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Integrating Network Pharmacology to Explore the Pharmacology Mechanisms of Meliae Cortex Against Hepatocellular Carcinoma

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Abstract: ***Objective:*** *Using network pharmacology to study the active ingredients and mechanism of action of Meliae Cortex in the treatment of Hepatocellular Carcinoma, providing theoretical basis for clinical application.* ***Methods:*** *Applying the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP) to obtain the active ingredients and targets of Meliae Cortex, Using Genecard (https://genecard, org/) database and Online Mendelian Inheritance in Man (OMIM) database (http://omim. org/), select " Hepatocellular Carcinoma " as the keyword to search for disease targets of Hepatocellular Carcinoma, After obtaining the intersection targets of Melia azedarach and Hepatocellular Carcinoma using Venny, a common target visualization network diagram was constructed using STRING database and Cytoscape. Realize gene (GO) enrichment analysis and (KEGG) enrichment analysis using the metascape database and microbiome platform.* ***Results:*** *Obtained 15 active ingredients from Meliae Cortex, 5024 Hepatocellular Carcinoma targets, and 32 common targets. GO analysis shows that biological processes mainly involve the regulation of responses to steroid hormones, estradiol, organic compounds, and various chemical and physical stimuli; KEGG enrichment shows that anti-cancer effects are mainly exerted through pathways such as AGE-RAGE and TNF.* ***Conclusion:*** *Using network pharmacology methods, it has been demonstrated that Meliae Cortex exerts therapeutic effects on Hepatocellular Carcinoma through multiple components, targets, and pathways, providing a theoretical basis for clinical application.*

Keywords: Meliae Cortex, Hepatocellular Carcinoma, Network Pharmacology.

1. Introduction

Hepatocellular Carcinoma is a common malignant tumor. According to global data statistics in 2020, Hepatocellular Carcinoma ranks third in cancer mortality and sixth in incidence rate [1]. And the incidence rate of primary Hepatocellular Carcinoma is increasing [2]. Due to the involvement of multiple mutated genes in Hepatocellular Carcinoma, intervention with a single target cannot be effective. The thinking mode of scientific research today has shifted from a single causal relationship to the interaction between multiple factors, as well as the network formed by this interaction, especially for traditional Chinese medicine with multiple components, targets, and pathways. CORTEX MELIAE is the dry bark and root bark of Melia toosendan sieb. et Zucc, a plant in the Meliaceae family. It is irregularly shaped and widely distributed in China. Meliae Cortex is a traditional Chinese medicine that can clear heat, dry dampness, and kill insects. It is recorded in "Bie Lu", "Ri Hua Zi Ben Cao", and "Yi Lin Ji Yao". In recent years, studies have found that Meliae Cortex has significant anti-tumor activity [4]. In addition to excellent insecticidal activity, TSN also has functions such as anti-inflammatory, anti botulinum toxin, antibacterial, antiviral, and analgesic [5]. In addition, recent studies have shown that TSN also has a broad-spectrum anti-cancer effect in lung cancer, gastric cancer, sarcoma, colorectal cancer, pancreatic cancer, glioma and other tumors [6-11] Because TCM treatment of diseases has the characteristics of multiple components, multiple targets, and multiple pathways working together, research on the biological mechanism of TCM treatment of diseases needs to take into account the integrity and systematicness. Network pharmacology is a new method for studying the interactions between active compounds, targets, and diseases in traditional Chinese medicine. Essentially, it is a systems biology based on big data information mining. This method has the characteristics of scientific and visual evaluation of drug efficacy and research on the mechanism of action. Based on this, this study aims to explore the potential mechanism of treating Hepatocellular Carcinoma by analyzing the corresponding targets and signaling pathways of the active ingredients of Meliae Cortex.

2. Materials and Methods

2.1 Using " Meliae Cortex " as a keyword, input it into the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw. com/tcmsp. php) to search for the corresponding compounds of the ingredients.

