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Exploration on the Mechanism of the Effect of Icariin on Hepatocellular Carcinoma Based on Network Pharmacology

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Abstract: Hepatocellular carcinoma is an important cause of human death, Icariin has been found to have anti-tumor effects, which have been demonstrated in a variety of oncology experiments. In order to explore the mechanism of Icariin's action on hepatocellular carcinoma, we have analyzed it by using several platforms. Finally, we obtained six targets, which are TNF, PRKCE, PRKCD, IL1B, NFKB1, and PTGS2. Among them, TNF is the key target of Icariin to inhibit hepatocellular carcinoma. Meanwhile, the study also found that Icariin's action on hepatocellular carcinoma may be closely related to immunomodulation, apoptosis, AGE-RAGE, NF-kappaB and other pathways.

1. Introduction

Tumors, especially malignant tumors, are common and frequent diseases that seriously endanger human health. With the development of society, changes in the living environment and daily diet, the overall morbidity and mortality of malignant tumors have continued to rise in recent years, and there is a trend of rejuvenation [1]. Studies have shown that cancer has afflicted multicellular organisms for more than 200 million years, and the cancer of human ancestors can be traced back to over a million years ago. Unlike infectious diseases, parasites and many environmental diseases, cancer is not primarily caused by some substances foreign to our bodies. Its destructive factors are human cells that lose control of proliferation and transform into pathological masses in some way [2].

Primary liver cancer refers to hepatocellular carcinoma (HCC), is one of the most common and invasive malignant tumors, which is a major cause of cancer-related deaths. The liver cancer situation in China is more severe, with approximately 380, 000 people dying from it every year, accounting for 51% of the global deaths. In modern medicine, the risk factors for liver cancer include hepatitis B, cirrhosis, family history of cancer, history of alcohol drinking and smoking, age over 40 and etc. And studies found that the incidence of liver cancer in people living in coastal areas is far higher than that in inland. It may be due to the hot and humid climate, which causes the breeding of carcinogens in food, such as "aflatoxin". The liver is the sixth most common site for primary cancers, and hepatocellular carcinoma (HCC) is the main form of primary liver cancer in adults. The development

of HCC is associated with various risk factors, including viral infections (hepatitis B virus (HBV) and hepatitis C virus), metabolic alterations (alcoholic steato hepatitis and non-alcoholic steatohepatitis, food impurities (e.g., aflatoxins), or parasitic infections (e.g., trematodes) [3]. Vaccination and new antiviral therapies can inhibit the increase of HBV-related or HCV-related hepatocellular carcinomas, but lifestyle factors, such as chronic alcohol consumption, dietary habits and sedentary lifestyles, can aggravate chronic inflammation and increase the incidence rate of liver tumors [4]. HCC is one of the tumors most closely related to environmental factors, the variable natural history, the unusually high number of environmental susceptibilities and individual genetic susceptibility to HCC are characterized by a high degree of tumor heterogeneity. Although modern medicine has made progress in identifying risk factors [5], the incidence rate of HCC continues to increase [5]. In addition, patients with this disease have limited treatment options and a low survival rate. Therefore, there is an urgent need for alternative and innovative therapeutic strategies.

At present, clinical treatments for liver cancer mainly include surgery, radiotherapy, chemotherapy and interventional therapy. Among them, surgery mainly includes hepatic resection and liver transplantation. Hepatic resection can prevent cancer cells from spreading again, which is especially for early stage of liver cancer. However, due to the large exposure of internal organs, there is a possibility of infection. At the same time, it needs to destroy the ligaments and nerves around the liver, which is not conducive to the recovery of patients after surgery and prone to adverse reactions. Liver transplantation can completely eliminate cancer cells and significantly improve the quality of life and survival time of patients, but it requires higher requirements on the basic physical condition of patients. Radiation is valuable for tumor control, symptom improvement, and survival rate, but can not improve symptoms like ascites and jaundice. Moreover, patients with severe complications and liver dysfunction usually can not use radiation therapy. Chemotherapy is often unsatisfactory because hepatocellular carcinoma is not sensitive to many chemotherapy drugs. And it often accompanied with cirrhosis, poor tolerance of chemotherapy and high toxicity reaction. Minimally invasive radiotherapy and ultrasound interventional therapy play important roles in local treatment and ablation of liver cancer. Nowadays, interventional therapy for liver cancer is usually used for liver cancer that cannot be surgically removed and cannot be cured or relapses after surgery. It can prevent tumor metastasis and consolidate treatment, but it may cause varying degrees of damage to liver function. Nowadays, it is advocated to combine traditional Chinese and Western medicine to treat liver cancer, which can obviously enhance the curative effect and reduce the toxic side effects.

