

# Prediction of the mechanisms of action of YinQiao powder in Influenza: A network pharmacology study

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## Research

**Keywords:** Pharmacological Mechanism, Symptom Mapping, Influenza, Yingqiao Powder

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# Abstract

**Objective** To predict the potential target and pharmacological mechanism of YinQiao Powder (YQP) in the treatment of influenza.

**Methods** The symptom mapping (SymMap) platform was retrieved to obtain the targets of all herbs in YQP. Influenza-related targets were obtained using the Comparative Toxicogenomics Database (CTD) and DisgeNet database. The String database was used to construct the protein-protein interaction (PPI) network; the Metascape platform was used to enrich key targets by GeneOntology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Data were visualized and analyzed using software such as Bioinformatics, Edrawmax, and Cytoscape.

**Results** A total of 263 potential targets of YQP for influenza treatment were screened. There are 256 nodes and 3697 edges in the PPI network. The key targets of the PPI network are AKT1, TP53, INS, IL6, MAPK3, VEGFA, etc. The results of Go enrichment showed that YQP can interfere with biological processes such as response to toxic substance, cytokine-mediated signaling pathway, response to lipopolysaccharide, response to extracellular stimulus, and positive regulation of cell death. The results of KEGG pathway enrichment showed that YQP could interfere with the infection process of the influenza virus by intervening PI3K/Akt, TNF, IL-17, MAPK, and other pathways.

**Conclusion** This study reflects the multi-target and multi-pathway action characteristics of YinQiaoSan, and its mechanism of action in the treatment of influenza may be related to the regulation of PI3K-Akt, NF-kappa B, and MAPK signaling pathways. However, further experimental studies are needed to verify the specific mechanism of YQP.

## 1. Introduction

Influenza is a highly infectious acute respiratory disease caused by influenza viruses. The World Health Organization estimates that about 1 billion people are infected with influenza each year, resulting in 300,000-500,000 deaths<sup>[1]</sup>. The virus is highly infectious and has a high mortality rate, so it is listed as one of the major infectious diseases that seriously threaten human health. The emergence of influenza-resistant strains has been accelerated by the large use of antiviral drugs such as oseltamivir<sup>[2]</sup>. Therefore, it is particularly urgent to develop safe and efficient new anti-influenza drugs.

In China, traditional Chinese medicine (TCM) has been used to treat influenza for thousands of years with good safety and remarkable efficacy<sup>[3]</sup>. YinQiao Powder (YQP) was originated from the book named "Treatise on differentiation and treatment of epidemic febrile disease" and has been used for hundreds of years. At the same time, it is also one of the TCM prescriptions recommended treating influenza in China<sup>[4]</sup>, and its anti-influenza efficacy has been confirmed by clinical and experimental studies<sup>[5]</sup>. In this study, network pharmacology was used to predict and screen the targets of YQP against the influenza

virus and explore the mechanism of YQP in the treatment of influenza<sup>[6]</sup>, which lay a theoretical foundation for the subsequent in vivo and in vitro experimental studies of YQP.

## 2. Materials And Methods

1. *2.1 Data collection.* On the symptom mapping (SymMap) platform (<https://www.symmap.org/>)<sup>[7]</sup>, the targets of each herb of YQP were obtained with FDR (Benjamini-Hochberg multiple testing correction) < 0.05. Influenza-related targets were obtained from the Comparative Toxicogenomics Database (<http://ctdbase.org/>)<sup>[8]</sup> and DisgeNet databases (<https://www.dissent.org/>)<sup>[9]</sup>. The above-collected drug targets and influenza-related targets were mapped using the bioinformatics (<http://www.bioinformatics.com.cn/>) platform. At the same time, the intersection of drug targets and influenza-related targets was taken as potential targets of YQP for influenza treatment<sup>[10]</sup>. Finally, all the target information was standardized by UniProt (<http://www.uniprot.org/>)
2. *2.2 Construction of Protein-protein interaction (PPI) network of key targets* The potential targets were imported into the STRING 11.0 database (<http://string-db.org/>)<sup>[11]</sup> to obtain PPI networks. PPI network analysis and visualization were performed with Cytoscape software (version: 3.7.2, <http://chianti.ucsd.edu/cytoscape-3.7.2/>), and network topological eigenvalues such as Degree, Betweenness Centrality and Closeness Centrality were analyzed with NetworkAnalyzer (default settings). Nodes with degree greater than average node degree were extracted to construct a subnetwork, and nodes with a degree more than 2 times the average degree in the network were regarded as key nodes of PPI network and were displayed in pictures by Cytoscape.
3. *2.3 Enrichment Analysis for Potential targets* Potential targets were input into the Metascape platform (<https://metascape.org/>)<sup>[12]</sup> for GO (Gene Ontology) enrichment<sup>[13]</sup> and KEGG pathway (Kyoto Encyclopedia of Genes and Genomes) enrichment<sup>[14]</sup>. In enrichment setting, species were set as "h. sapiens", P-value was ≤ 0.01, enrichment factor was 1.5, and the minimum count was 3. The enrichment results were arranged in descending order according to the number of hitlist targets, and the top-ranked results were screened. The results were visualized using bioinformatics (<http://www.bioinformatics.com.cn/>) and Edrawmax (<https://www.edrawmax.cn/online/>) online software. Cytoscape was used to construct the top 10 key pathways into a "target-pathway" network. Finally, KEGG mapper (<https://www.kegg.jp/kegg/mapper.html>)<sup>[15]</sup> was used to demonstrate potential targets enriched in the influenza disease pathway.