2.2 Active ingredient and target protein screening of drugs. Oral bioavailability (OB) of drugs is one of the important indicators in pharmacokinetic ADME parameters, which refers to the speed and degree of absorption of effective active ingredients into the human bloodstream. The lower the OB value, the worse the therapeutic effect of the drug may be. Drug like properties (DL) refer to the degree of similarity between a compound and a known drug. Typically, the lower the DL value, the lower the likelihood that the compound will be used as an active ingredient in a drug. Therefore, based on the criteria of OB $\geq$ 30% and DL $\geq$ 0.18, further screening was carried out to identify components with high biological activity. Match the ingredients with drug targets in TCMSP and correct the obtained targets to standard gene names through the Uniprot database (https://www.uniprot. org/).

2.3 Disease targets are searched and screened for known disease targets using the Genecard (https://genecard, org/) database and the Online Mendelian Inheritance in Man (OMIM) database (http://omim. org/) using the keyword "Hepatocellular Carcinoma". Duplicate targets are deleted to obtain known targets. Using R language to match and map the targets related to drug active ingredients and disease targets, and drawing a Venn diagram to obtain potential anti-Hepatocellular Carcinoma targets of the active ingredients in Melia azedarach peel.

2.4 Using the STRING database platform (https://stnng db. org/Version 10) 5) Predict protein-protein interaction relationships. Import the intersection targets of the anti Hepatocellular Carcinoma effect of Melia azedarach peel into the String database, select the studied species as Homo sapiens, obtain protein interaction relationships, and export the results in TSV format. The light blue line represents protein-protein interactions from the database, while the purple line represents experimentally validated protein-protein interactions.

2.5 Constructing a "Drug Component Target Disease" network using complex network visualization analysis and editing software Cytoscape (Version 3.8.0) to construct a "Drug Component Target Disease" network diagram. Using Betweenness, Closeness, and Degree values to sort and remove free targets, the core targets of the anti Hepatocellular Carcinoma effect of Melia azedarach peel were obtained. Afterwards, the core eight point STRING bubble chart will be imported into Cytoscape (Version 3.8.0) in TSV format. In the network, nodes represent the active ingredients, key target genes, and Hepatocellular Carcinoma of the bitter azalea bark drug. Edge represents the interaction relationship between bitter azalea bark and its corresponding active ingredients, active ingredients and target genes, and diseases and target genes.

2.6 Constructing a protein-protein interaction (PPI) network using the STRING platform (https://string-db.org/) Construct an intersection target protein-protein interaction (PPI) network, ranked by Degree value, to clarify the key targets of the main active ingredients in Melia azedarach on Hepatocellular Carcinoma.

2.7 Pathway enrichment analysis will import the obtained core genes into the Metascape database (https://metascape.org/) Perform gene ontology (GO) biological function enrichment analysis and Kyoto Encyclopedia of Genes and Ge nodes (KEGG) signaling pathway enrichment analysis. And with the help of the bioinformatics platform (bioinformatics. com. cn), the enriched results will be plotted into bubble charts and bar charts.

3. Results

3.1 Acquisition of Active Ingredients in Drugs

In the TCMSP database, a total of 43 compounds were obtained by searching for bitter azalea bark. After screening based on OB ≥ 30% and DL ≥ 0.18, a total of 15 active ingredients were obtained, as shown in Table 1.

Table 1: Active ingredients, oral bioavailability, and class drug properties of Meliae Cortex

Herb	number	Active ingredient name	MOL ID	OB (%)	DL
Meliae Cortex	1	(+)-Syringaresinol-di-O-β-D-glucosid _qt	MOL010628	34.99371	0.72284
	2	4,8-dimethoxy-1-vinyl-beta-carboline	MOL010629	66.77594	0.19657
	3	kulactone	MOL010631	45.43808	0.81495
	4	kulinone	MOL010632	44.87867	0.77489
	5	kulolactone	MOL010633	43.96732	0.81198
	6	nimbolin A	MOL010638	32.1122	0.34177
	7	nimbolin B	MOL010639	30.54288	0.29875
	8	sendanolactone	MOL010643	63.17715	0.9045
	9	trichilin A	MOL010646	39.58859	0.27589
	10	trichilinin B	MOL010647	30.74114	0.46813
	11	trichilinin D	MOL010648	33.51429	0.34287
	12	SMR000232316	MOL010650	35.43154	0.73898
	13	beta-sitosterol	MOL000358	36.91391	0.75123
	14	sitosterol	MOL000359	36.91391	0.7512
	15	Stigmasterol	MOL000449	43.82985	0.75665

3.2 Acquisition of Drug Targets

Using TCMSP for target screening of 15 active compounds, a total of 52 targets were obtained.

