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Recent Research Progress in Chinese Alternative Medicine in the Prevention and Treatment of Liver Cancer

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Abstract

The modernization of traditional/alternative Chinese medicine research provides diverse and comprehensive methods for disease treatment and health management by integrating the advantages of traditional medicine and modern science. Recent evidence has accumulated rich experience and outstanding achievements in the research of integrated traditional Chinese and Western medicine for the prevention and treatment of liver cancer. Their recent research is dedicated to the research and development of traditional Chinese medicine formulas, the discovery of treatment targets and predictive markers, providing new effective evidence for liver cancer prevention and treatment research, and expanding research directions. This article provides a review of the clinical and experimental research progress of this team in recent 5 years, in order to provide more ideas and insights for research in this field.

Keywords: Compound Chinese medicine formula; Novel biomarkers; Novel anticancer mechanisms; Traditional Chinese medicine; Hepatocellular carcinoma (HCC).

Introduction

Hepatocellular carcinoma (HCC) is the most prevalent type of liver malignancy, accounting for about 85% of cases [2]. It ranks third in terms of incidence and cancer-related mortality. In the United States, HCC is the fastest-growing cancer type, with its incidence doubling in the past two decades [3]. Infection is a primary risk factor for liver cancer, and lifestyle factors such as alcohol consumption, smoking, high-fat diets, and obesity can also increase the risk. In some regions, drinking water quality, potentially contaminated with chemicals like nitrosamines, may be linked to liver cancer risk. Early-stage HCC patients can be cured through local ablation, surgical resection, or liver transplantation. However, the recurrence rate remains high, with a post-surgery five-year survival rate around 35%. Over half of HCC patients are diagnosed at an advanced stage, limiting the effectiveness of available treatments. Recent developments in HCC treatment include the approval of multi-kinase inhibitors (MKIs) like Lenvatinib, Cabozantinib, and Regorafenib for advanced or metastatic HCC, but treatment options remain limited for these patients. HCC is considered an inflammation-related cancer, originating from chronic inflammatory liver damage, such as that caused by Hepatitis B virus. Therefore, it's theorized that HCC patients might benefit from immunotherapy. Despite significant progress in understanding the immunogenicity of HCC, clinical trials show limited efficacy of immune checkpoint inhibitors (ICIs) in treating HCC, benefiting only a minority of patients. Additionally, the benefits of combining ICIs with other anticancer agents have proven limited [4-8]. Thus, exploring new and effective combination therapies and biomarkers to improve clinical outcomes for liver cancer patients remains a key international task in the HCC treatment field.

The advantages of integrative traditional Chinese and Western medicine in the prevention and treatment of Hepatocellular Carcinoma (HCC)

It's noteworthy that increasing evidence suggests that the combination of traditional Chinese and Western medicine offers additional advantages in the prevention and treatment of liver cancer, potentially enhancing clinical benefits for patients with Hepatocellular Carcinoma (HCC) [9] for instance.

In terms of comprehensive treatment: This integrative approach combines traditional Chinese medical practices with modern Western medical techniques, offering a holistic treatment plan. This includes Chinese herbal medicine, acupuncture, Qi Gong, and other traditional Chinese therapies, alongside surgery, radiotherapy, and chemotherapy from Western medicine. Such a comprehensive approach better meets patient needs, providing personalized treatment plans. Regarding symptom relief: Traditional Chinese treatments like acupuncture and herbal medicine can alleviate symptoms such as pain, nausea, and fatigue, thus improving the quality of life. In enhancing immunity: Chinese medicine emphasizes balancing the body's natural equilibrium and boosting immunity. Through Chinese medicinal treatments, patients' immune systems can be strengthened, aiding them in better coping with liver cancer and the side effects of treatment. In reducing side effects: Chinese medical practices can alleviate the side effects of Western medical treatments like radiotherapy and chemotherapy, increasing patient tolerance and enabling more effective completion of treatment plans. For recurrence prevention: Traditional Chinese treatments often focus on addressing the root causes of diseases and overall conditioning, which can help maintain a healthy lifestyle after treatment and reduce the risk of liver cancer recurrence. In personalized treatment: The integration of Chinese and Western medicine allows for the creation of personalized treatment plans based on the specific conditions and physical characteristics of the patient, taking into account their overall health [9-11]. Chinese medicine, with its emphasis on holistic body regulation, has certain advantages in treating intermediate and advanced-stage tumors, enabling patients to live with the tumor. Therefore, the synergistic benefits of combining traditional Chinese and Western medicine could potentially improve cure rates and quality of life for cancer patients, leading to the development of a unique Chinese model in cancer treatment.

