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Mechanism of Hedysarum Multijugum Maxim. in Treatment of Bladder Cancer Based on Network Pharmacology and Molecular Docking Technology

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Abstract: To investigate the targets and mechanism of Hedysarum Multijugum Maxim (HMM) in treatment of bladder cancer (BC). Based on Traditional Chinese Medicine Systems Pharmacology (TCMSP) and gene databases, active substances and potential targets of HMM were screened, and the HMM-active substances-targets-BC (HATB) regulatory network and PPI network were constructed. Hub targets were screened by Cytoscape. The main active substances and Hub targets were molecularly docked with AutoDock and visualized by PyMOL. 12 Hub targets were screened. Molecular docking showed that active substances mainly acted on MAPK14, MAPK1 and CCND1. The bindings of calycosin to MAPK14, formononetin to MAPK14, and calycosin to CCND1 were stable.

Keywords: Network pharmacology; Molecular docking; HMM; BC; Targets

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1. Introduction

Bladder cancer is one of the most common cancers in the world, and its occurrence is increasing gradually. The pathogenesis of BC is complex and affected by many factors, including smoking, drinking water, diet, gender, body weight, genetic factors, occupation, air pollution, schistosomiasis infection, medical interventions, etc. The primary form of BC is non-invasive urothelial carcinoma, and about 30% of patients treated for this disease relapse after 5 years and develop muscle-infiltrating BC. For decades, the long-term survival rate of patients with muscle-infiltrating BC has not been effectively improved; about 50% of patients will develop cancer cell metastasis. Common BC treatments include surgery, chemotherapy, and radiotherapy, which are not only expensive but also have side effects on patients.

Traditional Chinese medicine(TCM) focuses on the overall and dialectical diagnosis and treatment. In the

treatment of many diseases, TCM has the advantages of high efficiency and low toxicity, which is expected to bring new ideas to the treatment of BC. HMM has been clinically applied to a variety of urological diseases, and is used in the treatment of BC as a high-frequency drug, but the specific mechanism of HMM against BC remains to be further studied. Network pharmacology is based on biological networks, which can study multi-target relationships between multi-active substances of TCM and organisms as a whole, and provides a new method for elucidating the pharmacological effects of TCM. Molecular docking is used to screen out the key active substances of HMM and corresponding targets of BC, based on 3D structures of ligands and spatial conformations of receptors.

This study used network pharmacology and molecular docking to identify active substances and targets of HMM in BC treatment, to provide a theoretical basis for its clinical application.

2. Materials and methods

2.1. Collection of active substances of HMM

The active substances of HMM were collected through the TCMSP database, a platform of pharmacology for TCM. According to the ADME model, the screening conditions were set as "oral bioavailability (OB) ≥ 30% and drug-like property (DL) ≥ 0. 18" to find active substances of HMM.

2.2. Drug targets prediction

The target proteins corresponding to active substances of HMM were found and downloaded in the TCMSP database. As the naming rules of these target proteins were different from PPI and other databases used in subsequent analysis, a Perl script was written to match the target protein names with the data in the UniProt database and convert them into corresponding gene names (Gene Symbol).

2.3. BC related targets prediction

The keyword "bladder cancer" was input into GeneCards and the OMIM database, respectively, to find target genes related to BC. The genes searched in the two databases are combined.

2.4. Obtaining intersection target genes of HMM and BC

Using Venny2. 1 tool (https://bioinfogp.cnb.csic.es/tools/venny/index.html), we constructed the intersection of target genes of HMM and BC and drew a Venn diagram.

2.5. Construction of the regulatory network of HATB

A Perl script is written to map the HMM active substances obtained by "2.1" and the intersection target genes of HMM and BC obtained by "2.4" to obtain three output files: a file of HMM active substances, a file of each node attribute in the regulatory network, and a file of the connection relationships in the regulatory network. The Cytoscape was used to construct a regulatory network of HATB. The nodes in the network respectively represented HMM, active substances, targets, and BC. The connection lines represented relationships between active substances and targets.