3.3 Acquisition of Hepatocellular Carcinoma Disease Targets

Using Genecard (https://genecard, org/) database and Online Mendelian Inheritance in Man (OMIM) database (http://omim.org/), "Hepatocellular Carcinoma" was selected as the keyword for disease target search and screening After taking two median values of the targets obtained from the Genecard database, the top 4671 targets were retained and added to the 519 targets obtained from the OMIM database. After removing duplicate targets, 5024 disease targets were obtained. Using R language to match and map drug active ingredient related targets and disease targets, and draw a Venn diagram (Venn) as shown in Figure 1. Obtaining potential targets for anti-Hepatocellular Carcinoma effects of active ingredients from Melia azedarach peel.

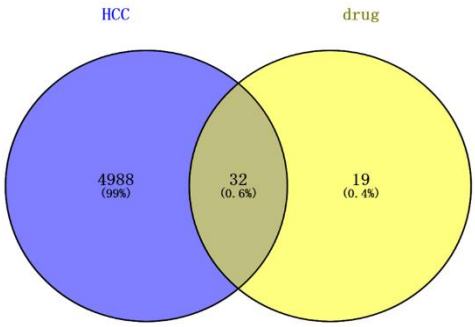

Figure 1: Venny diagram of the intersection of drug targets and disease targets

3.4 Acquisition of Common Targets

In order to further clarify the common targets of Melia azedarach and Hepatocellular Carcinoma, the intersection part in the Venny diagram was obtained, and the specific gene names are shown in Table 2.

Table 2: Intersection gene names

Gene name	Gene name	Gene name	Gene name
CASP8	PGR	PTGS1	IGHG1
TGFB1	PLAU	NCOA1	SLC6A3
BAX	CASP9	ADRB2	GABRA3
JUN	PIK3CG	MAOA	HTR2A
RXRA	RELA	NCOA2	SLC6A4
BCL2	PRKCA	AKR1B1	KCNH2
PTGS2	ADH1C	NR3C2	MAP2
CASP3	PON1	OPRM1	MAOB

3.5 Building a PPI Network

Input the obtained 32 intersecting target proteins into the String platform, set the confidence level to Highest ≥ 0.4, and construct a PPI network. See Figure 2.

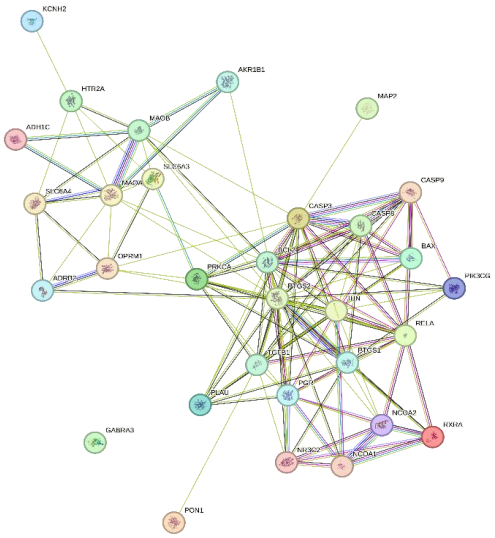

Figure 2: String of Intersection target

Import the PPI network into Cytoscape software, sort it by Degree value, and obtain 30 core proteins. The higher the Degree value, the more important the corresponding target is in the network, and the larger the graph, as shown in Figure 3.