In recent years, the development of traditional Chinese medicine (TCM) has gradually been widely recognized, and more and more experts have proposed the combination of Chinese and Western medicine in the treatment of cancer. Curcumin and allicin have shown some inhibitory effects on liver cancer cells. Epimedii Herba, commonly known as goat's horn herb or Yin Yang Huo [6], is an important Chinese herbal medicine widely used as a tonic, aphrodisiac and anti-rheumatism medicine in China and Korea. Icariin is a flavonoid and is the most abundant constituent of Epimedia, also known as Horny Goat Weed [6]. In the past few years, it has been widely studied and proven to have antioxidant, anti-neuroinflammatory and anti-tumor properties [7]. It is now considered a potential method for treating various diseases, from tumors to cardiovascular disease. In cultures and animal models of cerebral ischemia, depression, and Parkinson's, Icariin has been shown to attenuate the pro-inflammatory response of microglia. Icariin can also prevent hydrogen peroxide (H2O2), endoplasmic reticulum (ER) stress, and neurotoxicity induced by ribonucleic acid and homocysteine. Meanwhile, Icariin can stimulate osteoblast proliferation, and it has been shown that icariin have therapeutic benefits in preventing bone loss and osteoporosis [8].

2. Information and methods

2.1. Obtaining the target sites of Icariin

Firstly, we obtained the SMILE name of Icariin from PubChem, imported the SMILE name into SWISS Target Prediction database. And we obtained the gene name of the target of Icariin action, and deleted the target with zero probability.

2.2. Obtaining targets for liver cancer

We used GeneGards, DisGENET and Home-OMIM to download the related targets of liver cancer, and merged the targets from the three databases.

2.3. Get the intersection target of disease and drug

We used Microbiotics-Online Bioinformatics Analysis, Visualization Cloud Platform (www.bioinformatics.com.cn) to create Venn diagrams.

2.4. Construct protein interaction network diagram and screen core targets

We used the String database (https://string-db.org/), uploaded the obtained intersecting proteins and selected "Multiple proteins". Then we set the species as "Homo sapiens" and the minimum interaction requirement score as "Homo sapiens". The minimum interaction requirement score was set to 0.400, and the other values were kept as default. We obtained protein-protein interaction (PPI) and downloaded the data. We uploaded the data to Cytoscape 3.9.1, used it to calculate the BC value (Betweenness), parsed it to generate the visualized network. The larger the value of the node is, the more conducive it is for connecting and interacting with other nodes, which is more likely to play a critical role in the network.

2.5. GO enrichment analysis, KEGG pathway enrichment analysis

We imported the genes with BC values greater than 0 into metascape (https://metascape.org//), selected the species Homo sapiens and performed GO, KEGG analysis respectively.

3. Analyzing the results

3.1. Targets of Icariin

We screened 23 potential targets for icariin from Swiss Target Prediction database.

3.2. Hepatocellular carcinoma targets

12, 199 targets were obtained from GeneGards, 1, 377 targets were obtained from DisGENET, 173 targets were obtained from Home-OMIM, and 12, 361 disease targets were obtained after removing duplicates (Figure 1).

