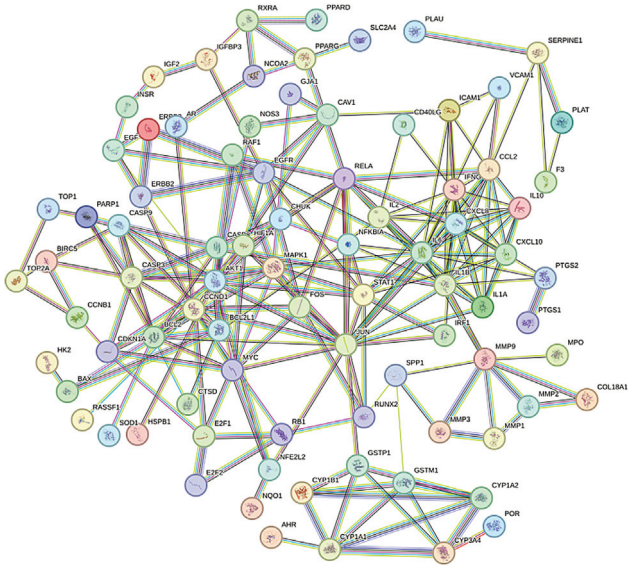

Fig. 2 PPI network map of oncotargets between quercetin and HR-HPV.

Results

Prediction of Quercetin Targets for the Treatment of Cervical High-Risk Human Papillomavirus

A total of 232 active constituents of quercetin were identified from the TCMSP database. Additionally, a total of 154 targets associated with "HR-HPV" and 3,450 targets associated with "high risk human papillomavirus" were obtained from GeneCards. After removing duplicate targets, a total of 3,420 unique targets were obtained. Using Veeny 2.1.0 software, 139 genes were found to be shared between the active ingredient targets of quercetin and the disease targets of HR-HPV, as depicted in ►**Fig. 1**.

Protein–Protein Interaction Network of Quercetin Targets for the Treatment of High-Risk Human Papillomavirus

The oncotargets were inputted into the String database and screened using a confidence score threshold >0.9. This process resulted in the construction of a PPI network map, consisting of 488 protein relations involving 139 protein targets. The average number of nodes in the network was 7.02. Bar charts were generated based on the TSV file of the PPI networks, as shown in ►**Fig. 2**. Core targets were

