Review

Chinese medicines for prevention and treatment of human hepatocellular carcinoma: current progress on pharmacological actions and mechanisms

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Abstract

Hepatocellular carcinoma (HCC) is one of leading causes of death in the world. Although various treatments have been developed, the therapeutic side effects are far from desirable. Chinese medicines (CMs, including plants, animal parts and minerals) have drawn a great deal of attention in recent years for their potential in the treatment of HCC. Most studies have shown that CMs may be able to retard HCC progression with multiple actions, either alone or in combination with other conventional therapies to improve quality of life in HCC patients. Additionally, CMs are used for preventing HCC occurrence. The aim of this study is to review the potential prophylactic and curative effects of CMs on human HCC and the possible mechanisms that underlie these pharmacological actions. Publications were collected and reviewed from PubMed and China National Knowledge Infrastructure from 2000 to 2014. Keywords for literature searches include “Chinese medicine”, “Chinese herb”, “traditional Chinese Medicine”, “hepatocellular carcinoma” and “liver cancer”. CMs in forms of pure compounds, isolated fractions, and composite formulas are included. Combination therapies are also considered. Both in vitro and in vivo efficacies of CMs are being discussed and the translational potential to bedside is to be discussed with clinical cases, which show the actions of CMs on HCC may include tumor growth inhibition, antimetastatic activities, anti-inflammation, anti-liver cancer stem cells, reversal on multi-drug resistance and induction/reduction of oxidative stress. Multiple types of molecules are found to contribute in the above actions. The review paper indicated that CMs might have potential to both prevent HCC occurrence and retard HCC progression with several molecular targets involved.

Keywords: medicine, Chinese traditional; liver neoplasms; hepatocellular carcinoma; recurrence; disease treating & preventing; molecular targets; review


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1 Introduction

Internationally, cancer is a leading cause of death and discomfort. In recent statistics, there were about 12.7 million cancer cases in the world, among which roughly 748 300 patients suffered from liver cancer[1]. Liver cancer is among the chief causes of cancer-related death in Asian countries, and hepatocellular carcinoma (HCC) accounts for more than 70% of these liver cancer cases[1].

In general, HCC results from several major sources: chronic hepatitis B/C virus (HBV/HCV) infection, exposure to aflatoxin B1, alcohol-related cirrhosis and possible non-alcoholic fatty liver disease[2]. Various treatments have been developed for HCC therapies, including chemotherapy, partial hepatectomy to remove the tumors, radiotherapy, cryo-ablation, liver transplantation and transcatheter arterial chemoembolization (TACE). However, due to unresponsiveness of HCC cells to most of these conventional therapies, clinical outcomes of HCC treatment are often poor. Moreover, HCC patients receiving the above conventional treatments also suffered from adverse effects such as nausea, vomiting, bone marrow suppression and/or hair loss. Thus exploring new strategies for the treatment of HCC is still necessary.

Complementary and alternative medicine (CAM) is one of the possible strategies for approaching HCC treatment. Chinese medicine (CM; derived from plants, animal parts and minerals) has been used for preventing and treating liver diseases in China for centuries[2–5], and is now considered an important component of the CAM system. Based on the theory of pathology in CM, liver cancer belongs to Zhengji (accumulation syndrome), resulting from Yu (stasis of blood or Qi), Du (toxicity) and Xu (deficiency). The principles of treating Zhengji in CM are as follows: (a) Huoxue Huayu (activating blood and resolving stasis), (b) Qingre Jiedu (clearing heat and resolving toxin) or (c) Jianpi Liqi (tonifying spleen and regulating qi)[6]. In recent years, more studies have highlighted the role of CM in retarding HCC progression in combination with other treatment modalities. CM has also been used for preventing HCC, either alone or in combination with other conventional therapies. More data have demonstrated that CM is an effective strategy to mitigate the financial and social burden associated by HCC[7]. However, the definition of CM’s role in HCC treatment and prevention requires further clinical research. In this report, we critically review the literature documenting the efficacy of CM in the treatment of HCC. The majority of this research focuses on pharmacological actions and mechanisms of the treatment. More than 200 publications were reviewed, spanning 2000 to 2014. The records were found in searches of the PubMed and China National Knowledge Infrastructure (CNKI) databases, using the key words mainly of “Chinese medicine”, “Chinese herb”, “traditional Chinese medicine”, “hepatocellular carcinoma (HCC)” and “liver cancer”. Some detailed keywords or phrases, such as “ginseng”, “coptis”, “Nrf2”, were added if needed. Here we discuss the actions and mechanisms of CM from in vitro, in vivo and clinical studies.

2 Classification of CMs in HCC prevention and treatment

CM covers 11 146 species of plants, 1 581 types of animal parts and 80 distinct minerals[7]. In terms of CM for liver cancer therapy, medicinal herbs are most commonly used, while a few animal and mineral-derived products are also included in some prescriptions. Chemicals isolated from CMs, CM fractions, and composite formulas are the major subjects in most studies, while the combination of therapies is also frequently assessed in the literature.

2.1 Pure products

Some studies have been conducted to specially address only pure substances from CMs. These studies illustrate the actions and mechanisms of these individual substances, and their effectiveness in the treatment of HCC are summarized in Table 1.

2.2 Isolated fractions

An area of particular interest in CM research investigates the activity of fractions isolated from CM herbs and compounds. The clinical practice of active fractions in pre-clinical study is in a close relationship with its dose form, and may therefore elucidate the action and mechanism of CM. One example can be found in the Chinese medicinal herb Huanglian (Coptis chinensis Franch., Coptis deltoidea C. Y. Cheng et P.K. Hsiao, or Coptis teeta Wall.), which has a significant effect on HCC. We found that extracts from Huanglian up-regulated miR21 and miR23a in HCC cells; these genes targeted the tumor suppressors tropomyosin 1 (TPM1) and programmed cell death 4 (PDCD4)[115]. Both Jie et al[83] and our studies[116] further showed that the herb and its active alkaloids could inhibit metastasis by blocking Rho/Rho-associated protein kinase (ROCK) and vascular endothelial growth factor (VEGF) in HCC cells. Synergy among all the active components in the un-extracted herb may result in greater activity[117]. The activities of various fractions of CM herbs and compounds against HCC are listed in Table 1.