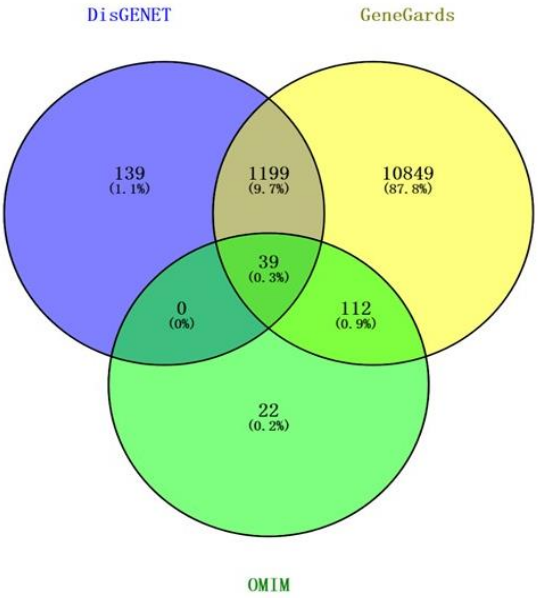


Figure 1: Disease targets

3.3. Disease-drug intersection targets

By removing duplicate values and intersecting disease targets with icarin targets, we obtained 18 common targets, including NQO2, ACHE, ADORA1, ADRA2C, AKR1B1, CA12, CA2, CD38, IL2, NOX4, PRKCA, PRKCB, PRKCD, PRKCE, PRKCH, PTGS2. RPS6KA3, TNF. (Figure 2)

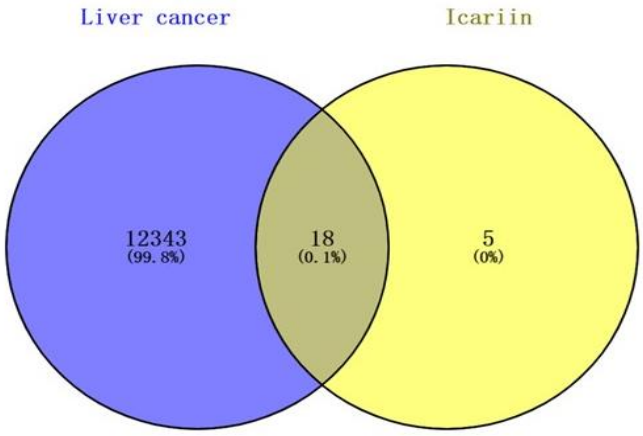


Figure 2: Common targets

3.4. Protein interactions network

Due to the number of cross targets was limited, we chose more proteins with strong correlations. It can be seen that there is a stronger correlation between NOX4, ADRA2C, IL2, PRKCA, PTGS2, and TNF (Figure 3).