## 3 Results

3.1 *Potential targets of YQP for influenza treatment* A total of 2151 targets of all traditional Chinese herbs in YQP were retrieved by the SymMap platform, and 381 targets were obtained after deleting duplicates (Table 1). The CTD database and DisgeNet database retrieved 8356 and 588 influenza-related targets, respectively. After removing duplicates, 8533 influenza-related targets were obtained. The target of YQP and influenza-related targets were genetically mapped, and the two were intersected to obtain a total of 263 potential targets (Fig. 1).

Table 1  
The number of active compounds and their targets contained in each herb of YinQiao Power(YQP).

	Compounds	Targets
Flos Lonicerae	320	323
Fructus Forsythiae	192	261
Phyllostachys nigra	N/A	N/A
Herba Schizonepetae	209	267
Fructus Arctii lappae	178	272
herba Menthae	209	232
Semen Sojae Praeparata	21	159
Radix Glycyrrhizae	283	286
Radix Platycodi	121	147
Rhizoma Phragmitis	111	204
Note: N/A indicates that the data is not detected in the database		

**3.2 Protein-Protein Interaction (PPI) network analysis of potential targets** PPI network was obtained through String online platform, and the minimum required interaction score was set to medium confidence (0.400). The network includes 256 nodes, 3697 edges, and the average node degree is 28.2. Nodes with a degree higher than average node degree were extracted to construct a sub-network, which had 93 nodes in total. Network analyzer plug-in was used to analyze network topology parameters. Node size and color are adjusted according to the degree of the node (Fig. 2). The degree of a node in the network represents the number of nodes that directly interact with the node. As shown in Fig. 2, the higher the degree, the larger the corresponding node size, the darker the color, and the greater the importance of the node in the network.

There are 37 nodes in the network whose degree is more than 2 times the average degree. Figure 3 shows the network topology parameters of the nodes whose degree ranks the top 30. These key nodes are AKT1, TP53, INS, IL6, MAPK3, VEGFA, CASP3, MYC, JUN, MAPK1, TNF, MMP9, PTGS2, PTEN, CCND1 in order of degrees from high to low.

**3.3 Results of GO Enrichment Analysis of Potential Targets** In the GO enrichment results on MetScape, the results of cluster analysis are removed, and the results are arranged in descending order according to the number of hitlist, and then the top ten results are selected for visualization. The results of GO enrichment include three parts: Biological Processes (BP), Molecular Functions (MF) and Cellular Components (CC). As shown in Fig. 4, YQP can interfere with biological processes such as response to toxic substance,

cytokine-mediated signaling pathway, response to lipopolysaccharide, response to extracellular stimulus, and positive regulation of cell death.

**3.4 Results of KEGG Pathway Enrichment Analysis of Potential Targets** Metascape platform will cluster the enrichment results of KEGG pathways, and then the overlapping or related pathways will be merged and displayed together. As shown in Fig. 5, potential targets are mainly enriched in Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, Fluid shear stress and atherosclerosis, Apoptosis and other signaling pathways. Besides, we selected the ten signaling pathways with the largest number of genes in the original data, and the disease signaling pathways were removed. The results are shown in Fig. 6.

The top ten pathways and potential targets enriched in the pathways were introduced into Cytoscape together to construct a "target-pathway" map. As shown in Fig. 7, Red Diamonds represent signaling pathways and blue circles represent potential targets. The more times the target appears in each pathway, the larger the circle area and the darker the color, that is, representing the target is an important target affecting multiple pathways. We mapped the disease pathway of influenza through KEGG Mapper to better show the intervention mechanism of YQP in the occurrence and development of influenza disease (Fig. 8).

## 4 Discussion

More and more studies have confirmed that TCM has a good effect on viral infectious diseases<sup>[16-18]</sup>. A prospective, randomized, controlled trial of 410 participants confirmed that YQP plus Maxingshigan decoction had shorter fever clearance time than oseltamivir in the treatment of influenza<sup>[5]</sup>. The challenge experiment of influenza A virus also confirmed that YQP had a protective effect on mice. YQP could prolong the survival time of infected mice and improve the survival rate<sup>[19]</sup>.

