E3S Web of Conferences **271**, 03036 (2021) https://doi.org/10.1051/e3sconf/202127103036
ICEPE 2021

Exploring the Potential Pharmacologic Mechanism of Heterophyllin B in the Treatment of Esophageal Cancer by Network Pharmacology

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Abstract. This study used the method of network pharmacology to preliminarily predict the mechanism of Heterophyllin B(HB) inhibiting Esophageal Cancer(EC). We found the HB targets in the TCMSP and PuChem databases, and searched all EC-related targets in the GeneCards database. Taken the intersection of HB and EC as potential targets for inhibiting EC, and used Cytoscape 3.7.1 software to perform topological analysis on potential targets to obtain core targets. Used the start Analysisi function in the DAVID database to analyzed the biological process of the core target, and visualized it with the the R language tool. As a result, 75 potential targets for inhibiting EC were obtained, of which MMP9, MMP2, CCND1, STAT3, CXCR4, BDKRB1and PTGS2 were the main core targets. HB inhibits the occurrence of EC through Pathways in cancer, TNF signaling pathway, Bladder cancer, Small cell lung cancer, Rheumatoid arthritis related pathways, mainly involving proteolysis, collagen catabolic process, extracellular matrix disassembly, positive regulation of cell proliferation, positive regulation of cytosolic calcium ion concentration biological processes. This study initially revealed the molecular mechanism of HB inhibiting EC, and provided a reference for HB to expand new indications.

1 Introduction

Esophageal Cancer （EC） is a common malignant tumor of the digestive tract, with a high mortality rate, among which men are higher than women[1], and its morbidity and mortality have been on the rise in recent years[2]. When carcinogens or external mechanical forces continuously stimulate the esophageal mucosa, EC will continue to develop[3-4]. The treatment of EC is still based on traditional radiotherapy and chemotherapy, and there is no particularly effective treatment for EC, which has also led to a continuous decline in the survival rate of patients[5]. In recent years, the development of anti-tumor drugs from Traditional Chinese Medicine (TCM) is a hot topic. Of course, TCM has also made significant contributions in the process of human anti-tumor[6].

Heterophyllin B （HB） is an important active substance in the plant Pseudostellaria heterophylla (Miq.) Pax et Hoffm. Studies have shown that HB has anti-inflammatory, anti-tumor, anti-fungal, and anti-bacterial activities[7-10]. HB has a certain inhibitory effect on a variety of tumor cells, but there are few reports on the effect of HB on EC, and its mechanism of action has not been elucidated.

Based on the analysis methods of network pharmacology and molecular informatics, this study initially discussed the molecular mechanism of HB inhibiting EC, and provided reference for the clinical application of HB.

2 Materials and methods

2.1 HB-related targets

We collect the targets of HB and establish the active ingredient target data set through TCMSP and SwissTargetPrediction(http://www.swisstargetprediction.ch/) database.

2.2 EC-related targets

With "Esophageal Cancer" as a key word, We search the EC-related targets and establish disease targets data set through the GeneCards database(https://www.genecards.org/).

2.3 Network construction

We use the intersection of drugs and disease targets to find potential targets for HB to inhibit EC. The PPI network of potential targets was constructed through the String database (https://string-db.org/), cytoscape 3.7.1 software visually analyzed the PPI network . In this way,

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the median values of "Degree", "Betweenness centrality" and "Closeness centrality" are calculated.

2.4 Bioinformatic analysis

Genetic ontology (GO) analysis of biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis through the DAVID (https://david.ncifcrf.gov/tools.jsp). The R language tool performs visual analysis, P≤0.05 as the screening criteria to screen out biological processes, cell compositions, molecular functions and signal pathways with extremely significant differences.

2.5 Pathway mapper construction

The core targets are mapped to related pathways mainly through KEGG (https://www.genome.jp/kegg/)Mapper function.

3 Results

3.1 Target prediction

We obtained 108 targets corresponding to HB in TCMSP and Swiss Target Prediction database, and 5801 EC targets were obtained in GeneCards database.The results are shown in Figure 1.