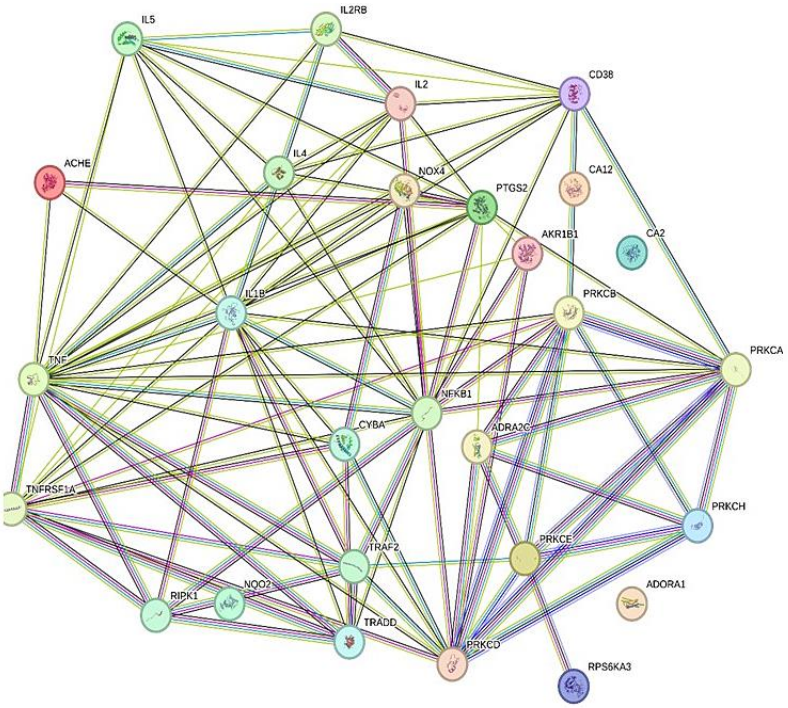


Figure 3: Related proteins

3.5. Core targets

We uploaded the data on STRING to Cytoscape 3.9.1, sorted them by using the BC value (Betweenness) and made a circle graph. The bigger the circle was, the bigger the BC value became, and