Recent explorations in drugs and related anticancer mechanisms

Clinical studies: In the realm of treatment, a recent clinical study by a team included 60 patients with Child C stage primary liver cancer. These patients were randomly divided into a treatment group of 40 and a control group of 20. The control group received best supportive care, while the treatment group received best supportive care plus oral administration of a traditional Chinese medicine formula, for a cycle of 21 days, observed over two cycles. The results showed that the treatment group had significant improvements in liver cancer-related symptoms, liver function Child-Pugh scores, and grading compared to before treatment ($P<0.05$), which was not significantly observed in the control group ($P>0.05$). The treatment group also showed better improvements in these aspects compared to the control group ($P<0.05$). This study found that the traditional Chinese medicine formula showed good efficacy in stabilizing tumors and protecting liver function in Child C stage primary liver cancer patients, improving cancer-related symptoms with minimal adverse reactions, making it a viable option for treatment [13].

Another team included 80 patients with liver cancer of the liver heat and blood stasis type, randomly divided into a control group and an observation group of 40 each. The control group received a combination of pirarubicin (THP), mitomycin C (MMC), carboplatin (CBP), and super iodinated oil for arterial chemoembolization. The observation group received the same treatment plus the traditional Chinese medicine formula, over a 30-day period. After treatment, both groups showed significant reductions in levels of ALT, AST, and LDH ($P<0.05$), with the observation group showing lower levels than the control group ($P<0.05$). Post-treatment, total bilirubin levels decreased, and albumin and prealbumin levels increased ($P<0.05$), with more significant improvements in the observation group ($P<0.05$). AFP, α-L-fucosidase, and carcinoembryonic antigen levels also decreased significantly post-treatment ($P<0.05$), with lower levels in the observation group ($P<0.05$). No significant adverse reactions were observed during the treatment in both groups. The study suggests that the combined treatment can effectively improve liver function and suppress tumor cell growth, offering a safe and reliable clinical effect [14].

Furthermore, a study included 85 HCC patients, randomly divided into a treatment group (n=52) and a control group (n=33). The treatment group received oral administration of the traditional Chinese medicine formula combined with local perfusion of hydroxycamptothecin via liver artery catheterization, while the control group received conventional treatment and transarterial chemoembolization (TACE). The clinical efficacy of both groups was evaluated based on changes in tumor size, with the Cox proportional hazards model analyzing factors related to prognosis and the Kaplan-Meier method analyzing survival rates. The results showed significant differences between the two groups in median survival time, and survival rates at 0.5, 1, and 2 years ($P<0.05$). The best prognosis was seen in patients with liver depression and spleen deficiency, followed by liver heat and blood stasis type, and the worst in liver and kidney yin deficiency. The study suggests that the combination of the traditional Chinese medicine formula with hydroxycamptothecin intervention treatment improves patient survival rates and duration compared to TACE alone, and that traditional Chinese medicine syndrome differentiation has a certain relationship with prognosis. This indicates that combining herbal medicine with systemic treatment is an effective measure for non-surgical treatment of large liver cancer [15].

In vivo studies: A recent study explored the mechanisms of Emodin, a compound derived from rhubarb, in inhibiting the growth of liver cancer cells both in vivo and in vitro. The team used RNA sequencing technology to identify differentially expressed genes (DEGs) in HepG2 cells induced by Emodin treatment. Specifically, they identified a total of 859 DEGs in Emodin-treated HepG2 cells, including 712 downregulated and 147 upregulated genes. Subsequently, they conducted functional and pathway enrichment analysis using DAVID. They constructed a protein-protein interaction (PPI) network using STRING and performed module analysis with Cytoscape, ultimately identifying 25 hub genes. Pathway analysis revealed that these genes were mainly enriched in neuropeptide signaling pathways, inflammatory responses, and positive regulation of cytoplasmic calcium ion concentration. Survival analysis indicated that LPAR6, C5, SSTR5, GPR68, and P2RY4 might be involved in the molecular mechanism of Emodin's therapeutic action against HCC. qRT-PCR testing showed that mRNA levels of LPAR6, C5,

SSTR5, GPR68, and P2RY4 were significantly reduced in Emodin-treated HepG2 cells. The study suggests that these DEGs and hub genes are key nodes in Emodin's anti-liver cancer action, providing new insights for further research into the molecular mechanisms of Emodin [16].