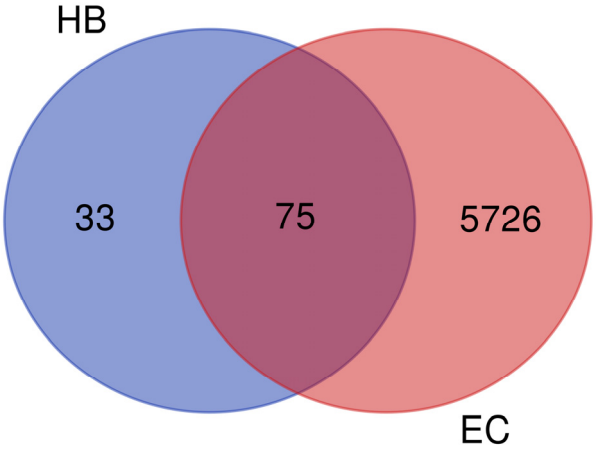

Fig. 1. Drug-disease intersection target Venn diagram

3.2 Screening of core targets

Cytoscape 3.7.1 software was used for visual analysis, and the core target was selected that satisfies the median value of more than three topological parameters at the same time. The median values of the three topological parameters are 11 (Degree), 0.0070 (Betweenness centrality), 0.4534 (Closeness centrality). The results are shown in Table 1 and Figure 3.

3.3 Drug-disease-core target network construction

Cytoscape 3.7.1 software was used constructed "drug-disease-core target" network, and the results are shown in Figure 2.

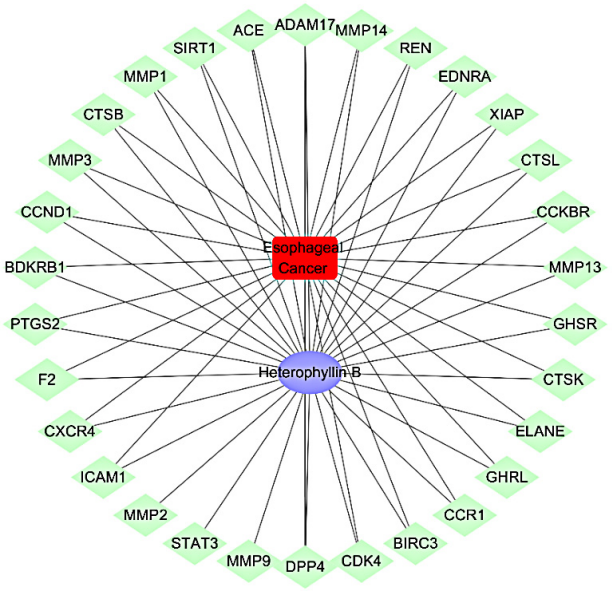

Fig.2. Drugs-diseases-core targets network

3.4 GO Biological process enrichment analysis

The top 10 biological processes, cell composition and molecular functions were selected from 86 biological processes.The results are shown in Figure 4. The results show that HB may inhibit EC through multiple biological processes such as proteolysis, collagen catabolic process, extracellular matrix disassembly, positive regulation of cell proliferation, positive regulation of cytosolic calcium ion concentration.

Table 1. Related topological parameters of the core target

UniProt CID	Gene name	Protein name	Degree	Closeness Centrality	Betweenness Centrality
P14780	MMP9	Matrix Metallopeptidase 9	35	0.6460	0.1087
P40763	STAT3	Signal Transducer And Activator Of Transcription 3	29	0.6134	0.1304
P08253	MMP2	Matrix Metallopeptidase 2	27	0.6033	0.0536
P05362	ICAM1	Intercellular Adhesion Molecule 1	26	0.5935	0.0852
P61073	CXCR4	C-X-C Motif Chemokine Receptor 4	25	0.5794	0.0882
P00734	F2	Coagulation Factor II, Thrombin	24	0.5573	0.0988
P35354	PTGS2	Prostaglandin-Endoperoxide Synthase 2	23	0.5703	0.0459

P46663	BDKRB1	Bradykinin Receptor B1	20	0.4679	0.0366
P24385	CCND1	Cyclin D1	20	0.5368	0.0468
P08254	MMP3	Matrix Metallopeptidase 3	18	0.5252	0.0097
P07858	CTSB	Cathepsin B	18	0.4679	0.0468
P03956	MMP1	Matrix Metallopeptidase 1	17	0.5214	0.0114
Q96EB6	SIRT1	Sirtuin 1	17	0.5177	0.0432
P12821	ACE	Angiotensin I Converting Enzyme	16	0.5105	0.0131
P78536	ADAM17	ADAM Metallopeptidase Domain 17	16	0.5105	0.0156
P50281	MMP14	Matrix Metallopeptidase 14	16	0.5368	0.0237
P00797	REN	Renin	15	0.5328	0.0290
P25101	EDNRA	Endothelin Receptor Type A	15	0.4740	0.0169
P98170	XIAP	X-Linked Inhibitor Of Apoptosis	15	0.4867	0.0162
P07711	CTSL	Cathepsin L	15	0.4534	0.0267
P32239	CCKBR	Cholecystokinin B Receptor	14	0.4534	0.0093
P45452	MMP13	Matrix Metallopeptidase 13	14	0.5177	0.0100
Q92847	GHSR	Growth Hormone Secretagogue Receptor	14	0.4620	0.0202
P43235	CTSK	Cathepsin K	14	0.4740	0.0087
P08246	ELANE	Elastase, Neutrophil Expressed	14	0.5034	0.0146
Q9UBU3	GHRL	Ghrelin And Obestatin Prepropeptide	13	0.4834	0.0299
P32246	CCR1	C-C Motif Chemokine Receptor 1	12	0.5141	0.0193
Q13489	BIRC3	Baculoviral IAP Repeat Containing 3	12	0.4834	0.0073
P11802	CDK4	Cyclin Dependent Kinase 4	12	0.4740	0.0174
P27487	DPP4	Dipeptidyl Peptidase 4	11	0.4932	0.0107