2.3 Composite formulas

Composite formulas have been clinically effective in treating liver cancer by regulating the balance of Yin and Yang, Qi and Xue and removing pathological factors[118]. CM philosophy treats the human body with a holistic approach, and composite formulas are constructed to balance the Yin and Yang of the whole body. An example of this treatment strategy in the treatment of HCC is Xiaoxiao...
Table 1 (to be continued)  Chinese medicines and their ingredients with antitumor activity in liver cancers

<table>
<thead>
<tr>
<th>Family name</th>
<th>Resource/Chinese name</th>
<th>Compounds</th>
<th>Minimum dose/duration</th>
<th>Targets</th>
<th>Mechanisms</th>
<th>Cell lines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compositae</td>
<td>Artemisia annua L. / Qinghao</td>
<td>Artemisinin</td>
<td>1 μmol/L/48 h (in vitro); 50 mg/(kg.d)/4 weeks (in vivo)</td>
<td>Arresting at G1 phase; anti-proliferation; inducing apoptosis</td>
<td>↓Cyclin D1; ↓cyclin E; ↓CDK2; ↓CDK4; ↓E2F1; ↑Cip1/p21; ↑Kip1/p27; ↑caspase-3; ↑Bax/Bcl-2; ↑PARP; ↑MDM2</td>
<td>HepG2, Hep3B, BEL-7404 and Huh-7 (in vitro and in vivo)</td>
<td>[8,9]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td>Salvia miltiorrhiza Bunge/Danshen</td>
<td>Tanshinone II-A</td>
<td>1 μg/mL/72 h (in vitro); 5 mg/(kg.d)/4 weeks (in vivo)</td>
<td>Arresting at G2/G0 phase; inducing apoptosis anti-invasion and anti-metastasis</td>
<td>↑Fas; ↑Ca2+, Bax and caspase-3; ↓MMP-2; ↓MMP-9; ↓NF-κB</td>
<td>HepG2, SMCC7721, BEL-7402 and BEL-7404 (in vitro); HCCLM3, HepG2 and J5 (in vivo)</td>
<td>[10–14]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td>Salvia miltiorrhiza Bunge/Danshen</td>
<td>Salvianolic acid B</td>
<td>10 mg/kg/6 weeks (in vivo); 60 mg/d/6 months (clinical trials)</td>
<td>Anti-fibrosis</td>
<td>↓HSC, ↓ALT, AST, TBIL, HA, LN, IV-C and PHIP; ↓NF-κB; ↓TGF-β1; ↓Smad</td>
<td>Rats (in vivo); clinical trial</td>
<td>[15–17]</td>
</tr>
<tr>
<td>Pinaceae</td>
<td>Pseudolarix kaempferi Gordon (Synonym of Pseudolarix amabilis (J. Nelson) Rehder)/ Jinqiansong (also Tujinpi)</td>
<td>Pseudolaric acid B</td>
<td>1 μmol/L/24 h</td>
<td>Reversing MDR; arresting at G2/M phase; inducing apoptosis</td>
<td>↓P-gp; ↑caspase-3</td>
<td>BEL-7402 (in vitro)</td>
<td>[18,19]</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td>Oldenlandia diffusa (Wild.) Roxb./ Baihuasheshao</td>
<td>Ursolic acid</td>
<td>15 μg/mL/12 h (in vitro); 50 mg/kg/14 d (in vivo)</td>
<td>Inducing apoptosis</td>
<td>↑Bak, Bax, and p53; ↑AIF; ↑PI3K and survivin</td>
<td>R-HepG2 (in vitro and in vivo)</td>
<td>[20]</td>
</tr>
<tr>
<td>Celastraceae</td>
<td>Tripterygium wilfordii Hook. f./Leigongteng</td>
<td>Triptolide</td>
<td>10 nmol/L/48 h</td>
<td>Inducing apoptosis; immuno-suppressing; anti-inflammatory</td>
<td>↑TNF-α; ↓IL-2; ↓T cells</td>
<td>SMMC-7721 (in vitro)</td>
<td>[15,21,22]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td>Isodon rubescens (Hems.l.) H. Hara. (Syn. Rabdosia rubescens (Hems.l.) Hara)/Donglingcao</td>
<td>Oridonin</td>
<td>5 μg/mL/24 h or 22 μmol/L/24 h</td>
<td>Enhancing apoptosis effects of Pishuang; inducing G2/M phase arrest</td>
<td>↑ROS, Bax and cytochrome c; ↑Akt, XIAP, NF-κB; ↑Hsp70.1, STRAP, TCTP, Stil and PPase; ↓hnRNP-E1</td>
<td>BEL-7402, HepG2, SMCC7721, and murine Hepa 1-6 cells (in vitro)</td>