2.6. PPI network and Hub targets analysis

The intersection target genes of HMM and BC obtained in "2.4" were imported into the Multiple Proteins option in the String database. The species was selected as "*Homo sapiens*" and the screening condition was Score > 0.9.

The nodes without connecting lines were hidden. Finally, interaction data between these targets was obtained. PPI networks were plotted using Cytoscape. The Molecular Complex Detection (MCODE) plug-in of Cytoscape was used to screen the Hub targets in the PPI network based on the number of interactions between proteins.

2.7. Molecular docking of active substances with Hub targets

The 3D structures of active substances corresponding to Hub targets in the format of "*.sdf" were obtained from the PubChem database and converted into "*.pdb" format by Open Babel software. The 3D structures of Hub targets in the format "*.pdb" were downloaded from the AlphaFold database. Molecular docking verification was performed using AutoDock software (V1.5.6). Among them, active substances were used as ligands, and Hub targets were used as receptors. Each pair of molecules was docked 10 times, and only the docking results with the lowest free energy (kcal/mol) were retained. The docking results were visualized by PyMOL software (V4.5.0).

3. Results

3.1. Screening of active substances of HMM

By setting the threshold of "OB ≥ 30% and DL ≥ 0.18," a total of 20 active substances of HMM were retrieved in the TCMSP database.

3.2. Prediction of HMM drug targets and BC-related targets

Through the TCMSP database, 180 drug targets of HMM were obtained. A total of 11,675 BC-related targets were obtained through the GeneCards database, and 708 targets were screened using Relevance score ≥ 20 as the threshold. 496 BC-related targets were identified in the OMIM database. The targets in GeneCards and OMIM databases were combined to remove the duplicate genes, and a total of 1072 genes were obtained.

3.3. Obtaining intersection targets of HMM and BC

Using Venny2. 1 online tool, 86 targets for the intersection of HMM and BC were obtained and the Venn diagram was drawn. The result is shown in **Figure 1**.

3.4. Construction of HATB regulatory network

The Cytoscape was used to construct a regulatory network of HATB, with 104 nodes and 264 edges. As shown in **Figure 2**, the blue node represents HMM, the purple nodes represent active substances of HMM, the green nodes represent intersection targets of HMM and BC, the red node represents BC, and edges represent relationships between active substances and targets.

3.5. Construction of PPI network

After 86 targets were imported into the String database and independent targets were excluded, interaction data of 82 targets were obtained. The data was imported into Cytoscape to draw relationships between 82 targets. As shown in **Figure 3A**, node size was proportional to the degree value of the protein in the PPI network. Using the MCODE plug-in in Cytoscape to identify the most important module in PPI network, 12 Hub targets (IL1A, HIF1A, MAP K14, RelA, CXCL8, MAP K1, IL10, CCND1, CCL2, IL6, IL1b, and IL4) of the sub-network can be obtained, as shown in **Figure 3B**.

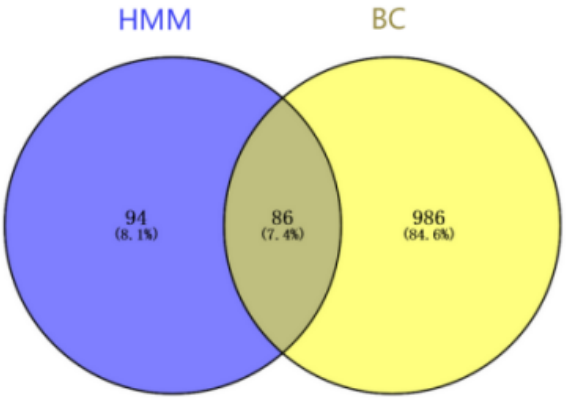

Figure 1. Venn diagram of the intersection targets of HMM and BC.