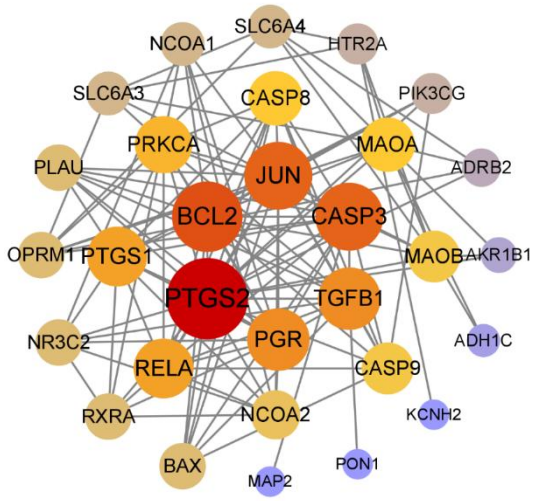

Figure 3: PPI network of Intersection target

3.6 Construction of Drug Component Target Disease Co expression Network

Produce files on the active ingredients, key target genes, interactions between key target genes and Hepatocellular Carcinoma, as well as node attributes of Meliae Cortex, and import them into Cytoscape to construct a "Drug Component Target Disease" network. See Figure 4. Among them, the top 5 drug components ranked from high to low in terms of their relationship with Hepatocellular Carcinoma are beta sitosterol at 21, Stigmasterol at 16, and (+) - Syringaresinol di-O- β-D-glucosid_qt is 4, sendanolactone is 4, and sitosterol is 4, as shown in Table 3.

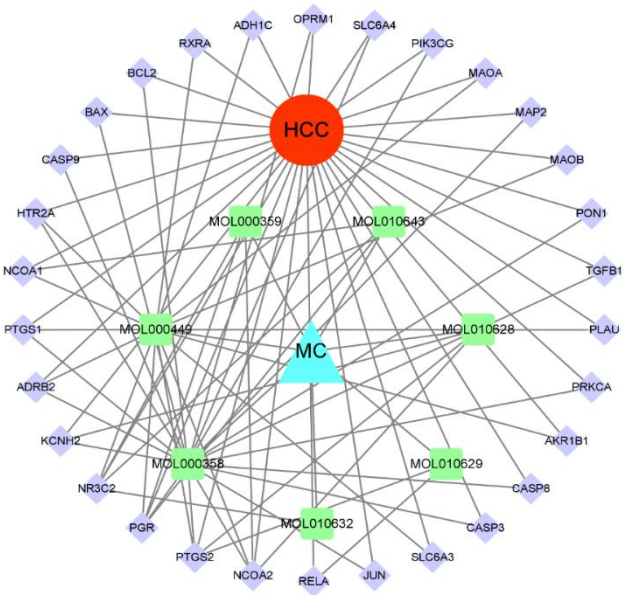

Figure 4: Drug component target disease co expression network diagram

Table 3: Top 5 Drug Active Ingredients with Degree Values

Active ingredient name	Degree	Source
beta-sitosterol	21	Meliae Cortex
Stigmasterol	16	Meliae Cortex
(+)-Syringaresinol-di-O-β-D-glucosid _qt	4	Meliae Cortex
sendanolactone	4	Meliae Cortex
sitosterol	4	Meliae Cortex

3.7 GO Enrichment Analysis

Using metascape, GO analysis was conducted on relevant target proteins in terms of molecular function, cellular components, and biological processes. After comprehensive correction of logP values and enrichment of genes in this function, the top ranked genes were selected and presented in a bar chart using the microbiome platform, as shown in Figure 5. The analysis results showed that the above proteins mainly involve reactions to steroid hormones, cells to organic cyclic compounds, and hormones in biological processes; In terms of cellular composition, it mainly affects the outer membrane of organelles and mitochondria; In terms of molecular function, it mainly participates in the regulation of cysteine endopeptidase activity in the apoptotic signaling pathway, cysteine endopeptidase activity in the apoptotic process, and transcription factor binding. It is speculated that Meliae Cortex may exert its anti-tumor effects in the treatment of Hepatocellular Carcinoma through the above functions.