the BC value of the red circle was bigger than the green circle. It can be seen that the protein with the largest BC value is TNF, followed by PRKCE, PRKCD, PTGS2, NFKB1, IL1B (Figure 4).

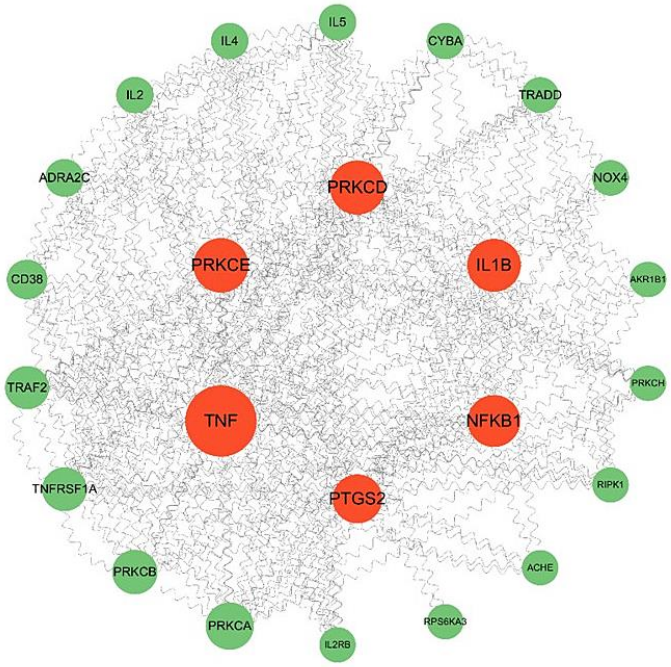
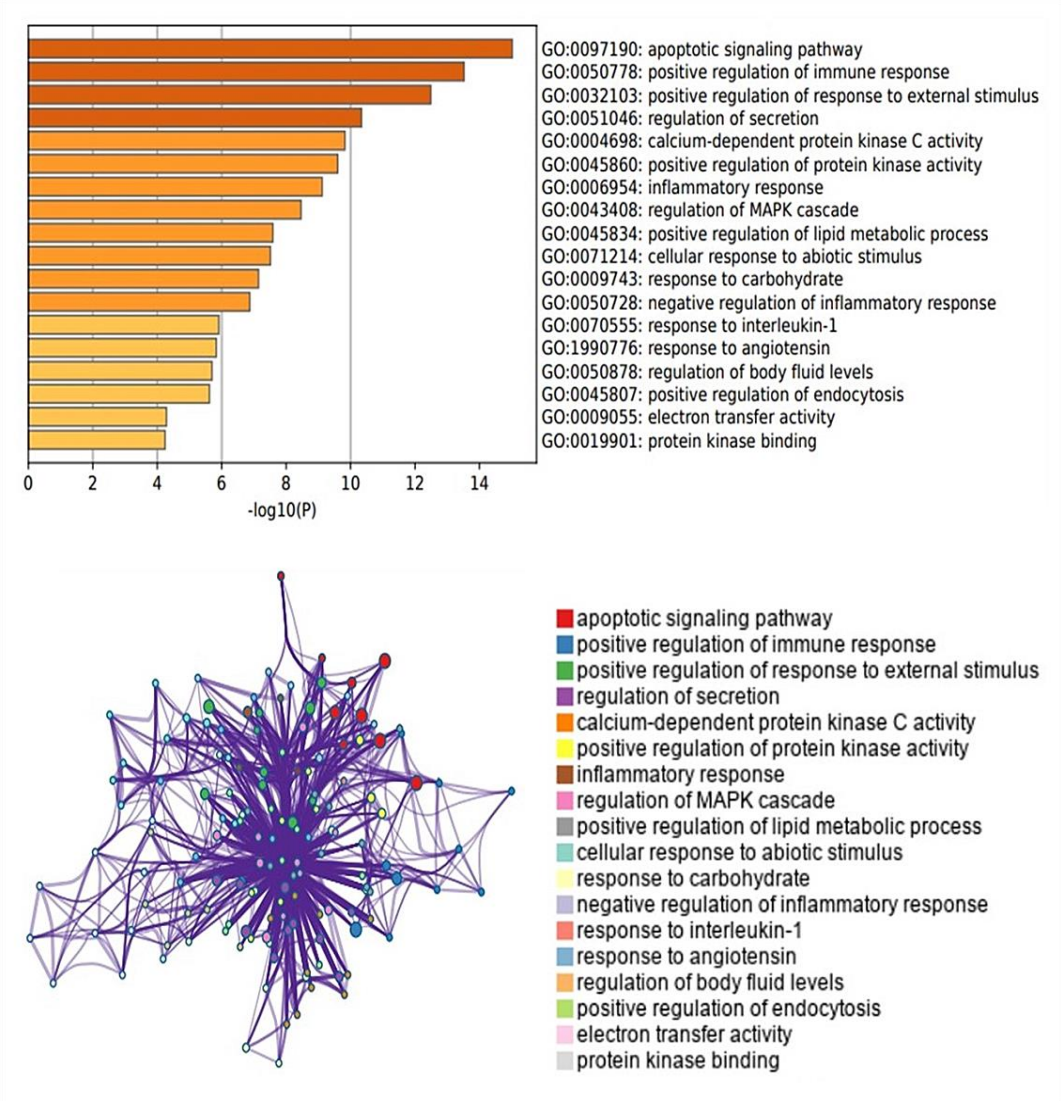