<td>[23–25]</td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Astragalus membranaceus Bunge./Huangqi</td>
<td>Astragalus polysaccharide</td>
<td>20 μg/mL (in vitro); 57.59 mg/(kg.d)/28 d (in vivo)</td>
<td>Anti-HBV/HCV; inducing apoptosis anti-invasion</td>
<td>↓Virus; ↓AFP; ↓TGF-β1</td>
<td>HepG2 (in vitro); C57_TgN (HBVadr2.0) SMMU mice (in vivo)</td>
<td>[26–28]</td>
</tr>
<tr>
<td>Family name</td>
<td>Resource/Chinese name</td>
<td>Compounds</td>
<td>Minimum dose/duration</td>
<td>Targets</td>
<td>Mechanisms</td>
<td>Cell lines</td>
<td>References</td>
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<tr>
<td>Araliaceae</td>
<td>Panax ginseng C.A. Mey./Renshen</td>
<td>Rh2, Rg3, Rb1, Rb2, Rc, Rd, Re, Rf, Rg2</td>
<td>5 μg/mL/48 h</td>
<td>Arresting at G1 phase; reducing metastatic growth; ↓Cyclin D1; ↓cyclin E; ↑p16 protein; ↑p21, cytochrome c, Bax, and caspase-3; ↓Bcl-2, NF-κB, and MMP-2/9</td>
<td>Jγ-GT, TAT</td>
<td>HepG2 and R-HepG2 (in vitro)</td>
<td>[29–31]</td>
</tr>
<tr>
<td>Orobanchaceae</td>
<td>Pedicularis striata Pall./Maxianhao</td>
<td>Isoverbascoside</td>
<td>10 μmol/L/6 d</td>
<td>Arresting at G0/G1 phase; inducing differentiation</td>
<td>↓γ-GT; ↑TAT</td>
<td>SMMC-7721 (in vitro)</td>
<td>[32]</td>
</tr>
<tr>
<td>Melanthiaceae</td>
<td>Paris polyphylla Sm./Chonglou</td>
<td>Polyphyllin D</td>
<td>10 μg/mL/24 h</td>
<td>Reversing MDR</td>
<td>↓P-gp</td>
<td>HepG2 and R-HepG2 (in vitro)</td>
<td>[33]</td>
</tr>
<tr>
<td>Cucurbitaceae</td>
<td>Gynostemma pentaphyllum (Thunb.) Makino/Jiaogulan</td>
<td>Gypenosides</td>
<td>300 μg/mL/24 h</td>
<td>Inducing apoptosis</td>
<td>↑Ca2+/ROS; ↓ΔΨm; ↓ERK; ↑Bax; ↓Bcl-2</td>
<td>Huh7 (in vitro)</td>
<td>[34]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td>Scutellaria baicalensis Georgi./Huangqin</td>
<td>Baicalin</td>
<td>5 mg/L/48 h</td>
<td>Anti-invasion</td>
<td>↓MMP-2/TIMP2; ↓E-cad; ↓RhoA/ROCK-1; ↓integrin β1</td>
<td>BEL-7402 (in vitro)</td>
<td>[35,36]</td>
</tr>
<tr>
<td>Rutaceae</td>
<td>Citrus reticulate Blanco./Chenpi</td>
<td>Hesperidin</td>
<td>50 μmol/L/24 h</td>
<td>Anti-invasion</td>
<td>↓MMP-9; ↓NF-κB; ↓AP-1; ↓IκB</td>
<td>HepG2</td>
<td>[37]</td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Glycyrrhiza uralensis Fisch./Gancao</td>
<td>Glycyrrhizin</td>
<td>2.7 μg/mL/72 h</td>
<td>Reversing MDR; anti-hepatitis; anti-fibrosis</td>
<td>↓Virus; ↑IFN; ↓HSCs; ↓ALT; ↓AST; ↓COX; ↓P-gp, MRP2 and MRP3</td>
<td>SMMC-7721; Huh7 HCC (in vitro)</td>
<td>[15,38–42]</td>
</tr>
<tr>
<td>Primulaceae</td>
<td>Ardisia pusilla A. DC.</td>
<td>Ardisipusilloside I</td>
<td>50 μmol/L/24 h</td>
<td>Antiproliferation; anti-metastasis and anti-invasion</td>
<td>↓Erk1/2 and Akt; ↓MMP-2/9; ↑Rac1 and E-cadherin</td>
<td>HepG2 and SMMC-7721 (in vitro)</td>
<td>[43]</td>
</tr>
<tr>
<td>Arecaceae</td>
<td>Areca catechu L.</td>
<td>Eugenol</td>
<td>20 mg/mL/24 h</td>
<td>Anti-invasion</td>
<td>↓Antiproliferation; anti-metastasis and anti-invasion</td>
<td>HepG2 and SMMC-7721 (in vitro)</td>
<td>[44]</td>
</tr>
<tr>
<td>Theaceae</td>
<td>Camellia sinensis (L.) Kuntze</td>
<td>Green tea polyphenols</td>
<td>25 μmol/L/16 h</td>
<td>Reversing MDR; anti-oxidative stress; anti-inflammatory</td>
<td>↑MAPK, and caspase-3</td>
<td>HepG2-C8</td>
<td>[45–46]</td>
</tr>
<tr>
<td>Family name</td>
<td>Resource/Chinese name</td>
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<td>Minimum dose/duration</td>