3.8 KEGG Pathway Enrichment Analysis

Perform KEGG analysis on 30 key anti-cancer genes, as shown in Figure 6. There are a total of 94 pathways, sorted according to the LogP value, and the top 20 pathways are selected to draw bubble charts for display, mainly involving pathways in cancer, Small cell lung cancer, Lipid and astroclear, AGE-RAGE signaling pathway in diabetic composites, Apoptosis, Colorectal cancer, TNF signaling pathway, and other signaling pathways.

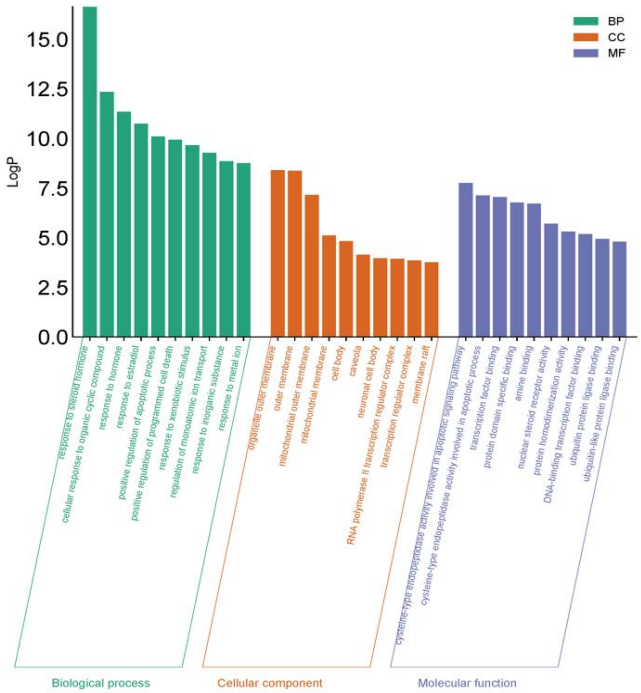

Figure 5: GO enrichment analysis

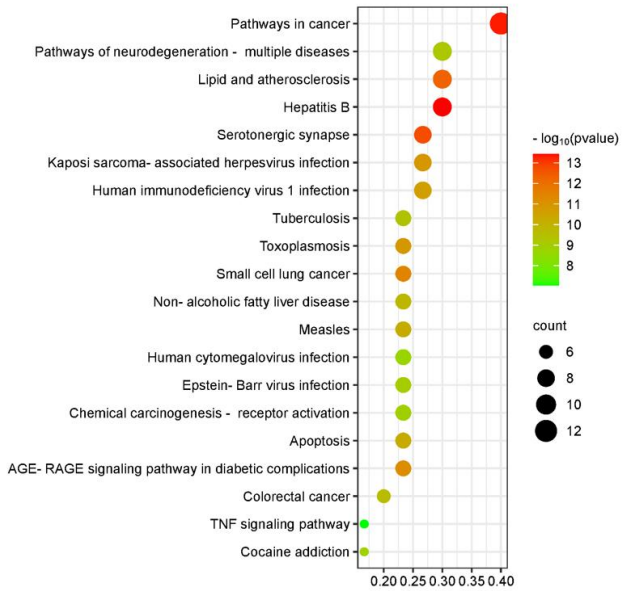

Figure 6: KEGG pathway enrichment analysis

4. Discussion

Hepatocellular Carcinoma is one of the most common malignant tumors in China, and its incidence rate and mortality are only second to those of gastric cancer and lung cancer. Hepatocellular Carcinoma can occur at any age stage, but is more common between the ages of 31 and 50. Most Hepatocellular Carcinoma patients are diagnosed in the advanced stage, and surgical opportunities are often missed. The modern comprehensive treatment methods that can be used are often limited to radiotherapy, chemotherapy, and immunotherapy. However, radiotherapy and chemotherapy have significant toxic side effects on the treatment of this disease, reduced indications, and poor efficacy. Hepatocellular Carcinoma is a disease that has been recorded as early as the Neijing; Throughout history, it has been referred to as Feiqi, Piqi, and Jiqi. Hepatocellular Carcinoma is a malignant disease characterized by deficiency of qi and blood in the organs, with qi, blood, dampness, heat, stasis, and toxin as the main indicators. It accumulates in the liver and gradually becomes a symptom accumulation. The basic pathogenesis is liver failure and laxity, with swelling, hard pain, emaciation, loss of appetite, fatigue, or jaundice or coma in the right flank as the main manifestations. Therefore, the important treatment principles for Hepatocellular Carcinoma include clearing dampness and heat, supplementing qi and promoting blood circulation, promoting blood circulation and removing stasis, purging fire and detoxifying, and regulating liver and qi. Melia azedarach peel has the effects of clearing heat, drying dampness, and repelling insects. Its main components, beta sitosterol, Stigmasterol, and Toosendanin, all have good anti-tumor effects. In recent years, various studies have shown that TSN can inhibit growth and induce apoptosis in