# Deciphering key targets and associated potential pathway of Sanguisorba Officinalis acting on hepatocellular carcinoma: Coupling Network Pharmacology With Targetable Screening From the Cancer Genome Atlas

**Background:** Sanguisorba Officinalis (SO), natural herb, have recently emerged as a promising strategy for cancer treatment, but little is known concerning their effects on hepatocellular carcinoma (HCC) tumorigenesis.

**Methods:** We obtained breast cancer genetic data from The Cancer Genome Atlas (TCGA) database, network pharmacology to further clarify its biological properties. Survival analysis and molecular docking techniques were implemented for the final screening to obtain key target information. Our experiments focused on the detection of intervention effects of SO on HCC cells (MCF-7 and MDA-MB-231), and quantitative RT-PCR (qRT-PCR) WB was used to assess the expression of key targets.

Results: A total of 1,439 differentially expressed genes (DEGs) were identified by TCGA and used to build disease networks. Module analysis, gene ontology and pathway analysis revealed characteristic of the DEGs network. Topological properties were used to identify key targets, survival analysis and molecular docking finally found that the targets of SO regulation of HCC cells may be CCNB1, CDC6, and p53. Through cell viability, migration and invasion assays, we found that SO interferes with the development of breast cancer in MCF7 and MDA-MB-231 cells in a dose-dependent manner. Furthermore, qRT-PCR verification suggested that the expression of CCNB1 and CDC6 in breast cancer cells was significantly downregulated in response to SO, while expression of the tumor suppressor gene P53 was significantly increased.

Conclusion: Results of this study suggest therapeutic potential for SO in HCC treatment, possibly through interventions with CCNB1, CDC6, and P53. Furthermore, these findings illustrate the feasibility of using network pharmacology to connect large-scale target data as a way to discover the mechanism of natural products interfering with disease.

Keywords: Sanguisorba Officinalis, breast cancer, network pharmacology, proliferation inhibition, TCGA

## Introduction

## Results

### Identification of Differentially Expressed Genes of ccRCC

We downloaded 418 RNA expression datasets and identified DEGs by calculating the difference in gene expression between 50 normal samples and 368 HCC samples. Using the LIMMA software package, differentially expressed data were extracted and analyzed. Using | log 2-fold change | ≥ 2.5 and P-value <0.01 as screening cutoff conditions, 568 DEGs were screened, containing 290 upregulated genes and 278 downregulated genes (Figures 1,2).

### DEGs Enrichment Analysis and PPI Network Analysis

To further analyse DEGs in HCC, we used the DAVID database to perform GO analysis and pathway enrichment analysis on DEGs. GO analysis, including molecular function, biological process and cellular component (Figure 3A), was performed on 568 DEGs, with p < 0.05 as the cut-off criterion. Enrichment results show that: Molecular function participates in cellular activities, such as calcium ion binding, transcription factor activity, sequence-specific DNA binding, cytoskeletal protein binding and peptidase activity; Biological process is mainly enriched in cell-cell signaling, cell cycle, ion transport, and cell adhesion and other physiological processes related to cell growth, division, and proliferation; Cellular component is associated with the extracellular space, plasma membrane binding, extracellular regions, and components of the plasma membrane.

KEGG pathway enrichment analysis of the 568 DEGs was also performed in DAVID, for which the cut-off criterion was P < 0.05. Pathway enrichment shows that the biological processes involved in these DEGs are mainly neuroactive ligand-receptor interaction, cytokine-cytokine receptor interaction, calcium

signaling pathway, and cell cycle signaling pathway (Figure 3B).

Construction of a protein interaction network is a way to quickly analyse interactions between DEGs. We constructed a PPI network with 517 nodes and 6477 edges using String and Cytoscape software (Figure 4A).

The Cytoscape plug-in MCODE was used to perform module analysis on the PPI network. We selected the most meaningful modules for analysis and used DAVID to perform pathway enrichment analysis on the nodes of the module. The Hub Module contains 95 nodes and 3963 edges (Figure 4B). Through pathway enrichment analysis, DEGs of the Hub Model were shown to be mainly enriched in cell cycle, p53 signaling pathway, progesterone-mediated oocyte maturation and oocyte meiosis signaling pathway.

Cytoscape’s plug-in CytoNCA performs topology analysis on PPI networks. According to the results of the topological analysis based on comprehensive ranking of screening criteria, such as “degree,” the nodes with the best meaning in the network map were screened out, and the first ten DEGs were identified as targets for continued screening, including DNA topoisomerase 2-alpha (TOP2A), insulin (INS), interleukin-6 (IL6), mitoticspecific cyclin-B1 (CCNB1), histone H3-like centromeric protein A (CENPA), cyclin-A2 (CCNA2), aurora kinase B (AURKB), DNA repair protein RAD51 homolog 1 (RAD51), cell division control protein 6 homolog (CDC6), and polo-like kinase 1 (PLK1) CDK1

CCNB1, CDC20, CCNA2, BUB1, BUB1B, AURKB, CCNB2, CDCA8, TOP2A (Table 1).

### Survival Analysis

To investigate the prognostic value of these 10 DEGs, the Kaplan-Meier plotter bioinformatics analysis platform was used. Analysis showed that from the 10 identified DEGs are all statistically relevant to the survival of HCC. We found that high expression of the 10 DEGs was associated with unfavorable RFS and OS in HCC patients, suggesting the remaining 10 DEGs can be used as biomarkers for HCC (Figure 5).

### Molecular Docking Model

Molecular docking is a theoretical simulation method for studying the interaction between molecules, such as ligands and receptors, and for predicting their binding mode and affinity. In recent years, molecular docking has become an important technology in the field of computer-aided drug research (26). In this study, to further explore the mechanism of interaction between APS and the 10 DEGs, we constructed a molecular docking model of APS and the DEGs. We found that CCNB1 (PDB ID: 2JGZ, docking score: 5.2146) and CDC6 (PDB ID: 4I5N, docking score: 5.7514) have a stable binding site in the APS small molecule model, and the residues of APS interact with hydrogen bonds in the binding site (Figure 6).

### Analysis of Key Target Characteristics

Box plot showed a significant upregulation of CCNB1 and CDC6 in BC samples (Figure 7). Based on GSEA, we observed that the differential regulation of CCNB1 and CDC6 was significantly enriched in P53 signaling pathway (Figures 8A,B). And interestingly, both CCNB1 and CDC6 showed a significantly

### Active compounds in Sanguisorba Officinalis

### Putative targets of Sanguisorba Officinalis

### GO enrichment and KEGG pathway analysis of the putative targets

### Pharmacological mechanisms of Sanguisorba Officinalis acting on hepatocellular carcinoma

### Molecular docking validation

## Material and Methods

Active compounds of Sanguisorba Officinalis

### Data Source and Processing

DNA expression data for BC were downloaded from the TCGA database (https://cancergenome.nih.gov/). DNA expression data for 1,208 samples were obtained, including 112 normal samples and 1,096 BC samples. These samples have complete survival data and are histologically typed as BC. Since the information was retrieved from TCGA database, a public data set, further ethical approval was not needed for our research. Data collection and processing procedures were in accordance with TCGA data access and policies for protecting human subjects (<http://cancergenome.nih.gov/> publications/publicationsguidelines). Subsequently, based on the edgeR software package in the R platform, downloaded DNA data were normalized and analyzed for differences to identify differentially expressed genes.

### Construction of a Known Drug-Target Network of Sanguisorba Officinalis

### Network construction

### Analysis of the target-target network

### Molecular docking simulation

## Discussion

## Conclusions

## References