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<tr>
<td>Pinaceae</td>
<td><em>Pinus massoniana</em> Lam. / Maweisong</td>
<td>Procyanidine</td>
<td>1.0 μmol/L/90 min</td>
<td>Reversing MDR</td>
<td>↓P-gp</td>
<td>RBMECs (<em>in vitro</em>)</td>
<td>[47]</td>
</tr>
<tr>
<td>Ginkgoaceae</td>
<td><em>Ginkgo biloba</em> L. / Yinxing</td>
<td>Quercetin</td>
<td>2 g/kg/1 week</td>
<td>Reversing MDR</td>
<td>↓P-gp</td>
<td>Rats (<em>in vivo</em>)</td>
<td>[46,48]</td>
</tr>
<tr>
<td>Zingiberaceae</td>
<td><em>Curcuma longa</em> L. / Jianghuang</td>
<td>Curcumin</td>
<td>1 mg/kg/6 d (<em>in vivo</em>)</td>
<td>Anti-fibrosis; anti-inflammatory; anti-oxidative stress; enhancing resveratrol toxicity</td>
<td>↓HSC; ↑PPARγ; ↓lipooxygenase; ↓COX-2; ↓IL-1β, -6, -8; ↓MIP-1; ↓MCP-1; ↓NF-κB, Bel-2, Bel-XL, and Akt; ↓TNF-α; ↑Nrf2; ↑GST; ↑NQO; ↑ROS; ↓XIAP; ↓survivin</td>
<td>Mice and rats (<em>in vivo</em>); Hepal-6 (<em>in vitro</em>)</td>
<td>[15,49–55]</td>
</tr>
<tr>
<td>Polygonaceae</td>
<td><em>Rheum palmatum</em> L. / Dahuang</td>
<td>Emodin</td>
<td>57.59 mg/(kg·d)/3 weeks (<em>in vivo</em>); 20 mol/L/48 h (<em>in vitro</em>)</td>
<td>Inducing apoptosis; anti-HBV/HCV</td>
<td>↓ΔΨm; ↑caspase-9; ↑caspase-3; ↑p53; ↑Fas; ↑p21; ↑ERK; ↑Bax; ↓Bcl-2; ↓virus</td>
<td>Mice (<em>in vivo</em>); SMMC-7721, Mahlavu, PLC/PRF/5 and HepG2</td>
<td>[26,56,57]</td>
</tr>
<tr>
<td>Compositae</td>
<td><em>Silybum marianum</em> (L.) Gaertn./Shuifeiji</td>
<td>Silibinin</td>
<td>180 μmol/L/24 h or 10 μg /24 h</td>
<td>Arresting at G2/M phase; inducing apoptosis</td>
<td>↓PCNA; ↓Ki-67</td>
<td>HuH7, HepG2, Hep3B, and PLC/PRF/5 (<em>in vitro</em>)</td>
<td>[58,59]</td>
</tr>
<tr>
<td>Clusiaceae</td>
<td><em>Garcinia hanburyi</em> Hook. f. / Tenghuang</td>
<td>Gambogenic acid</td>
<td>0.5 μmol/L/24 h (<em>in vitro</em>); 2 mg/kg, 3 times/week/3 weeks (<em>in vivo</em>)</td>
<td>Inducing apoptosis</td>
<td>↓Telomerase; ↓Bcl-2; ↑Bax and caspase-3</td>
<td>HepG2 (<em>in vitro</em>); SMMC-7721 (<em>in vitro and in vivo</em>)</td>
<td>[60–63]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Scutellaria baicalensis</em> Georgi / Huangqin</td>
<td>Wogonin</td>
<td>20 μmol/L/24 h</td>
<td>Inducing apoptosis; arresting at G0/G1 or G2/M phase</td>
<td>↓Bcl-2; ↓pro-caspase-3; ↑Bax; ↑p53; ↑p21</td>
<td>SK-HEP-1 and SMMC-7721 (<em>in vitro</em>)</td>
<td>[64,65]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Scutellaria baicalensis</em> Georgi / Huangqin</td>
<td>Oroxylin A</td>
<td>27 μmol/L/24 h</td>
<td>Inducing apoptosis; arresting at G0/G1 or G2/M phase</td>
<td>↓Bcl-2; ↓pro-caspase-3; ↑Bax</td>
<td>HepG2 (<em>in vitro</em>)</td>
<td>[66]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Scutellaria baicalensis</em> Georgi / Huangqin</td>
<td>Total extracts</td>
<td>40, 80 and 120 μg/mL</td>
<td>Inhibiting tumorigenesis in the liver</td>
<td>↓MDA; ↑SOD</td>
<td>Diethylnitrosamine-induced rat tumorigenesis</td>
<td>[67]</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Scutellaria baicalensis</em> Georgi / Huangqin</td>
<td>Total flavonoids</td>
<td>40, 80 and 120 μg/mL</td>
<td>Reducing the metastatic capability</td>
<td>↓MMP-2; ↓MMP-9; ↑TIMP-1; ↑TIMP-2</td>
<td>MHCC97-H (<em>in vitro</em>)</td>
<td>[68]</td>
</tr>
</tbody>
</table>
Table 1 (continuation 3) Chinese medicines and their ingredients with antitumor activity in liver cancers