Figure 4: The protein with the BC value

3.6. Enrichment analysis results

Enrichment analysis of proteins with BC score greater than 0 in Cytoscape 3.9.1 showed that they are closely associated with immune regulation, secretion regulation, inflammatory response, lipid metabolism, apoptosis, AGE-RAGE, NF-kappaB and other pathways, and they also regulate protein kinase C activity, interleukin IL-17, and angiotensin. (Figure 5 and Figure 6)



Figures 5: The results of GO enrichment

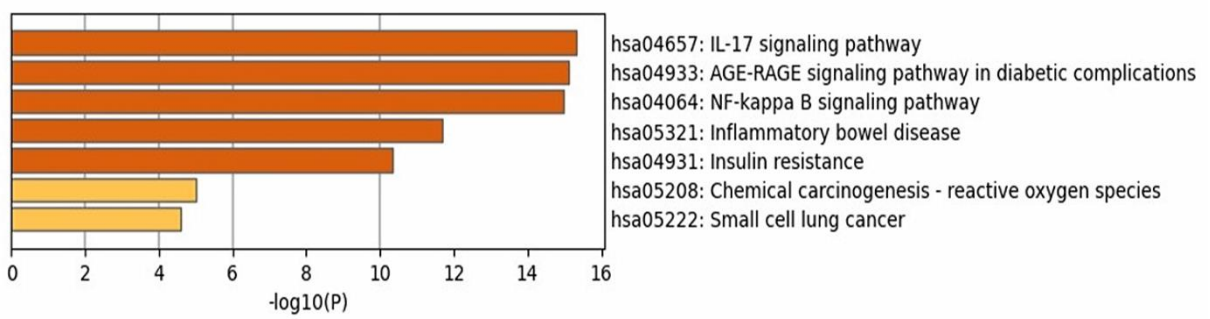


Figure 6: The results of KEGG enrichment

4. Discussion

As a major disease affecting human health, cancer has always been treated with various methods in clinical practice, including systemic chemotherapy, hormone therapy, targeted therapy, immunotherapy, local therapy, surgical resection and liver transplantation [9]. Although there are many treatment methods, due to various limitations, the prognosis of most cases is very poor [10]. For many years, the medical community has been fighting against cancer, seeking better ways to treat it. At present, the combination of traditional Chinese and Western medicine in combating cancer has become a hot topic in recent years, and traditional Chinese medicine assisted treatment of cancer can reduce side effects to a certain extent. As an emerging anti-tumor drug, there are not many studies on the inhibitory effect of Icariin on hepatocellular carcinoma, but some experiments have shown that Icariin can significantly inhibit tumor growth and regulate the bone marrow. However, it has been demonstrated that Icariin can significantly inhibit tumor growth in murine model of liver cancer, regulate the percentage of CD4+and CD8+cells in the bone marrow and CD19+cells in the blood. Icariin has demonstrated anticancer efficacy both in vitro and in vivo. The mechanism of action may be related to its anti-angiogenic and anti-proliferative effects in tumors. Icariin has tumor-suppressing efficacy on both tumor weight and tumor volume. Moreover, Icariin had no significant side effects, and the patient's body weight, spleen weight, blood parameters are normal [11]. It was shown that Icariin inhibits proliferation and induces apoptosis of HCC cell lines in a dose-dependent manner. Icariin enhances the antitumor activity of arsenic trioxide in HCC through a mechanism that may be related to the generation of intracellular ROS and the inhibition of NF-jB activity [12]. In the study of the anticancer effect of icariin on human hepatocellular carcinoma SMMC-7721 cells, it was found that icariin triggers the mitochondrial/caspase apoptotic pathway by enhancing the Bax-to-Bcl-2 ratio, loss of mitochondrial membrane potential, cytochrome c release, and the caspase cascade reaction. Meanwhile, icariin also induces a sustained activation of c-Jun N-terminal kinase (JNK) phosphorylation, indicating that icariin inhibits mitochondrial apoptosis through ROS/ JNK dependent mitochondrial pathway to induce cell apoptosis. In his study, it was concluded that the core target of Icariin action in hepatocellular carcinoma is TNF.

As the target with the highest BC value, TNF may have certain research value. Tumor necrosis factor (tumor necrosis factor, TNF) is a multi-directional pro-inflammatory cytokine secreted by various cells, with low expression under normal physiological conditions, which contributes to the enhancement of body immunity. When the body is in a pathological state, such as being diagnosed with tumors, its high expression can be induced, and the cellular cascade effect can be activated. It can lead to an increased inflammatory response in the body, result in various pathological damages and lead to or enhance the proliferation of tumor cells. Tumor necrosis factor (TNF) is a small molecule protein secreted by macrophages, mainly including TNF-α and TNF-β. TNF-α is mainly secreted by monocyte-macrophages, while TNF-β is mainly secreted by activated T-lymphocytes. TNF-α was identified as having the ability to induce rapid hemorrhagic necrosis in experimental cancers [13], and TNF-α is the first cytokine to be used in cancer therapy. It exerts anti-tumor activity through a complex mechanism of inducing inflammatory and immune responses, apoptosis/necrosis of tumor cells, extensive thrombosis, and tumor vascular disruption. To date, many studies have been conducted to evaluate the anticancer effects of TNF-α in various types of tumors, some of which have even entered clinical trials [14].

5. Conclusion

The present study suggests that Icariin may act on hepatocellular carcinoma cells by participating in the pathways of immunomodulation, apoptosis, AGE-RAGE, NF-kappaB, etc. Protein kinase C, interleukin IL-17, and angiotensin may be affected to a certain extent as well. In studying the effect

of Icariin on hepatocellular carcinoma cells, we can take TNF and apoptosis and immune response as the key research direction.

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