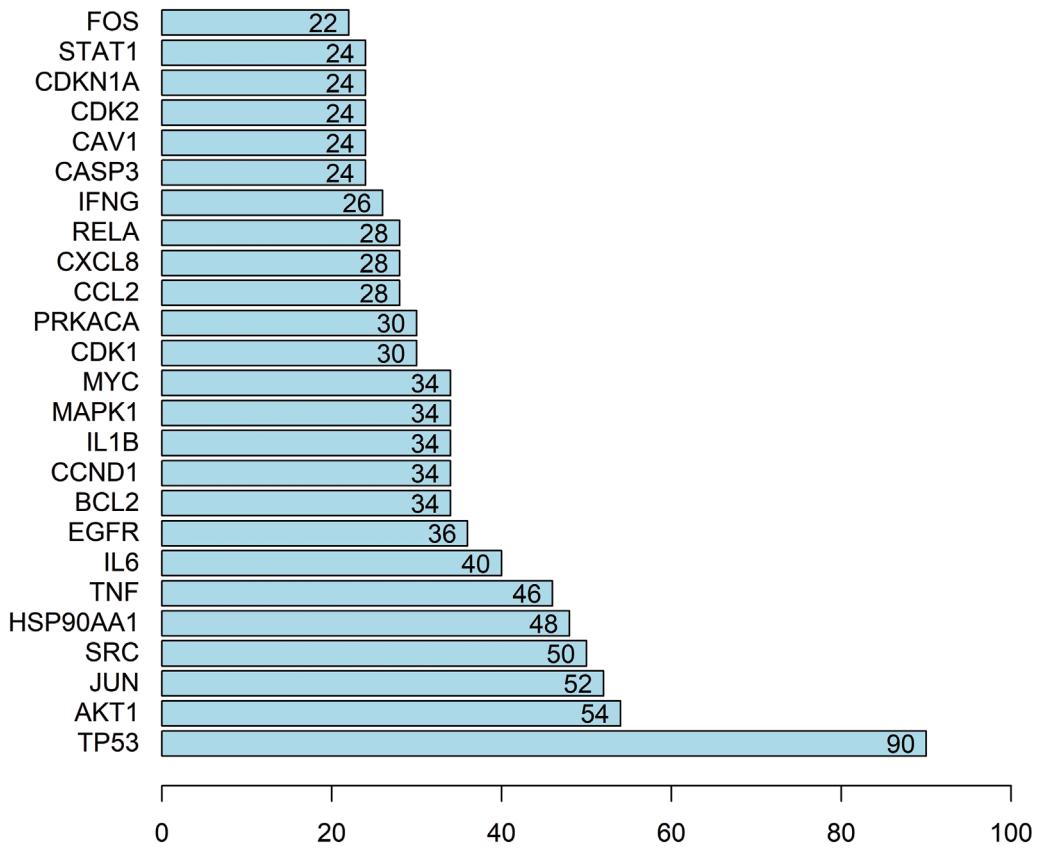

Fig. 3 PPI interactions network map of oncotargets between quercetin and HR-HPV.

identified based on the screening condition of count three or more times. The results suggest that threonine kinase Akt1 (AKT1), mitogen-activated protein kinase1 (MAPK1), human IL-6 protein (IL6), signal transducer and activator of transcription 3 (STAT3), and epidermal growth factor receptor (EGFR) may serve as potential targets for quercetin in the treatment of HR-HPV, as demonstrated in ►**Fig. 3**.

Gene Ontology Enrichment Analysis and Kyoto Encyclopedia of Genes and Genomes Pathway Analysis

A comprehensive examination of 139 genes was conducted through GO functional analysis, revealing that 82 of these genes were associated with biological processes (BP). The subsequent GO enrichment analysis yielded 150 entries, with 82 falling under BP, 18 under cellular component (CC), and 50 under molecular function (MF). The primary BP encompassed biological regulation, cellular process, metabolic process, regulation of biological process, and single-organism process.

The primary CC encompassed biological regulation, cellular processes, metabolic processes, regulation of BP, and single-organism processes. The primary MF comprised myosin light chain binding, protein kinase activity, and endopolyphosphatase activity. A smaller *p*-value corresponded to a stronger tendency for the point's color to be red, a higher number of enriched genes, and a larger point area. KEGG enrichment analysis yielded 120 pathways, primarily including the Th17 signaling pathway, IL-17 signaling pathway, Toll signaling pathway, PI3K-Akt signaling pathway, and others. The construction of the top 20 main pathways through GO analysis (►**Fig. 4**) and KEGG analysis (►**Fig. 5**) was facilitated using the Bioconductor package within the R software.