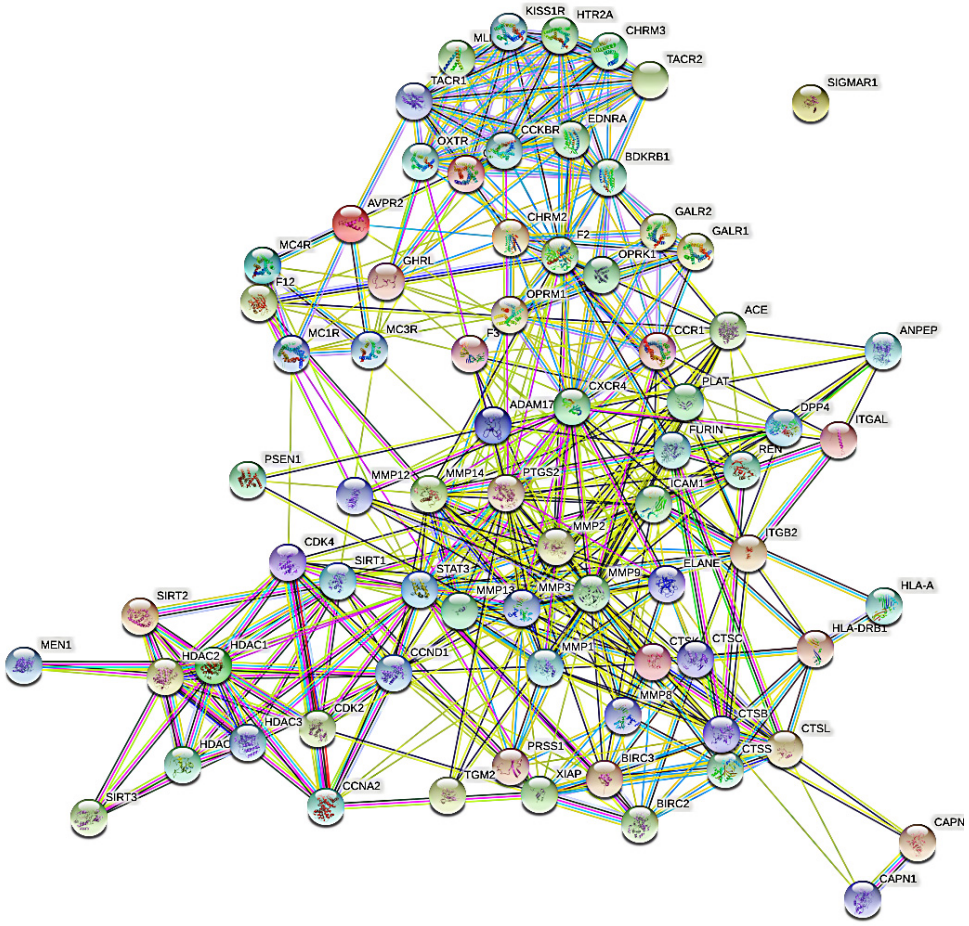

Fig. 3. The protein interaction network of drug-disease intersection target

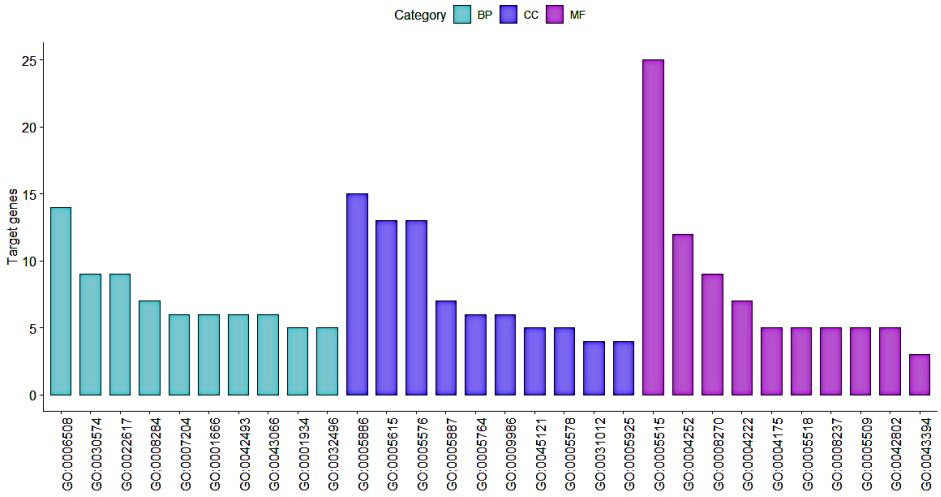

Fig. 4. Top 10 biological process enrichment results

3.5 KEGG Pathway enrichment analysis

The 13 signal pathways were enriched and analyzed by KEGG, and the results are shown in Figure 5. The main signaling pathways include Pathways in cancer, TNF signaling pathway, Bladder cancer, Small cell lung cancer, Rheumatoid arthritis related pathways.

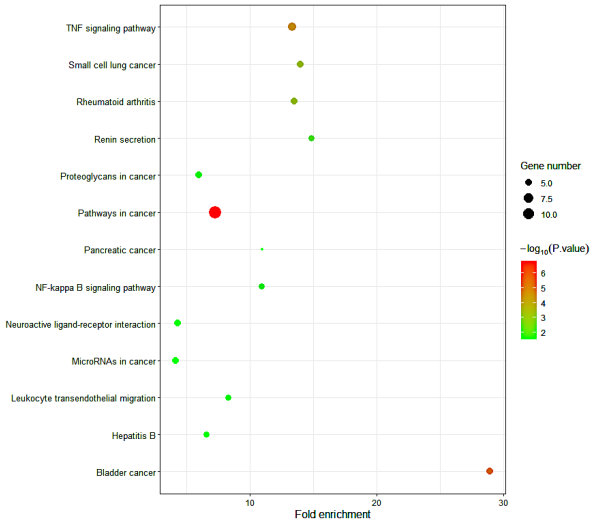

Fig. 5. The 13 pathways enriched by major hubs

3.6 Pathway annotation diagram of HB's anti-EC effect

We input the intersection target and mark the number of targets in each pathway through the KEGG Mapper function of the KEGG database. 12 target proteins are involved in the Pathways in cancer.

4 Discussion

Through the analysis of network pharmacology, MMP9, MMP2, CCND1, STAT3, CXCR4, BDKRB1, PTGS2, may be the potential targets for HB in the treatment of EC. In the expression of MMPs-related genes, MMP9 and MMP2 are closely related to the metastasis of a variety of human carcinoma, including esophageal cancer[11-12].

GO biological process enrichment analysis demonstrates that HB's anti-EC effect involves biological processes such as collagen catabolic process, extracellular matrix disassembly, proteolysis, positive regulation of cytosolic calcium ion concentration. The main biological process may include proteolysis, collagen catabolic process, extracellular matrix disassembly, positive regulation of cell proliferation, positive regulation of cytosolic calcium ion concentration.