<table>
<thead>
<tr>
<th>Family name</th>
<th>Resource/Chinese name</th>
<th>Compounds</th>
<th>Minimum dose/duration</th>
<th>Targets</th>
<th>Mechanisms</th>
<th>Cell lines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leguminosae</strong></td>
<td><em>Sophora flavescens</em> Aiton/Kushen</td>
<td>KS-Fs (Kushen flavonoids), including kurarinone, norkurarinone, 2′-methoxy-kurarinone and other flavonoids</td>
<td>60 mg/(kg·d) (<em>in vivo</em>)</td>
<td>Antiproliferation; inducing apoptosis; ↓NF-κB; ↓Bel-2; ↑caspase-8 and -3</td>
<td>H22 (<em>in vivo</em>)</td>
<td>[69]</td>
<td></td>
</tr>
<tr>
<td><strong>Leguminosae</strong></td>
<td><em>Sophora flavescens</em> Aiton/Kushen</td>
<td>Matrine</td>
<td>50 mg/L/48 h (<em>in vitro</em>); 50 mg/kg/48 h (<em>in vivo</em>)</td>
<td>Reversing MDR; inducing apoptosis</td>
<td>↓P-gp; ↑Akt</td>
<td>HepG2, Hep3B, SMMC-7721 (<em>in vivo</em>), H22 murine hepatocarcinoma cell (<em>in vitro or in vivo</em>)</td>
<td>[70–74]</td>
</tr>
<tr>
<td><strong>Leguminosae</strong></td>
<td><em>Sophora flavescens</em> Aiton/Kushen</td>
<td>Oxymatrine</td>
<td>1 mmol/L/24 h (HepG2); 1.0 mg/mL/48 h (SMMC-7721)</td>
<td>Anti-HBV/HCV; anti-fibrosis/cirrhosis; arresting cell cycle</td>
<td>↑INF-γ and IL-2; ↓TNF-α and IL-10; ↑p53 and bel-2</td>
<td>HepG2 and SMMC-7721 (<em>in vitro</em>)</td>
<td>[15,75–77]</td>
</tr>
<tr>
<td><strong>Ranunculaceae</strong></td>
<td><em>Coptis chinensis</em> Franch., <em>Coptis deltoidea</em> C. Y. Cheng et P.K. Hsiao, or <em>Coptis teeta</em> Wall./Huanglian</td>
<td>Berberine</td>
<td>0.125 mg/mL/24 h (coptischinensis extracts); 3.125 μmol/L/24 h (berberine)</td>
<td>Inhibiting carcinogenesis; arresting at G2/M phase; anti-inflammation; inducing apoptosis and autophagy; inhibiting metastasis</td>
<td>↓GGT and GST; ↑Akt; ↑p38; ↓NF-κB; ↓CD147; ↑Fas; ↑Bel-2; ↑Bax; ↑caspase-3, -8,-9; ↑PARP; ↑cytochrome c; ↓mTOR; ↑Beclin-1; ↑p53; ↑ROS; ↑miR21; ↑miR23a; ↑Rho/ROCK; ↑VEGF</td>
<td>HepG2, SMMC-7721 and MHCC97-L (<em>in vitro</em>)</td>
<td>[77–85]</td>
</tr>
<tr>
<td><strong>Cornaceae</strong></td>
<td><em>Camptotheca acuminate</em> Decne./Xishu</td>
<td>Camptothecin; hydroxycamptothecin</td>
<td>5 mg/L/6 d</td>
<td>Arresting at G2/M phase</td>
<td>↓Topo I; ↓AFP; ↑ALB</td>
<td>HepG2 (<em>in vitro</em>)</td>
<td>[86,87]</td>
</tr>
<tr>
<td><strong>Menispermaceae</strong></td>
<td><em>Stephania tetrandra</em> S. Moore/Fangji</td>
<td>Tetrandrine; fangcholine</td>
<td>5 μg/mL/48 h (HepG2); 1.25 μg/mL/48 h (BEL-7402); 5 μmol/L/24 h (HepG2, BEL-7402 and Huh7); 4 μmol/L/6 h (PLC/PRF/5, Hep3B and HepG2)</td>
<td>Inhibiting liver fibrosis/cirrhosis; reversing MDR; inducing apoptosis; arresting at S and G2/M or G1 phase</td>
<td>↓HSCs and PDGF; ↓Ca; ↓ALT and AST; ↓NO and iNOS; ↓P-gp; ↑ROS; ↑Akt; ↑Bax; ↑Bel-2; ↑p53 and/or p21; ↑cyclin D1; ↑APO-1 (CD95, Fas); ↑caspase-3; ↑PA28γ; ↓RPS12; ↓TAL; ↓PGAM1 HepG2, BEL-7402, Hep7, PLC/PRF/5 and Hep 3B (<em>in vitro</em>);</td>
<td>[15,88–91]</td>
<td></td>
</tr>
<tr>
<td>Family name</td>
<td>Resource/Chinese name</td>
<td>Compounds</td>
<td>Minimum dose/duration</td>
<td>Targets</td>
<td>Mechanisms</td>
<td>Cell lines</td>
<td>References</td>
</tr>
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</tr>
<tr>
<td>Apiaceae</td>
<td><em>Ligusticum chuanxiong</em> Hort.<em>Chuanxiong</em></td>
<td>Tetramethylpyrazine</td>
<td>16 μg/mL/72h</td>
<td>Reversing MDR</td>
<td>↓P-gp; ↓MRP2; ↓MRP3; ↓MRP5</td>
<td>HepG2/ADM and BEL-7402/ADM</td>
<td>[92,93]</td>
</tr>
<tr>
<td>Papaveraceae</td>
<td><em>Corydalis yanhusuo</em> W. T. Wang ex Z. Y. Su et C. Y. Wu/Yanhusuo</td>
<td>Tetrahydropalmatine</td>
<td>5 μg/mL/24 h</td>
<td>Anti-γ-radiation-induced damage</td>
<td>↓ROS; ↓MDA; ↓LDH; ↑GSH; ↑SOD; ↑Δψm; ↓caspase-3; ↓cytochrome c; ↓Bax/Bcl-2</td>
<td>Human endothelial cells (<em>in vitro</em>)</td>
<td>[94]</td>
</tr>
<tr>
<td>Araliaceae</td>