Related mechanism Studies: To explore the underlying mechanisms, a team utilized the integrative pharmacology platform of traditional Chinese medicine and the DAVID database to conduct an integrative pharmacological analysis of a traditional Chinese medicine formula. They built related networks to predict and analyze the anti-cancer mechanism of this formula from multiple perspectives. It was found that the formula contains a rich variety of anti-cancer medicinal components, with key targets including ADORA3, RHOA, AKT1, KRAS. Key pathways involved in the formula's action include the T-cell receptor signaling pathway, cancer pathways, and chemokine signaling pathways, and the formula potentially treats cancers like colorectal cancer, pancreatic cancer, and chronic myeloid leukemia. It is believed that the formula exhibits characteristics of multi-component, multi-target intervention in tumor diseases. Further network pharmacology research suggested that key components of the formula might include quercetin, luteolin, sinapic acid, and key targets might be protein kinase B, caspase-3, vascular endothelial growth factor A, v-jun sarcoma virus oncogene homolog, and v-myc avian myelocytomatosis viral oncogene homolog. The formula's mechanism of action might be related to hepatitis B and C, phosphatidylinositol-3-kinase/protein kinase B signaling pathway, p53 signaling pathway, microRNAs in cancer, programmed cell death ligand 1 and programmed cell death protein 1 checkpoint pathways, apoptosis, hypoxia-inducible factor-1 signaling pathway, interleukin-17 signaling pathway, and forkhead box O signaling pathway. The formula is thought to have a multi-target, multi-pathway mechanism of action in treating HCC. Additionally, recent in vitro experiments by their team found that the formula, as well as its alcohol-extracted supernatant and precipitate, significantly inhibited the activity of human liver cancer cells HepG2. Inhibitors like LY294002, Wortmanin, Akti, MEKi, JNKi, Z-VAD, Ferrostatin-1, and Necrostatin-1 did not significantly affect the formula's inhibition of HepG2 cell activity. The formula was also found to promote the release of LDH in HepG2 cells, causing cytotoxicity, inhibiting the expression of pyroptosis markers IL-1β and cleaved caspase-1, reducing Akt protein expression, and increasing caspase-3 protein expression in HepG2 cells. Therefore, the formula is believed to inhibit the activity of liver cancer cells, and its mechanism of action may be related to regulating the expression of Akt and promoting the expression of caspase-3. At the same time, it can reduce the expression of pyroptosis markers IL-1β and cleaved caspase-1, likely acting by blocking the pyroptosis process [17-19].

In the exploration of new biomarkers and targets: A study extracted primary Hepatocellular Carcinoma (HCC) data from the Cancer Genome Atlas (TCGA). The study applied Wilcoxon signed-rank tests, Kruskal-Wallis tests, and logistic regression analysis to assess the relationship between E2F2 expression and clinical pathological features. Cox regression and Kaplan-Meier methods were used to evaluate the correlation between clinical pathological features and survival rates. The biological functions of E2F2 were annotated through Gene Set Enrichment Analysis (GSEA). The study found a significant increase in E2F2 expression in liver cancer tissues. Elevated E2F2 expression in liver cancer samples was significantly associated with histological grading (G3-4 vs. G1–2, OR=2.62, p<0.001), clinical staging (Stage III-IV vs. I-II, OR=1.74, p=0.03), tumor size (T3–4 vs. T1–2, OR=1.64, p=0.04), tumor status (presence vs. absence of tumor, OR=1.88, p<0.001), and plasma alpha-fetoprotein (AFP) levels (for AFP≥400 vs AFP<20, OR=3.18, p<0.001; for 20≤AFP<400 vs AFP<20, OR=2.50, p<0.001). High E2F2 expression was associated with adverse overall survival (OS) (p<0.001), progression-free interval (PFI) (p<0.001), disease-free interval (DFI) (p=0.001), and disease-specific survival (DSS) (p<0.001). Elevated E2F2 was independently associated with OS (p=0.004, Hazard Ratio [HR]=2.4 [95% CI: 1.3-4.2]), DFI (p=0.029, HR=2.0 [95% CI: 1.1-3.7]), and PFI (p=0.005, Risk Ratio [RR]=2.2 [95% CI: 1.3-3.9]). Furthermore, nucleotide excision repair, ubiquitin-mediated proteolysis, and the citric acid (TCA) cycle were significantly enriched in high E2F2 expression phenotypes. The team believes that high expression of E2F2 could be a promising independent prognostic biomarker and therapeutic target for HCC. Additionally, cell cycle, pyrimidine metabolism, DNA replication, p53 signaling pathway, ubiquitin-mediated proteolysis, and the TCA cycle might be key pathways in E2F2's involvement in the initiation and progression of HCC. E2F2 can be regulated by various traditional Chinese medicine components, suggesting new approaches in the treatment of liver cancer with traditional Chinese medicine [20-30].