At present, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database<sup>[6]</sup> is the most frequently used database for screening drug components and targets in network pharmacology research. The general method of network pharmacology research is to screen the components satisfying oral bioavailability (OB) and drug similarity (DL) in the TCMSP database and then screen their corresponding targets. The SymMap platform integrates target information from HIT, TCMSP, HPO, DrugBank, and NCBI databases, and is one of the largest target databases to date. However, only OB data of components are provided by this database, while DL data do not, and the corresponding targets of components cannot be obtained directly. Therefore, we skipped the step of screening active ingredients, and the target with FDR (Benjamini-Hochberg multiple testing correction) < 0.05 corresponding to each traditional Chinese medicine was directly included.

In this study, we performed PPI network analysis on potential targets of YQP for influenza treatment, which exhibited the characteristics of the YQP multi-target antiviral effect. A total of 37 key targets, including AKT1, TP53, INS, IL6, MAPK3, were screened according to the PPI network with a degree greater

than twice the mean. TP53 is a major transcriptional regulator that is activated in various cellular stress responses and regulates a wide range of biological processes, such as cell cycle arrest, apoptosis and senescence<sup>[20, 21]</sup>. In addition, TP53 plays an important role in innate host immune control of viral infection and is the main target of viruses during infection<sup>[22, 23]</sup>. The Influenza virus can regulate the expression and post-transcriptional activity of TP53, which is helpful to maintain a cell state conducive to viral replication<sup>[24, 25]</sup>.

In addition, we performed functional enrichment analysis, including GO and KEGG pathway, to elucidate the multiple mechanisms of YQP treatment for influenza. The results of GO enrichment include three parts: BP, MF, and CC. The top 10 GO terms with the largest number of hitlist are shown by us. Biological processes such as response to toxic substance, cytokine-mediated signaling pathway, response to lipopolysaccharide, response to extracellular stimulus, and positive regulation of cell death are closely related to influenza virus infection, replication and the generation of influenza symptoms. YQP may achieve the efficacy of treating influenza by intervening in these biological processes.

Metascape has a unique KEGG pathway clustering algorithm, so we show the results of KEGG enrichment after clustering, as well as enrichment results that are not clustered and disease pathways are removed. YQP can interfere with the infection process of the influenza virus by intervening PI3K/Akt, TNF, IL-17, MAPK, and other signaling pathways. PI3K/Akt signaling pathway plays an important role in many physiological processes, and its activation is a marker of cell survival<sup>[26]</sup>. After the influenza virus infects host cells, its non-structural protein NS1 can directly bind to the p85 $\beta$  subunit on the PI3K of host cells, thereby activating the PI3K/Akt pathway<sup>[27, 28]</sup>. Viruses reduce the apoptotic rate of host cells by utilizing the PI3K/Akt signaling pathway, thereby increasing the replication time of viruses<sup>[29]</sup>.

Finally, the disease pathway map of influenza was drawn by us using KEGG Mapper, and potential targets enriched in this pathway were presented in the map. YQP can inhibit virus proliferation and alleviate clinical symptoms by interfering with the release of inflammatory factors mediated by the NF- $\kappa$ B signaling pathway and the expression of viral proteins mediated by the MAPK pathway. YQP was used by Fu to treat wild-type and TLR7 KO C57BL/6 mice infected with influenza virus FM1, respectively. The expression of TLR7, MyD88, IRAK4, and NF- $\kappa$ B was significantly decreased in the YQP group, but the therapeutic effect of YQP on TLR7 KO mice was poor. This suggests that YQP may play an anti-influenza role by regulating TLR7/NF- $\kappa$ B<sup>[30]</sup>. As for the MAPK pathway, some studies have reported that Raf/MEK/ERK signaling plays an important role in influenza virus proliferation. Mutation of Raf and ERK or treatment with MEK inhibitor (U0126) could significantly inhibit virus propagation<sup>[31, 32]</sup>. Berberine is a major component of a variety of traditional Chinese medicines, and it can hinder the replication of influenza A by inhibiting the nucleolar export of viral ribonucleoproteins mediated by the ERK pathway in vitro<sup>[33]</sup>.

# **Declarations**

## **Ethics approval and consent to participate**

Not applicable

## **Consent for publication**

Not applicable

## **Availability of data and materials**

The datasets analysed during the current study available from the corresponding author on reasonable request.

## **Competing interests**

The authors declare that they have no competing interests

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## **Authors' contributions**

LLH collected and analyzed the data. FYF analyzed the data and participated in the writing of the manuscript. XS performed data analysis and interpretation and was a major contributor to the writing of the manuscript. All authors read and approved the final manuscript.