<td><em>Eleutherococcus senticosus</em> (Rupr. &amp; Maxim.) Maxim./Ciwujia</td>
<td>Isofraxidin</td>
<td>33 μmol/L/24 h</td>
<td>Anti-invasion</td>
<td>↓MMP-7</td>
<td>Huh-7 and HepG2 (<em>in vitro</em>)</td>
<td>[95]</td>
</tr>
<tr>
<td>NA</td>
<td>Silkworm pupas/Chanyong</td>
<td>Selenium-rich amino acids</td>
<td>0.5 μmol/L/24 h</td>
<td>Inducing apoptosis</td>
<td>↑ROS</td>
<td>SMMC-7721 (<em>in vitro</em>)</td>
<td>[96]</td>
</tr>
<tr>
<td>NA</td>
<td>Bee</td>
<td>Fengdu (melittin)</td>
<td>40 μg/(kg·d)</td>
<td>Anti-migration</td>
<td>↓VEGF; ↓bFGF; ↓NF-κB</td>
<td>BEL-7402 (<em>in vivo</em>)</td>
<td>[97]</td>
</tr>
<tr>
<td>Cucurbitaceae</td>
<td><em>Momordica charantia</em> Linn./Kugua</td>
<td>MAP30</td>
<td>2.0 mg/kg/13 d</td>
<td>Arresting at S-phase; inducing apoptosis and necrosis;</td>
<td>↓P53; ↑caspase-8,-9 and PARP; ↑Bcl-2; ↑p-Akt, p-p38 and p-ERK</td>
<td>HepG2 (<em>in vivo</em>)</td>
<td>[98]</td>
</tr>
<tr>
<td>NA</td>
<td>Pishuang</td>
<td>Arsenic trioxide</td>
<td>0.1 μmol/L/24 h (<em>in vitro</em>); 50 μg/mL/2 d (<em>in vivo</em>); 0.16 mg/(kg·d) (clinical trial)</td>
<td>Antiproliferation; inhibiting metastasis; inducing apoptosis; enhancing immunotherapy</td>
<td>↓Hedgehog/GLI; ↓MMP-2; ↑nm23-M1; ↑FOXO3α; ↑PSK/Akt; ↑JNK; ↑caspase-3; ↑B7H3; ↑ROS</td>
<td>HepG2, Hep7, SMMC7721, Hep3B, B7H3, SK-Hep-1 and mouse hepatoma H22 (<em>in vitro</em>); mouse hepatoma H22 (<em>in vivo</em>); patients (phase II clinical trial)</td>
<td>[99–106]</td>
</tr>
<tr>
<td>Orchidaceae</td>
<td><em>Dendrobium chrysotoxum</em> Lindl./Guchuishi</td>
<td>Erianin</td>
<td>100 mg/kg/4 h</td>
<td>Anti-metastasis</td>
<td>↓F-actin and β-tubulin</td>
<td>BEL-7402 (<em>in vitro and in vivo</em>)</td>
<td>[107]</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Cleistocalyx operculatus</em> (Roxb.) Merr. et L. M. Perry/Shuiweng</td>
<td>ON-III</td>
<td>2.5 μg/mL (<em>in vitro</em>); 5.0 mg/kg/4 d/week</td>
<td>Anti-metastasis</td>
<td>↓KDR; ↓MAPK and Akt</td>
<td>BEL-7402 (<em>in vivo</em>)</td>
<td>[108]</td>
</tr>
<tr>
<td>Crassulaceae</td>
<td><em>Sedum sarmentosum</em> Bunge/Chuipencao</td>
<td>Extracts</td>
<td>25 μg/mL/24 h</td>
<td>Inducing apoptosis</td>
<td>↓Bcl-2; ↓VEGF; ↓p-STAT3</td>
<td>HepG2 (<em>in vitro</em>)</td>
<td>[109]</td>
</tr>
<tr>
<td>Family name</td>
<td>Resource/Chinese name</td>
<td>Compounds</td>
<td>Minimum dose/duration</td>
<td>Targets</td>
<td>Mechanisms</td>
<td>Cell lines</td>
<td>References</td>
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<tr>
<td>Bufonidae</td>
<td><em>Bufo bufo</em> gargarizans Cantor or <em>Bufo melanostictus</em> Schneider/Chansu</td>
<td>Bufalin</td>
<td>1, 10, and 100 nmol/L/24 h</td>
<td>Inhibiting proliferation, invasion, migration and adhesion; inducing autophagic cell death</td>
<td></td>
<td>HepG2 and HepB2 (in vitro); Hep3B, HepG2 and HA22T (in vitro)</td>
<td>[110,111]</td>
</tr>
<tr>
<td>Bufonidae</td>
<td><em>Bufo bufo</em> gargarizans Cantor or <em>Bufo melanostictus</em> Schneider/Chansu</td>
<td>Arenobufagin</td>
<td>20, 200, and 500 nmol/L</td>
<td>Inducing apoptosis and autophagy</td>
<td>↑Bax/Bcl-2; ↓Δψm; ↑caspase-3; ↑caspase-9; ↑Bax/Bcl-2</td>
<td>HepG2/ADM (in vitro); HepG2 and Bel7402 (in vivo)</td>
<td>[112]</td>
</tr>
<tr>
<td>Rosaceae</td>
<td><em>Rubus alceaefolius</em> Poir.</td>
<td>Total alkaloids</td>
<td>3 g/kg/21 d</td>
<td>Tumor growth inhibition; inducing apoptosis</td>
<td>↓Δψm; ↑caspase-3; ↑caspase-9; ↑Bax/Bcl-2</td>
<td>HepG2 (in vitro); tumor-bearing mice (in vivo)</td>
<td>[113]</td>
</tr>
<tr>
<td>Zingiberaceae</td>
<td><em>Rhizoma curcumae</em> or <em>Ezhu</em></td>
<td>Germacrone</td>
<td>80, 160, 200, and 240 μmol/L/24 h</td>
<td>Growth inhibition; G2/M cycle arrest; inducing apoptosis</td>
<td>↓Cyclin B1; ↓CDK1; ↑Bax/Bcl-2; ↓Δψm; ↑p53; ↑p21</td>
<td>HepG2 and Bel7402 (in vitro)</td>
<td>[114]</td>
</tr>
</tbody>
</table>