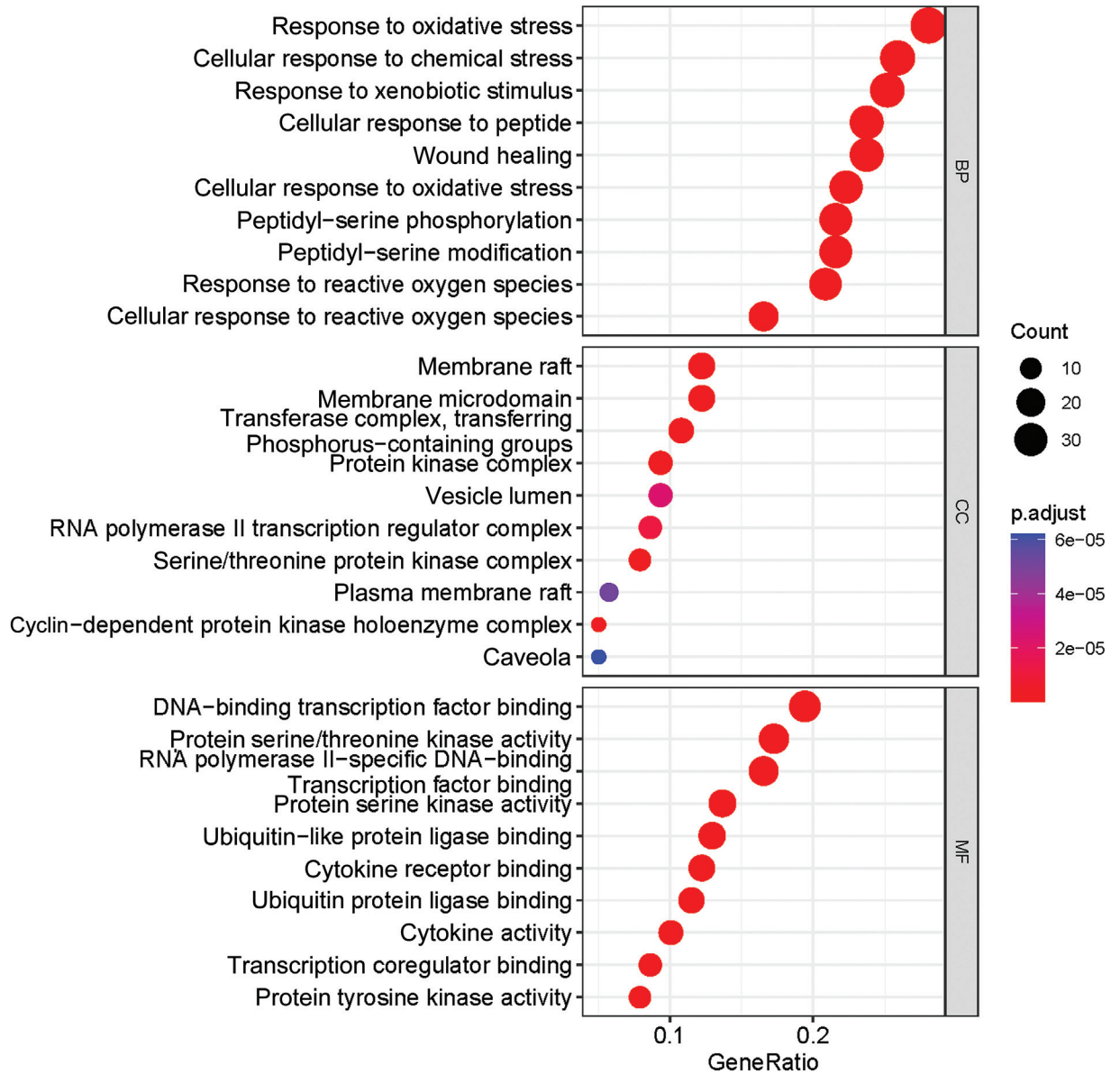

Fig. 4 GO enrichment analysis.