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Not applicable

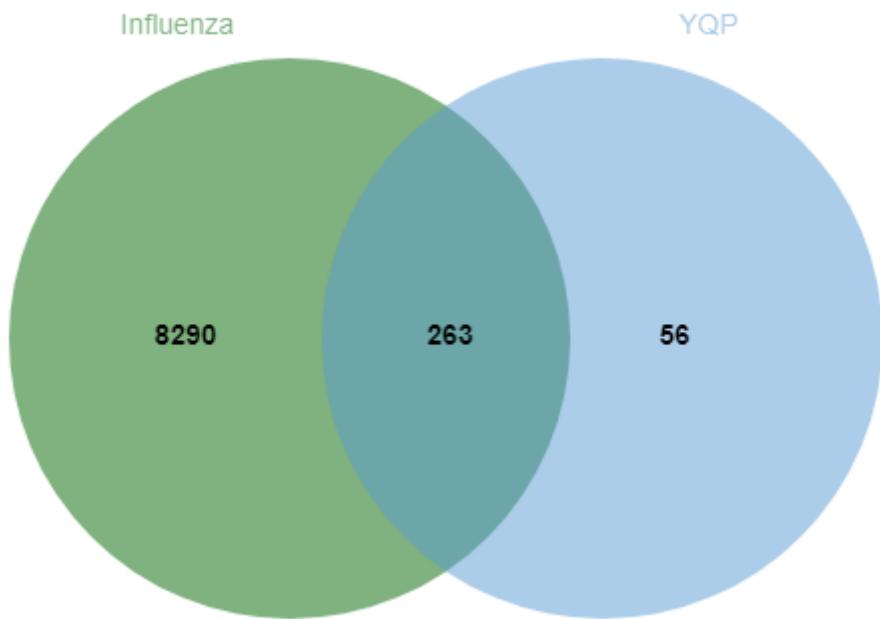
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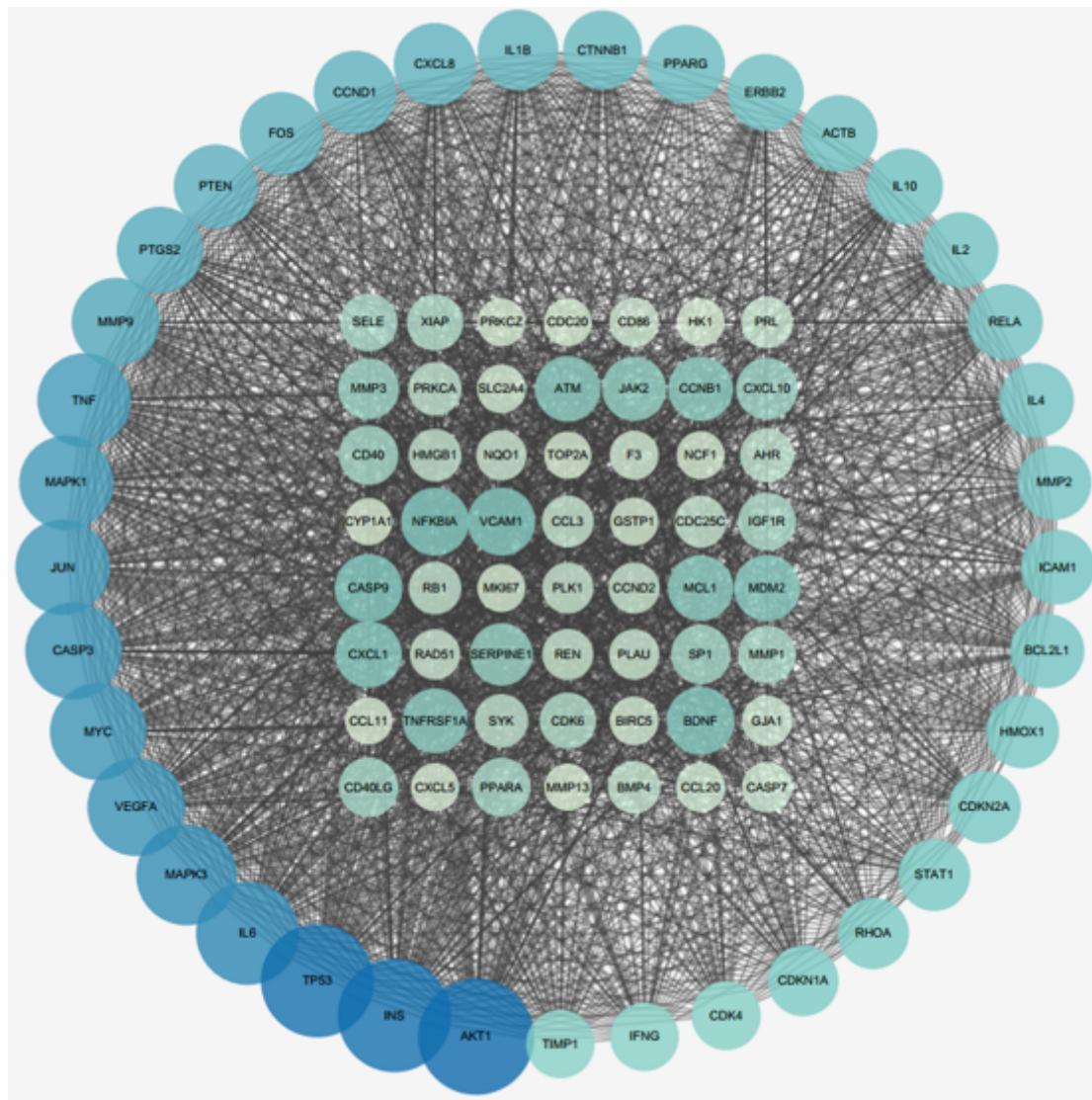
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## Figures