gastric cancer, osteosarcoma, glioblastoma, non-small cell lung cancer, colorectal cancer, Hepatocellular Carcinoma, pancreatic cancer and other tumor cells [12]. The chemical isomers of TSN also have a good inhibitory effect on the growth of various cancer cells, and its IC50 value is much lower than that of normal human liver L-02 cells. TSN and ITSN can inhibit the growth of breast cancer cells, lung cancer cells, gastric cancer cells and colorectal cancer cells. These results indicate that TSN and ITSN have great potential for

development as anticancer candidate drugs [13].

4.1 Core Active Ingredients of Drugs

By obtaining the active ingredients of Meliae Cortex and according to the network diagram of "drug ingredient target disease", the main active ingredients of Meliae Cortex, beta sitosterol and Stigmasterol, are the compounds with the highest degree of anti-Hepatocellular Carcinoma target in the topological network, that is, the most core node, indicating that these two active ingredients play an important role in the treatment of Hepatocellular Carcinoma. Beta sitosterol is a natural compound with anticancer properties against various types of cancer. β-Glutinosterol downregulates the expression of FOXM1 in vitro and in vivo. FOXM1 overexpression alleviated β- The inhibitory effect of sitosterol on HepG2 cells. In addition, β-Glutinosterol inhibits epithelial mesenchymal transition (EMT) in HepG2 cells, while overexpression of FOXM1 promotes EMT. Mechanistically speaking, β-Sitosterol inhibits Wnt by downregulating FOXM1/β-Catenin signaling regulates the transcription of target genes related to HepG2 cell proliferation and metastasis. β-Downregulation of sitosterol through FOXM1 and Wnt/ β-Catenin signal inhibition has shown great potential as a candidate therapeutic drug for inhibiting HCC growth and metastasis [14]. After treatment with Stigmasterol in mice, significant upregulation of Caspase3, Bax, and P53 expression was observed, as well as a decrease in cyclin D1 expression, ultimately leading to a reduction in tumor volume. In addition, stigmasterol can alter the gut microbiota α and β Diversity, and significantly increase the abundance of Lactobacillus johnsonii, Lactobacillus murinus, and Lactobacillus reuteri, which can reduce the proportion of regulatory T cells (Tregs) and CD8+T cells in the intestinal tract and tumor tissue, thereby enhancing the immune response of tumor microenvironment (TME) of HCC host [15].