Chai Hu Tang (Sho-saiko-to in Japanese), a formula composed of seven herbs\(^1\)\(^2\)\(^3\)\(^4\).

### 2.4 Combination therapy

CMs have also been integrated with conventional liver cancer therapies. The combination of CM with resection, chemotherapy, radiotherapy and TACE has been found to increase the efficacy and decrease adverse drug reactions (ADRs)\(^5\)\(^6\)\(^7\)\(^8\).

### 3 In vitro anti-tumor effects of CMs on HCC

#### 3.1 CMs inhibit in vitro growth of HCC cells

A number of CMs directly inhibit proliferation of HCC cells by reverting some cancerous properties, such as sustaining proliferation signaling, evading growth suppressors and resisting cell death. Cell cycle could be restored\(^9\)\(^10\)\(^11\). CMs can also induce cell death by breaking down the intracellular mitochondrial transmembrane potential (ΔΨm), and activating caspase-9, caspase-3 down the intracellular mitochondrial transmembrane potential of metastasis of HCC. Guo et al\(^12\) concluded that there is a great interest in understanding the process of metastasis of HCC.

#### 3.2 CMs inhibit angiogenesis and metastasis of cancer in vitro

More than 90% of mortality from cancers is attributed to metastasis. Furthermore, metastasis of a solid tumor depends on angiogenesis and vascular permeability.\(^13\)\(^14\).

Thus there is a great interest in understanding the process of metastasis of HCC. Guo et al\(^15\)\(^16\) revealed that baicalin (from Huangqin, Scutellaria baicalensis Georgii) blocks the invasion and adhesion of Bel-7402 cells by decreasing matrix metalloproteinase-2 (MMP-2) expression, and significantly inhibits the invasion ability of these cells. However, it may not be as effective as the combination of CMs with other therapies.

#### 3.3 CMs inhibit inflammation in in vitro models

Inflammation plays a key role in HCC carcinogenesis, in which activation of NF-κB initiates production of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, C-reactive protein (CRP), cyclooxygenase-1 (COX-1), COX-2, macrophage inflammatory protein-1 (MIP-1α) and 5-lipoxygenase (5-LO). Curcumin was previously reported as an inhibitor of NF-κB, and has been shown to reduce the production of various cytokines including TNF-α and IL-1β. This suggests the possible prophylactic effect of curcumin on HCC by inhibiting inflammation\(^17\) (Table 1).

#### 3.4 CMs inhibit liver cancer stem cells

Liver cancer stem cells (LCSCs) have recently been introduced as a new area of focus in liver cancer therapy. Although they only comprise 1% or less of the cells in a malignance, they are often resistant to chemotherapy or radiation-induced apoptosis, and have higher self-renewal and differentiation abilities\(^18\)\(^19\)\(^20\). Side population cells are also partially regarded as LCSCs and possess stem cell-like properties\(^21\)\(^22\). Some molecules have been identified for LCSC markers although it is still unclear whether all LCSCs share the same markers. To date, the factors that serve as markers of LCSCs/SP include one or two of the following: CD133, EpCAM (CD326), CD44, CD90, OV6 and CD24\(^23\)\(^24\)\(^25\)\(^26\). Other findings suggest that the use of only one or two markers is insufficient to identify a type of LCSC/SP\(^27\).

Cao et al\(^28\) reported that c-kit+ and CD133+ cell populations in mice with HCC had a strong reduction after treatment with either of the two Chinese formulas, Jiedu Xiaozheng Yin (4 Chinese medicinal herbs) and Fuzheng.
Yiliu Fang (4 Chinese medicinal herbs) (Table 2). Thus, some CMs might decrease the expression of cancer stem cell markers, however, to the best of our knowledge, there are no other reports supporting the ability of CMs to target LCSCs.

3.5 CMs reverse multidrug resistance

The development of multidrug resistance (MDR) in cancer cells causes a significant impediment to the advance and application of chemotherapeutical techniques. Mechanisms proposed to explain the rapid and independent development of drug resistance include changes at the cellular level that decrease rates of drug uptake, increase rates of drug efflux and promote changes to markers targeted by chemotherapeutic drugs. ATP-binding cassette (ABC) family members have been implicated as the dominant molecular causes of MDR at the cellular level[154].

Many Chinese medicinal products and their components have been shown to affect MDR in cancer cells through the inhibition of P-gp, MRP2, MRP3, MRP5, glutathione S-transferase (GST) or total protein kinase (PKC) (Table 1). To address some concerns about the ability of CMs to reverse MDR, Efferth et al[163] screened the activity of 22 CMs with MDR human CCRF-CEM leukemia cells. They found that artesunate was able to inhibit only MRP1, while bufalin could inhibit both P-gp and MRP1. He et al[164] further compared the ability of components isolated from CMs on P-gp. They ranked the effectiveness of these components based on their ability to inhibit P-gp as follows: tetrandrine > berbamine > daurisoline > berberine > tetrahydropalmatine > tetramethylpyrazine. The study did not extrapolate their findings to the potential effects on HCC cells.

Although there are many CMs that can reverse MDR in vitro, other CMs may induce MDR due to their interaction with the cytochrome (CYP) metabolic system. The nuclear receptor, pregnane X receptor (PXR), is a key modulator with the cytochrome (CYP) metabolic system. The nuclear receptors EREBP/SP1 (Keap1) no longer interacts with Nrf2, allowing Nrf2 to promote antioxidative activity, protecting liver cells from injury. Therefore, Nrf2-inducing CMs can play an important role in either protecting the liver from injury or preventing early oncogenesis[181], which is consistent with the traditional Chinese medicine (TCM) theory of Zhiweibing (prevention by CMs)[175].

3.6.1 Dual roles of CM-induced ROS generation in HCC

Hu et al[166] reported that silkworm pupae, rich in selenium, could dose-dependently increase the generation of ROS, leading to induction of apoptotic cell death in SMMC-7721 cells. Arsenic trioxide-induced ROS generation in the HCC cell line SK-Hep-1 is linked to the decrease in production of GSH by the up-regulation of serine/threonine-protein kinase OSR1 and ubiquinol-cytochrome c reductase complex core protein 1 (UQRC1), as well as the suppression of glutathione synthetase, Q6ZR44, sepiapterin reductase and UQRC1[163]. Furthermore, its combination with oridonin could enhance the effects of arsenic trioxide-induced apoptosis in HCC[23].