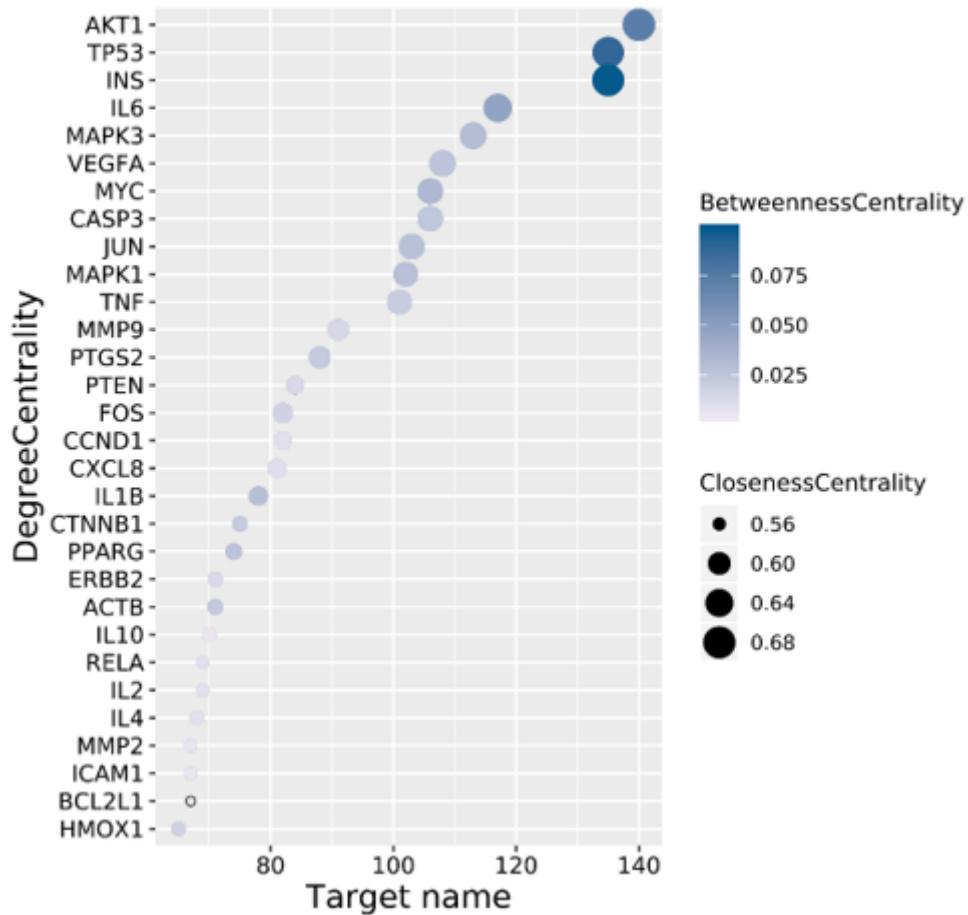
**Figure 1**

The intersection between the targets of YQP and influenza-related targets.