4.2 Key Target Genes

Analysis of the PPI network revealed that PTGS2, BCL2, JUN, and CASP3 genes have the closest interaction relationship and are at the core of the network, which can be regarded as key therapeutic targets. 1) The anti apoptotic BCL2 and the prostaglandin endoroxide synthese-2 (PTGS2) are abundant in gastric cancer and are associated with low patient survival rates, closely related to cisplatin resistance. PTGS2 plays an important mediating role in the development of cisplatin resistance by mediating the inhibitory effect of cisplatin on BCL2 expression. Mechanistically speaking, cisplatin passes through ROS/NF- κ B pathway induces PTGS2 expression. In addition, PTGS2 mediates cisplatin induced BCL2 expression and subsequently resists cell apoptosis through the PGE2/EP4/MAPKs (ERK1/2, P38) axis. Clinical specimen analysis shows that PTGS2 and BCL2 are positively correlated with human gastric cancer. In addition, in xenograft models, the inhibition of PTGS2 by celecoxib significantly enhanced the cytotoxicity of cisplatin in drug-resistant gastric cancer by inhibiting the expression of PTGS2 and BCL2 regulated by the ERK1/2 and P38 signaling axes, indicating that PTGS2 may be used as an adjuvant therapeutic target to reverse chemotherapy resistance in the cisplatin resistant gastric cancer subgroup. The prostaglandin endoroxide synthase-2 (PTGS2) gene encodes the main enzyme responsible for converting arachidonic acid to prostaglandins, which is associated with the survival of GC patients. PTGS2 mediates cisplatin induced BCL2 expression and subsequently exerts resistance to cell apoptosis through a PGE2/EP4/MAPKs (ERK1/2, P38) dependent mechanism. Changes in PTGS2 expression trigger changes in BCL2 expression and resistance to cisplatin [16]. C-JUN protein is a type of activated protein-1 (AP-1) transcription complex, which is a transcription factor with transcriptional activity in the AP-1 complex. Various physical, chemical, biological stimuli, and cellular stress responses can promote the expression and activation of C-JUN protein, thereby regulating biological processes such as cell proliferation and apoptosis.

The c-Jun protein also participates in the growth regulation of tumor cells under various extracellular stimuli such as growth factors, cytokines, and stress. The expression and activity regulation of c-Jun protein is regulated by multiple protein kinases, and it is a mechanism for multiple signaling molecules to activate and regulate multiple signaling pathways. The expression of Fos and Jun oncogenes is caused by various extracellular stimuli, which are related to neuronal mitosis, differentiation, or depolarization [17]. Caspase-3 initiates the clearance of GSDME in response to a variety of cells.

4.3 GO and KEGG Enrichment Analysis

GO enrichment analysis showed that Meliae Cortex mainly exerts its anti Hepatocellular Carcinoma effect by regulating functional processes such as steroid hormone and receptor binding, DNA transcription activation, and oxidative stress response.

The KEGG pathway results showed that multiple signaling pathways related to cell apoptosis and autophagy were designed during the treatment process of Melia azedarach. 1) AGEs interact with their receptor RAGE to activate genes and proteins involved in multiple signaling pathways. The activation of AGE-RAGE signaling also disrupts cellular redox balance and regulates various cell death pathways. During the development of malignant tumors, programmed cell death signals often undergo changes. AGEs combined with RAGE can activate various signaling pathways related to cell survival, inflammation, and cancer progression, including MAPK, ERK1/2, PI3K, Akt, JAK-STAT, and NF-KB [18]. RAGE and its ligands not only promote cell survival and inflammation around the tumor microenvironment, but also promote angiogenesis, cell migration, cell proliferation, invasion, and metastasis by limiting cell death caused by apoptosis RAGE can also accumulate due to hypoxia caused by tumors, and the involvement of the AGE-RAGE axis has been shown to promote autophagy flux while inhibiting apoptosis signals in cancer cells. [19] 2) TNF signaling pathway: TNF signaling pathway: tumor necrosis factor α (TNF)- α) It is the most powerful anti-tumor cytokine discovered so far. TNF- α By activating the SAKP/JNK signaling pathway, the sensitivity of lung cancer cells to radiotherapy is enhanced and cancer metastasis is inhibited.

In summary, this study used network pharmacology methods to obtain the core active ingredients and key target proteins of

Meliae Cortex in the treatment of Hepatocellular Carcinoma. GO and KEGG enrichment analysis was performed on the intersecting targets, elucidating that Meliae Cortex acts on Hepatocellular Carcinoma through multiple compounds, targets, and pathways, providing a scientific theory for clinical application. However, due to the limitations of this study based on large database mining, clinical and in vitro and in vivo studies are still needed to validate our findings.

Fund Project

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