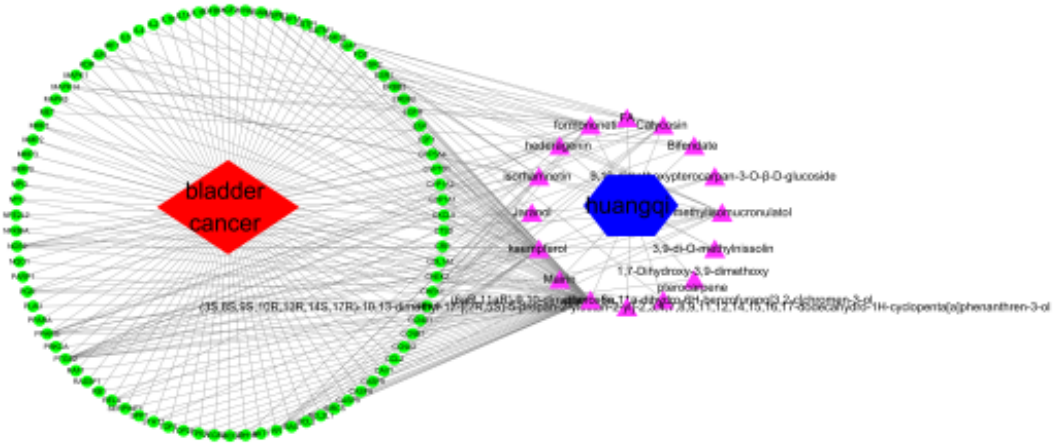

Figure 2. HATB regulatory network diagram.

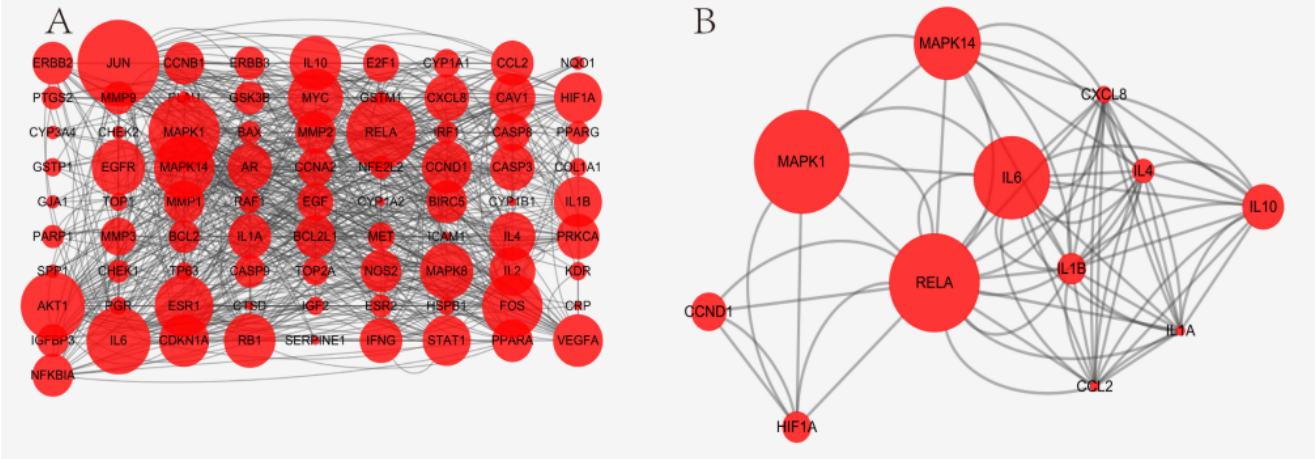

Figure 3. PPI network diagram. A is an interaction network of 82 targets; B is an interaction sub-network of 12 Hub targets.