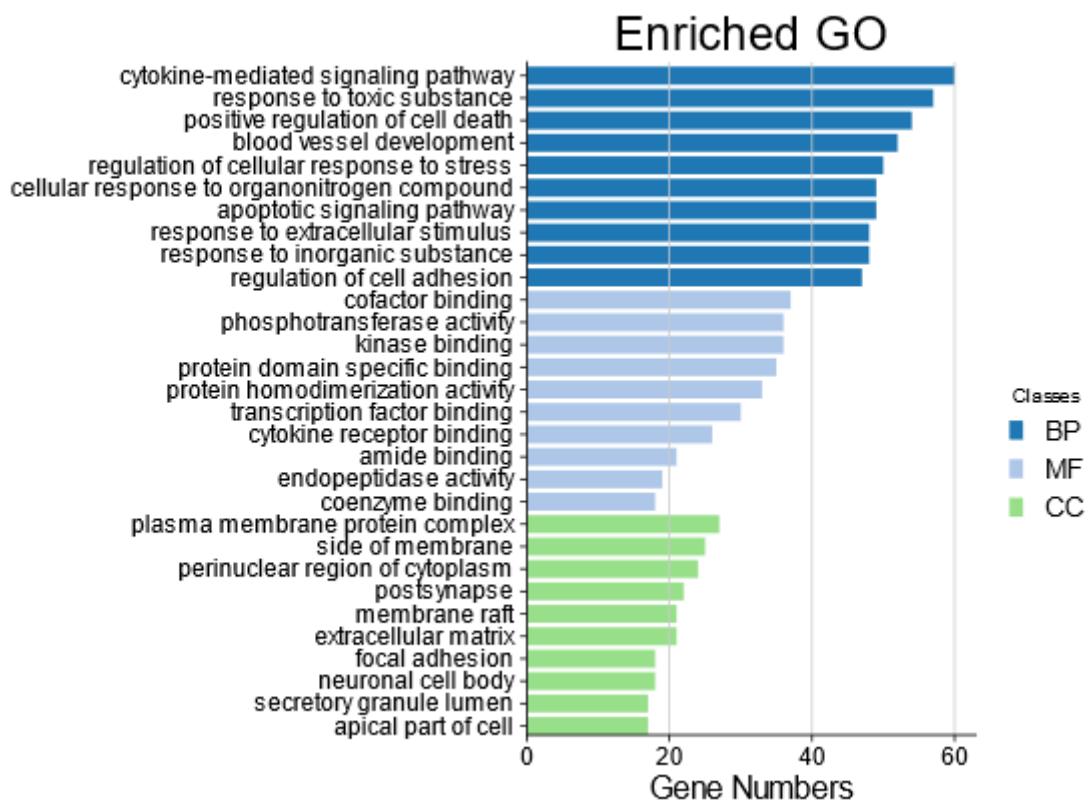
**Figure 2**

Protein protein interaction network. The potential target of YQP in the treatment of influenza is used to construct PPI network. The circle is the target and the connection between two nodes is the interaction. The area size and color depth of nodes are positively correlated with the degree of nodes.



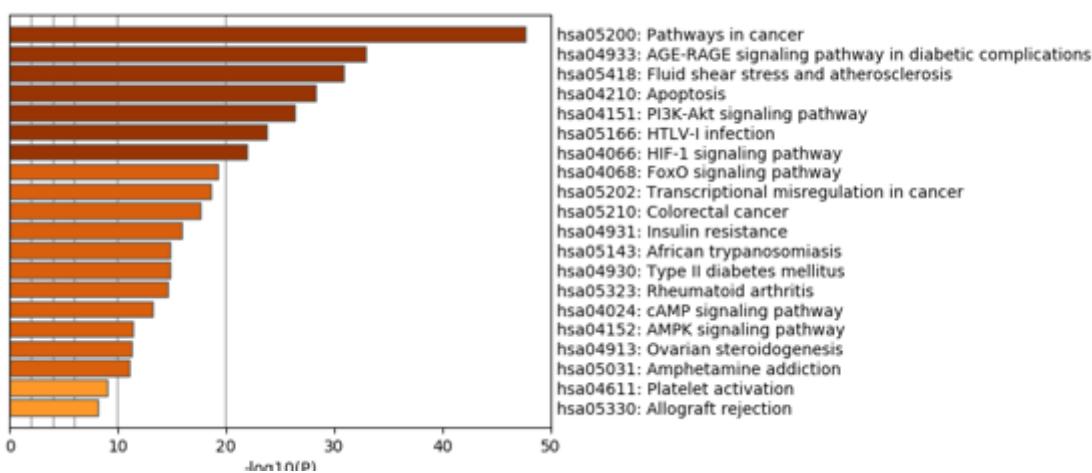
**Figure 3**

Network topology parameters of key targets of PPI network. Degree, betweenness centrality, and closeness centrality were used to evaluate the importance of key targets in PPI network, reflecting the changes of x-axis value, node color and node size.