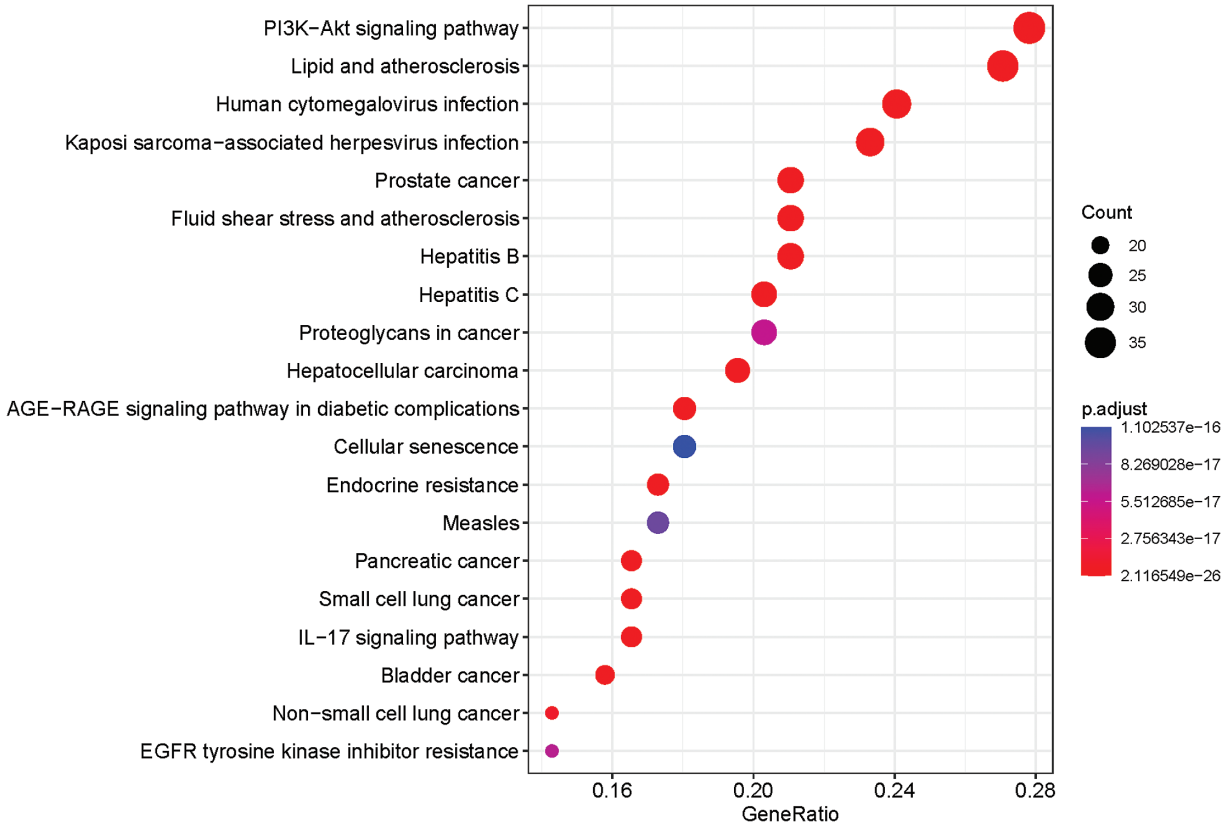

Fig. 5 KEGG pathway analysis.

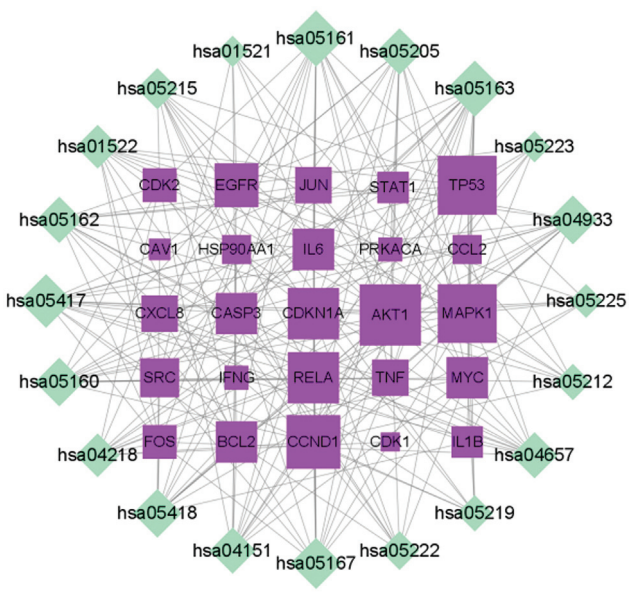

Fig. 6 "oncotargets target-pathway" network map.