Additionally, Cyclin-Dependent Kinase 5 (CDK5) is a unique member of the serine/threonine kinase cyclin-dependent kinases (Cdks) family. CDK5 plays a critical role not only in the physiological and pathological processes of the nervous system but also regulates cell apoptosis and aging, and is involved in various tumors. Recent research has discovered that CDK5 drives G1-S transition and RB phosphorylation in a model of medullary thyroid carcinoma. Its activity depends on binding with activators. P35, one of the two activators of CDK5, is encoded by CDK5 regulatory subunit 1 (CDK5R1), making CDK5R1 crucial for CDK5's normal activity. Previous studies have reported that overexpression of CDK5 and CKD5R1 (P35) can promote cancer progression and metastasis, with similar results observed in melanoma, pancreatic cancer, large B-cell lymphoma, and head and neck squamous cell carcinoma. However, the role and clinical significance of CDK5 and CKD5R1 (P35) in Hepatocellular Carcinoma (HCC) have not been adequately assessed. Thus, primary data on HCC was downloaded from the Cancer Genome Atlas (TCGA) database. Wilcoxon signed-rank tests, Kruskal–Wallis tests, and logistic regression were applied to study the correlation between CDK5R1 expression in HCC and clinical pathological features. Kaplan–Meier and Cox regression analyses were also used to examine the relationship between clinical pathological features and survival rates. Gene Set Enrichment Analysis (GSEA) was applied to annotate the biological functions of CDK5R1. The results indicated that CDK5R1 is abnormally overexpressed in liver cancer tissues. High expression of CDK5R1 in HCC tissues was significantly associated with tumor status (P=0.00), new tumor events (P=0.00), disease-free interval (DFI; P=1.785e-05), and progression-free interval (PFI; P=2.512e-06). Furthermore, univariate and multivariate Cox regression analyses found that increased CDK5R1 independently predicts poor overall survival (OS) (P=0.037, Hazard Ratio [HR]=1.7 [95% CI: 1.0-2.7]), DFI (P=0.007, HR=3.0 [95% CI: 1.4-6.7]), and PFI (P=0.007, HR=2.8 [95% CI: 1.3-5.9]). GSEA found that the Notch signaling pathway and non-small cell lung cancer were significantly enriched in high CDK5R1 expression phenotypes. It is believed that elevated CDK5R1 could be a promising independent prognostic factor for poor survival in HCC, providing a new candidate for the prognostic assessment

of HCC [21-30].

Another study employed high-throughput sequencing methods to screen for differentially expressed genes (DEGs), hub genes, and enriched signaling pathways. Various databases were used to analyze the association between the expression of hub genes, their aberrant expression, and their impact on genetic variation rates, overall survival (OS), relapse-free survival (RFS), progression-free survival (PFS), disease-specific survival (DSS), as well as pathological staging and grading in patients with Hepatocellular Carcinoma (HCC). A total of 1582 DEGs were identified. Gene ontology analysis revealed that these DEGs were primarily involved in processes such as "oxidation-reduction," "steroid metabolic process" and "fatty acid β-oxidation". Further enrichment analysis indicated that DEGs were mainly associated with "metabolic pathways," "PPAR signaling pathway", "fatty acid degradation", and "cell cycle". Based on this, the study identified eight hub genes. Additionally, the abnormal expression levels of these hub genes were closely associated with patients' OS, RFS, PFS, DSS, pathological staging, and grading. In all samples, the abnormal expression levels of these eight hub genes exceeded 30%. Through connectivity map analysis, several small molecule compounds were identified that might reverse the changes in DEGs, including benzoxybenzylamine, GW-8510, resveratrol, 0175029-0000, and rosiglitazone. Considering the critical role that dysfunctions in fatty metabolism pathways, cell cycle, oxidation-reduction processes, and viral carcinogenesis may play in the development of HBV-related early HCC, the study proposed that the identified eight hub genes (CDC20, CCNB1, AURKB, CCNA2, CDK1, APOA1, UBB, EHHADH) could serve as potent biomarkers and therapeutic targets for diagnosis and prognosis [31].