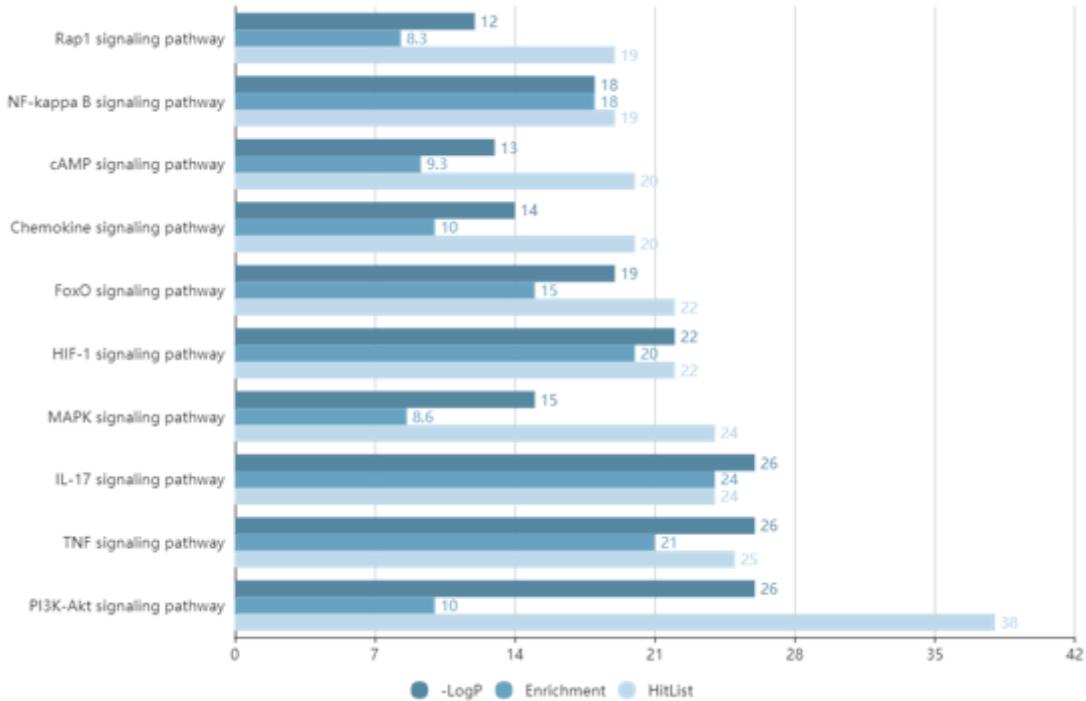
**Figure 4**

The top 10 GO enrichments in BP, MF, and CC. The number of genes enriched in each term was used to rank in descending order.



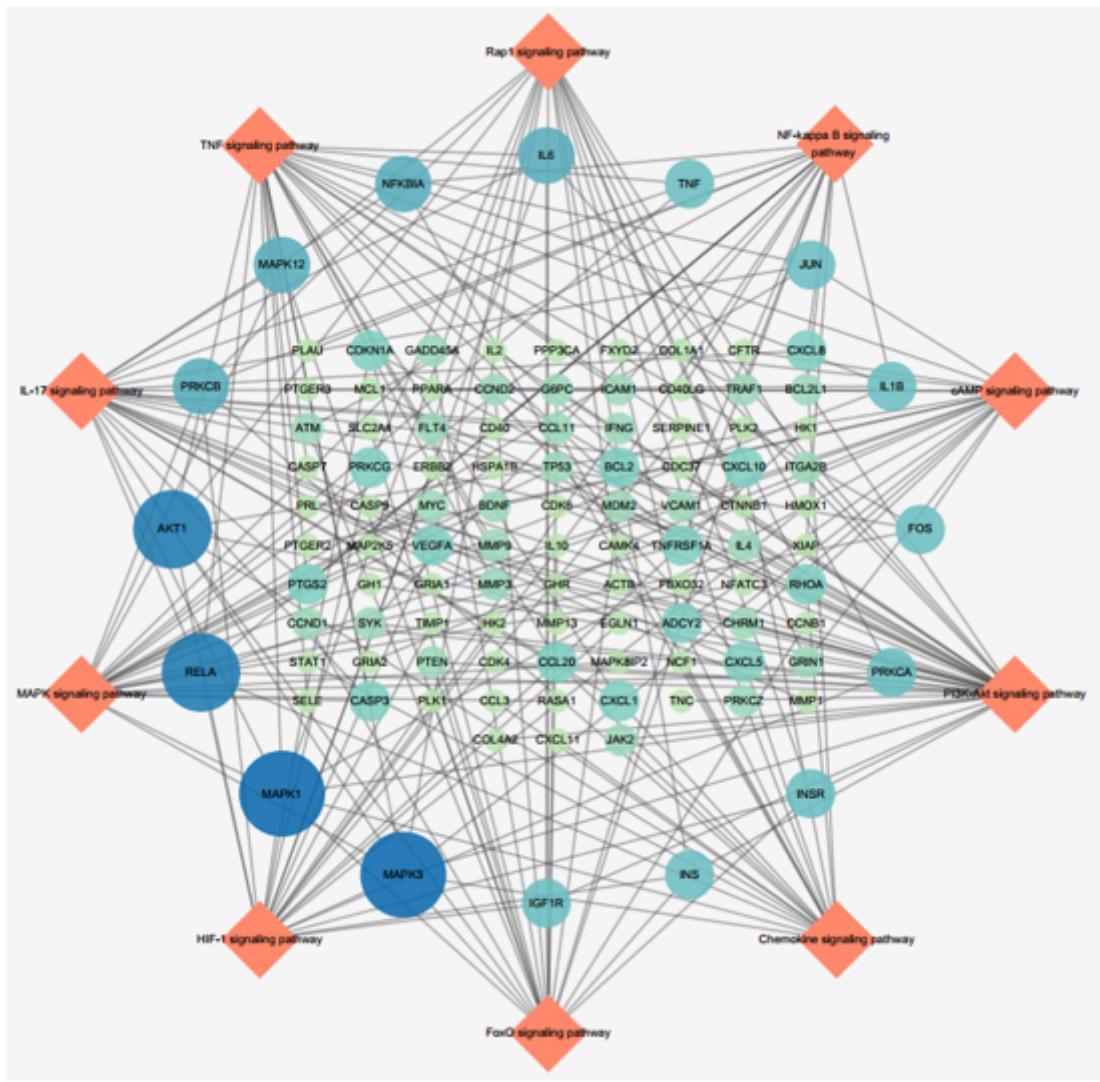
**Figure 5**

KEGG enrichment analysis and pathway mapping (ranked by  $-\log_{10}(p)$ ).