3.6. Molecular docking

Molecular docking of the screened six active substances, including quercetin, formononetin, calycosin, kaempferol, isorhamnetin, and 7-O-methylisomucronulatol, with 12 Hub targets was conducted. It is generally believed that the smaller the binding energy of the ligand to the receptor is, the more stable the binding conformation will be. As shown in **Figure 4A**, the active substances of HMM concentrated on MAPK14, MAPK1, and CCND1. The first three highest binding scores were calycosin and MAPK14 (-7.71 kcal/mol), formononetin and MAPK14 (-7.13 kcal/mol) and calycosin and CCND1 (-7.04 kcal/mol), respectively. The docking results were visualized using PyMOL, as shown in **Figure 4B–4D**. As shown in **Figure 4B**, calycosin and amino acid residues VAL-83, LYS-165, HIS-80, MET-78, LEU-75, LEU-86, and VAL-349 of MAPK14 formed a hydrogen bond interaction. As shown in **Figure 4C**, formononetin formed a hydrogen bond interaction with amino acid residues PHE-169, LYS-53 and MET-109 of MAPK14. As shown in **Figure 4D**, calycosin and CCND1 amino acid residues ASN-83, ASN-198, ILE-196, SER-197, PHE-195, ARG-57 and ALA-190 formed hydrogen bond interaction.

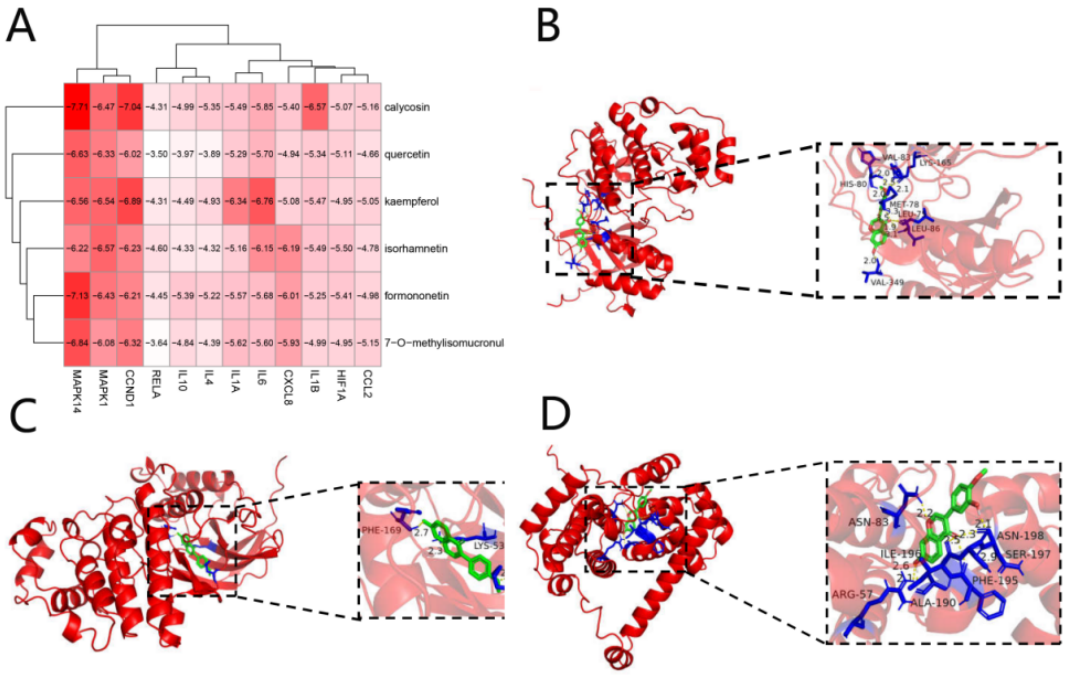

Figure 4. Molecular docking results. (A) Heat map of cluster analysis for molecular docking results. (B) The binding mode map of calycosin to MAPK14. (C) The binding mode map of formononetin to MAPK14. (D) The binding mode map of calycosin to CCND1.

4. Discussion

BC is one of the most common cancers in the urinary system, and the current methods of surgery, radiotherapy and chemotherapy cannot effectively inhibit BC recurrence [1]. TCM has advantages of wide resources and low cost, and its extract has a plurality of components, wide targets, small toxic and side effects [2]. TCM is a feasible method for BC treatment. At present, multiple components of HMM, such as astragalus polysaccharide and astragaloside, have been confirmed to be related to anti-tumor activity [3,4].