KEGG pathway enrichment analysis showed that HB anti-EC effects through regulating Pathways in cancer, TNF signaling pathway, Bladder cancer, Small cell lung cancer, Rheumatoid arthritis related pathways. The expression of MMP2 and MMP9 levels is mediated by multiple signals, PI3K/AKT is one of the main pathways[13]. HB inhibits the adhesion and invasion of ECA-109 cells mainly through the PI3K/AKT/β-catenin pathway that down-regulating the expression of MMP2 and MMP9[14].

5 Conclusions

A total of 75 potential targets were obtained, 30 of which are core targets for HB to inhibit EC. GO biological process enrichment analysis and KEGG pathway enrichment analysis revealed 10 biological processes, cell composition, molecular functions and 13 pathways are closely related to the occurrence and development of inhibiting EC, mainly involving Pathways in cancer, TNF signaling pathway, Bladder cancer, Small cell lung cancer, Rheumatoid arthritis. This study preliminarily explored the mechanism of HB inhibiting EC at the molecular level, and provided a reference for the discovery of new targets for EC.

Acknowledgments

The authors would like to thank the financial support from the Scientific Research Foundation of Hunan Provincial Education Department (20B415), the Technology Project Foundation of Tongren ([2018]48), the Technology Project Foundation of Tongren ([2019]55).

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