Analysis of Core Targets by "Oncotargets Target-Pathway"

The top 25 targets of the 2.2 PPI network were mapped to the top 20 KEGG pathways to construct a network map of "oncotargets target-pathway." As depicted in ►**Fig. 6**, AKT1, MAPK1, IL6, STAT3, and EGFR emerged as the central potential targets.

Molecular Docking Verification of Key Targets with Quercetin

The molecular docking of quercetin and the core targets AKT1, MAPK1, IL-6, EGFR, and STAT3 were conducted using the AutoDock Vina software. It is widely acknowledged that a lower docking score indicates a stronger affinity between the ligand and the receptor within the complex. The binding affinities of target-compound permutations, ranging from small to large, were analyzed. The results showed that the binding affinities of EGFR and quercetin (-7.1 kcal·mol^{-1}), MAPK1 and quercetin (-6.4 kcal·mol^{-1}), AKT1 and quercetin (-6.2 kcal·mol^{-1}), STAT3 and quercetin (-5.5 kcal·mol^{-1}), and IL6 and quercetin (-5.1 kcal·mol^{-1}) were demonstrated in ►**Table 1**. Among these, EGFR exhibited the strongest binding affinity. The conformation with the lowest affinity value was selected, and the docking conformation was visualized using PyMol software, as shown in ►**Fig. 7**.

Discussion

In this study, a network pharmacology analysis was conducted to screen a total of 134 key targets. The core potential targets, namely AKT1, MAPK1, IL6, STAT3, and EGFR, were identified through visualization of a PPI network. AKT1, a serine/threonine protein kinase, plays a crucial role in regulating various cellular processes such as metabolism, proliferation, cell survival, growth, and angiogenesis.[10] AKT1, a crucial protein in the P13K/AKT signaling pathway, serves as the target of quercetin.

Table 1 Results of molecular docking verification of key targets with quercetin

Core target	Binding affinity/(kcal·mol^{-1})
	Quercetin
AKT1	−6.2
EGFR	−7.1
IL6	−5.1
MAPK1	−6.4
STAT3	−5.5

Abbreviations: AKT1, threonine kinase Akt1; EGFR, epidermal growth factor receptor; IL6, human IL-6 protein; STAT3, signal transducer and activator of transcription 3.

Research findings indicate that there are positive associations between PI3K and AKT1 in HPV16 infection. The persistence of HPV infection may lead to the sustained activation of the PI3K–AKT signaling pathway, ultimately resulting in the development of cervical lesions.[11] Additionally, the *MAPK1* gene, responsible for encoding a member of the MAPK family, plays a role in cell proliferation, differentiation, and apoptosis.[12] Levels of IL-6, a multifunctional cytokine, exhibit antiapoptotic effects. IL-6 activates PI3K to inhibit transforming growth factor and induce Bcl-2 to exert its effect. In the context of HR-HPV infection, levels of IL-6 in cervicovaginal lavages are positively associated with the HR-HPV infection status.[13,14] EGFR serves as the receptor for epidermal growth factor (EGF) and triggers various intracellular signal transduction pathways involved in cell differentiation and proliferation. STAT3 plays crucial roles in cell proliferation and differentiation. The expression of STAT3 was found to be significantly higher in the HPV-positive group as compared to the HPV-negative group, as reported by a previous study.[15]

The KEGG enrichment analysis revealed the involvement of 120 KEGG pathways. Notably, the IL-17 signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway, and EGFR tyrosine kinase inhibitor resistance were found to be enriched in the KEGG analysis. The IL-17 signaling pathway plays a significant role in the immune response and inflammation within the body. Upon binding with its receptor, IL-17 activates the STAT3 transcription factor signaling pathway, leading to the release of proinflammatory cytokines through the MAPK pathway and NF-κB pathway. This amplifies the inflammatory response, as evidenced by previous research.[16]