Additionally, changes in DNA methylation patterns are considered early events in the development of Hepatocellular Carcinoma (HCC). However, their mechanisms and significance remain to be elucidated. A study employed Whole-Genome Bisulfite Sequencing (WGBS) to assess genome-wide methylation in HCC and to construct a prognostic model associated with differentially methylated regions (DMRs). Based on the analysis of four clinical samples, they found that HCC tissues exhibited a genome-wide hypomethylation pattern compared to adjacent peritumoral tissue (APT) and cirrhotic tissue. Furthermore, they identified 590 hypermethylated genes and 977 hypomethylated genes that were differentially expressed across the three groups. They also combined these differentially expressed genes (DEGs) and differentially methylated genes (DMGs) from the TCGA database to obtain DEGs with high methylation and low expression, as well as low methylation and high expression. Moreover, they integrated these key DEGs with clinical data to construct a prognostic model comprising 13 differentially methylated DEGs. The accuracy of this model was then evaluated, and the genes within the model were validated through several external databases. This process identified several key genes that might be involved in the onset and progression of HCC, including BFSP1, DEAF1, ECE2, FBXL7, GNA12, LPCAT1, NINJ2, PFKP, PTK7, RAP-1GAP, RNF220, RRM2, TXNRD1. The team believes this prognostic model could become a powerful tool for predicting the prognosis of HCC patients. Therefore, they suggest that their findings can better aid in understanding the mechanisms of epigenetic regulatory mechanisms across the HCC genome, contributing to early diagnosis and prognosis [32].

Furthermore, Chaperone-Mediated Autophagy (CMA) is an autophagic lysosomal pathway and a proteolytic system that aids in the degradation of intracellular proteins in lysosomes. Previous research has found that CMA can regulate tumor cell proliferation by targeting RND3 in cancer. Additionally, KEGG analysis indicates that RND3 is a recognized CMA substrate, and the inhibition of CMA in fibroblasts can also enhance the efficiency of cell transformation driven by MYC/c-MYC. The activation of CMA mediated by SNX10, leading to the reduction of p21Cip1/WAF1, facilitates the proliferation and survival of HCT116 cells. Moreover, TPD52 has been found to enhance CMA activation by interacting with HSPA8/HSC70 and enhancing substrate degradation in prostate cancer. Building on this, Professor Zhou's team further assessed the role of CMA substrate proteins in cancer, identifying PKM2 and HK2 as key enzymes in glycolysis that contribute to the Warburg effect in malignant tumors. They propose that targeting CMA substrate proteins offers a new direction for cancer treatment. The selective regulation of cancer-related CMA substrate proteins can also help in understanding the molecular mechanisms of tumorigenesis. For instance, PKM2 is a key downstream protein of mammalian target of rapamycin (mTOR). Disruption of mTOR could inhibit carcinogenic PKM2-mediated tumorigenesis. Furthermore, crosstalk between macrophage autophagy and CMA has been discovered, with mTOR playing a crucial role in regulating macrophage autophagy. PKM2 is considered a key protein between macroautophagy and CMA for further cancer-related research. Post-translational modifications in the selective regulation of cancer-related CMA proteins show potential for cancer treatment [37].

Discussion

Chinese alternative/traditional medicine has accumulated substantial experience in the field of integrative medicine, particularly in the prevention and treatment of diseases like liver cancer. The development of new traditional Chinese medicine formulations and the discovery of new therapeutic targets and prognostic markers are of great significance in the treatment of liver cancer. They also provide more directions for research in this field. Based on the current research achievements and learning, the future research directions in this field are relatively clear, such as: 1. Personalized Treatment Strategies: Deepening the understanding of individual differences among patients and the stages of disease progression to devise more personalized treatment plans. Integrating Chinese and Western medical concepts to optimize treatment plans based on the patient's constitution and disease characteristics; 2. Advancing Traditional Chinese Medicine Research: Continuing to explore the potential of traditional Chinese medicine in combating liver cancer, discovering more effective herbal components, and delving into their molecular mechanisms to develop more effective Chinese medicine treatment schemes; 3. Integrative Treatment Models of Chinese and Western Medicine: Studying the synergistic effects of Chinese and Western medicine in treating liver cancer and exploring how to combine modern medical precision treatment with the holistic adjustment of traditional Chinese medicine to improve treatment effectiveness; 4. Strengthening Biomarker Research: Identifying reliable biomarkers for early diagnosis of liver cancer and monitoring disease progression to enable more timely treatment interventions; 5. Psychological Support and Quality of Life: Researching how psychological support and improving the quality of life of patients can enhance the effectiveness of treatment and reduce the psychological and lifestyle pressures brought by the treatment; 6. Big Data and Artificial Intelligence: Utilizing big data and artificial intelligence technologies to analyze patient data and disease progres-

sion trends, providing more decision-making support for doctors and optimizing treatment plans; 7. Health Education and Prevention: Strengthening health education about liver cancer to raise public awareness of the risk factors and early symptoms of liver cancer, promoting early diagnosis and prevention.

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