The Hub targets screened in this study were mainly concentrated in the immune system (such as IL1A, IL1B, IL6, IL4, and IL10), cell signaling (such as MAPK1 and MAPK14), transcription factors (such as RELA and HIF1A), cytokines (such as CXCL8 and CCL2), and cyclins (such as CCND1).

Molecular docking results showed that the six active substances of HMM were mainly and stably bound to MAPK14, MAPK1 and CCND1, and all binding free energies (kcal/mol) were less than -6. MAPK14, as a member of MAP kinase family, plays an important role in cell proliferation, differentiation, transcriptional regulation, etc. The binding free energies of calycosin, formononetin with MAPK14 were all low, suggesting that HMM might play a role in inhibiting BC through these two pharmacodynamic substances. Liu et al. found that calycosin could reduce the expression of MAPK14 in patients with nasopharyngeal carcinoma [5]. Studies have found that after treatment of BC cells with formononetin, the expression of p38 protein kinase in the cells increased in a dose-dependent manner. The p38α is one of the isoforms of p38 protein kinase; its gene is MAPK14, which may be one of the main mechanisms of formononetin inducing apoptosis in BC cells [6,7]. The free energy of binding of calycosin to CCND1 was also less than -7, indicating that the binding was stable. Many studies have shown that CCND1 expression in BC cells can be increased [8–10]. However, there are no reports related to the discovery of how calycosin binds to CCND1.

5. Conclusion

In summary, network pharmacology and molecular docking were used in the present study to explore the mechanism of HMM against BC, predict some possible targets and related signaling pathways, and find some main chemical components, which provide a new theoretical basis and new ideas for the study of HMM in the treatment of BC.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] Dobruch J, Oszczudłowski M, 2021, Bladder Cancer: Current Challenges and Future Directions. Medicina, 57(8): 749.

[2] Huang C, Zheng C, Li Y, et al., 2014, Systems Pharmacology in Drug Discovery and Therapeutic Insight for Herbal Medicines. Briefings in Bioinformatics, 15(5): 710–733.

[3] Zhu Y, Li G, Sun W, et al., 2023, Huangqi (Radix Astragali) Inhibits the Migration and Invasion of Bladder Cancer Through the Janus Kinase/Signal Transduction and Transcription Activation Factor Signaling Pathway Inhibited by MicroRNA-133A. Anhui Medicine, 27(1): 55–59 + 217.

[4] Tongfu K, Yin X, Zhang Z, et al., 2023, Inhibitory Effect of Astragaloside II on the Growth of Renal Clear Cell Carcinoma Cell and Its Mechanism. Journal of Shandong University (Medical Edition), 61(1): 10–16.

[5] Liu F, Pan Q, Wang L, et al., 2020, Bioinformatic and Experimental Findings to Indicate Anti-Cancer Targets and Mechanisms of Calycosin Against Nasopharyngeal Carcinoma. Research Square, 1–14.

[6] Zhang X, Liang M, Huang W, et al., 2015, Effects of Formononetin on Apoptosis of Bladder Cancer Cells. China Public Health, 31(3): 314–317.

[7] Kudaravalli S, Hollander P, Mani S, 2022, Role of p38 MAP Kinase in Cancer Stem Cells and Metastasis. Oncogene, 41(23): 3177–3185.

[8] Yuan L, 2011, Study on CCND1 Gene Polymorphism and Genetic Susceptibility of Bladder Cancer in China Han Population. Nanjing Medical University, 2011.

[9] Chen X, Wang P, Wang S, et al., 2019, CIZ1 Knockdown Suppresses the Proliferation of Bladder Cancer Cells by Inducing Apoptosis. Gene, 719: 143946.

[10] Watters A, Latif Z, Forsyth A, et al., 2002, Genetic Aberrations of c-myc and CCND1 in the Development of Invasive Bladder Cancer. British Journal of Cancer, 87(6): 654–658.