The TNF pathway plays an important role in various cellular processes such as cell growth, proliferation, inflammation, and immunity. TNF-α, a well-known proinflammatory cytokine, is a key component in this pathway. Within the TNF-α signaling cascade, TNF receptor 2 (TNF-R2) activates the NF-κB and MAPK pathways, which are essential for virus clearance and antitumor immunity. Furthermore, it has been observed that the expression of TNF-α is significantly higher in HPV16-positive high-grade squamous intraepithelial lesion patients compared to HPV16 negative individuals.[17] Th17 cells are known to secrete IL-17A, IL-17F, and TNF-α, which have inflammatory effects and are considered the underlying cause of cervical cancers.[18] The EGFR signaling pathway plays a crucial role in various cellular processes, such as proliferation, migration, growth, and differentiation. EGFR, also known as EGF receptor, serves as a receptor for proliferation signaling. The activation of EGFR occurs through the binding of ligands, subsequently initiating downstream signaling pathways including PI3K/AKT, MAPK, and JAK/STAT.[19] The PI3K/AKT signaling pathway is a well-established pathway involved in the regulation of cell proliferation, differentiation, and apoptosis. The aberrant activation of signaling pathways is intricately associated with the proliferation, programmed cell death, and spread of tumor cells.[20] The activation of the PI3K/Akt pathway due to persistent HPV infection contributes to the initiation and progression of cervical lesion molecular docking analysis demonstrates that quercetin exhibits favorable binding activity with HR-HPV targets, with the highest affinity observed for EGFR.[13]

Conclusion

This study aimed to investigate the mechanism of quercetin in the treatment of HR-HPV using network pharmacology and molecular docking techniques. The study constructed a "compound-potential target network" and "target-pathway" to analyze the interactions. The findings revealed a strong association between HR-HPV and cellular immunity and demonstrated that quercetin can inhibit HPV multiplication by regulating multiple pathways. This study provides a system-

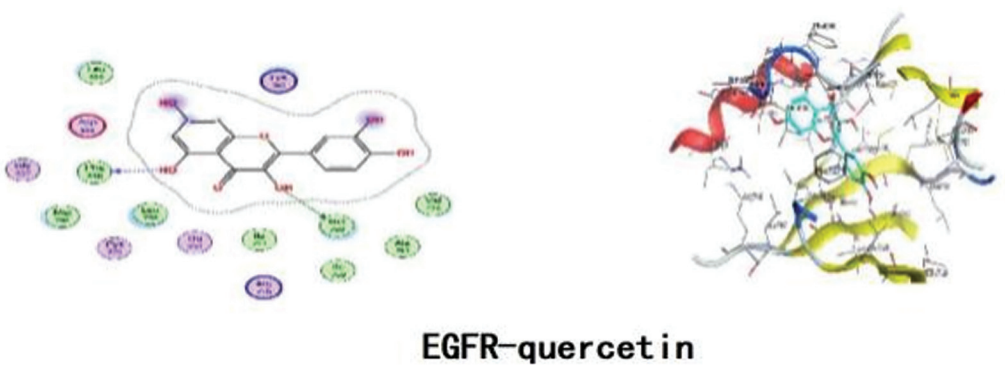

Fig. 7 Molecular docking mode.

level perspective on the effects of quercetin on HR-HPV, considering its multimolecule, multitarget, and multipathway actions. The research findings presented in this paper can serve as a valuable reference for future studies in this field.

CRediT Authorship Contribution Statement

Shanyun Wang: Visualization, funding acquisition, data curation, project administration, and writing-original draft. Jing Xiao: Resources. Jianfeng Zeng: Supervision, writing-review and editing. Huisi Hong: Formal analysis. Yiming Yuan: Mehtodology. Yinhao Yin: Validation and writing-review & editing.

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Conflict of Interest

The authors declare no conflict of interest.

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