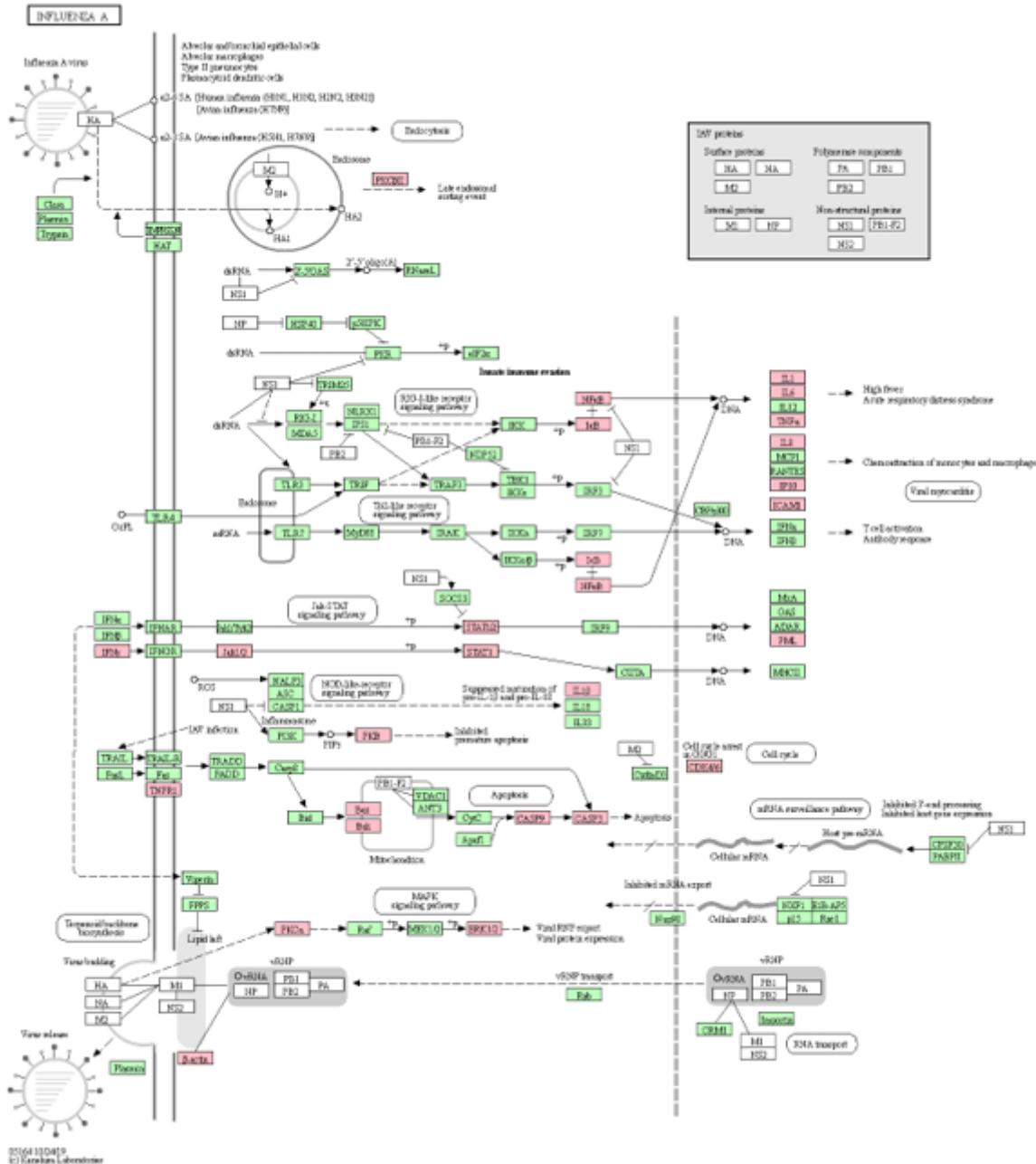
**Figure 6**

KEGG enrichment analysis and pathway mapping. The disease pathway was removed and ranked by the number of genes enriched in the pathway.



**Figure 7**

Target-pathway network of YQP. The diamond is the pathway, the circle is the target, and the connection between the two nodes is the interaction. The area size and color depth of the circle were positively correlated with the degree of nodes.



**Figure 8**

KEGG pathway: map05164. Pink rectangles represent proteins or pathways that are affected by YQP.