However, CM-induced ROS generation could result in some side effects. For example, curcumin was confirmed to have anticancer activity and has been approved in clinical trials[15,51–53,55,182], but has raised its potential carcinogenic effects with enhancing ROS formation[181,183].

3.6.2 Dual roles of CMs-blocked ROS generation and CMs-induced Nrf2 release

Qin et al[166] reported that a mixture of the extracts of CMs, ANTIOXIN, could protect hepatocytes from adriamycin-induced oxidative stress. Compared with the adriamycin-treated group, the mixture significantly decreased the
<table>
<thead>
<tr>
<th>Formula</th>
<th>Content/Chinese name (Latin name)</th>
<th>Minimum dose/duration</th>
<th>Targets</th>
<th>Mechanisms</th>
<th>HCC cell lines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xiaochaihu Tang (Sho-saiko-to in Japanese)</td>
<td>Chaihu (<em>Bupleurum chinense</em> DC.), Banxia (<em>Pinellia ternata</em> Druce), Huangqin (<em>Scutellaria baicalensis</em> Georgi), Dazhao (<em>Ziziphus jujuba</em> Lam.), Renshen (<em>Panax ginseng</em> C.A. Mey.), Gancao (<em>Glycyrrhiza uralensis</em> Fisch.) and Shengjiang (<em>Zingiber officinale</em> Roscoe)</td>
<td>20 μg/mL/3 d (<em>in vitro</em>); 50 μg/mL (clinical trial)</td>
<td>Anti-HBV/HCV; inhibiting proliferation; arresting at G1/G0 phase; inducing apoptosis; regulating immune function; anti-inflammation</td>
<td>↑IFNs and G-CSF; ↓KIM-1; ↑IL-10; ↑TNF-α; ↑CD4/CD8</td>
<td>HepG2 (<em>in vitro</em>); clinical trial</td>
<td>[119,127–130]</td>
</tr>
<tr>
<td>Huanglian Jiedu Tang (Japanese name: oren-gedoku-to)</td>
<td>Huanglian (<em>Coptis chinensis</em> Franch., <em>Coptis deltoidea</em> C. Y. Cheng et P.K. Hsiao, or <em>Coptis teeta</em> Wall.), Huangqin (<em>Scutellaria baicalensis</em> Georgi), Huangbo (<em>Phellodendron amurense</em> Rupr.) and Zhizi (<em>Gardenia jasminoides</em> J.Ellis)</td>
<td>500 μg/mL/48 h (<em>in vitro</em>) and 1 000 mg/kg/12 h</td>
<td>Arresting at cell-cycle S-G2/M; inducing apoptosis</td>
<td>↑phospho-Cdc2; ↑phospho-Cdc25C; ↑cyclin A; ↑cyclin B1; ↑Cdk2; ↑Cdc25C; ↑Bax; ↑Bak; ↓Bcl-2; ↓Bcl-XL; ↑IκBα; ↓NF-kB</td>
<td>HepG2 and PLC/PRF/5 (<em>in vitro</em>)</td>
<td>[126]</td>
</tr>
<tr>
<td>Longdan Tang</td>
<td>Longdan (<em>Gentiana scabra</em> Bunge), Zhizi (<em>Gardenia jasminoides</em> J.Ellis), Zhexie (<em>Alisma plantago-aquatica</em> subsp. <em>Orientalis</em> (Sam.) Sam.), Chaihu (<em>Bupleurum chinense</em> DC.) and Huangqin (<em>Scutellaria baicalensis</em> Georgi)</td>
<td>NA</td>
<td>Inducing apoptosis; arresting at G2/M phase</td>
<td>↑Caspase-3</td>
<td>Hep3B</td>
<td>[124]</td>
</tr>
<tr>
<td>Jiedu Xiaozheng Yin and Fuzheng Yiliu Fang</td>
<td>Baihuasheshecao (<em>Oldenlandia diffusa</em> (Willd.) Roxb.), Shancigu (<em>Cremastrea appendiculata</em> (D. Don) Makino), Xiakucao (<em>Prunella vulgaris</em> L.), Kushen (<em>Sophora flavescens</em> Aiton), Huangqi (<em>Astragalus membranaceus</em> Bunge.), Lingzhi (<em>Ganoderma lucidum</em> (Leyss. ex Fr.) Karst.) and Shanyao (<em>Paeonia lactiflora</em> Pall.)</td>
<td>0.54 g/kg/20 d (<em>Jiedu Xiaozheng Yin</em>) and 0.645 g/kg/20 d (<em>Fuzheng Yiliu Fang</em>)</td>
<td>Inhibiting CSCs</td>
<td>↓c-Kit; ↓CD133</td>
<td>H22 mice hepatoma cells</td>
<td>[131]</td>
</tr>
<tr>
<td>Fuzheng Yiliu Granule</td>
<td>Huangqi (<em>Astragalus membranaceus</em> Bunge.), Nüčhenzi (<em>Ligustrum lucidum</em> Ait.), Lingzhi (<em>Ganoderma malacudum</em> (Leyss. ex Fr.) Karst.) and Shanyao (<em>Paeonia lactiflora</em> Pall.)</td>
<td>3.6 g/kg/5 d (<em>in vivo</em>); drug-containing rat serum/48 h (<em>in vitro</em>)</td>
<td>Antiproliferation; inducing apoptosis; regulating immune function</td>
<td>↑NK cells; ↑IL-2 and TNF-α</td>
<td>H22 (<em>in vivo</em>); HepG2 (<em>in vitro</em>)</td>
<td>[132]</td>
</tr>
<tr>
<td>Fuzheng Xiaozheng capsules</td>
<td>Tusizi (<em>Cascuta chinensis</em> Lam.), Renshen (<em>Panax ginseng</em> C.A. Mey.), Sanqi (<em>Panaxnoto ginseng</em> (Burkill) F.H.Chen), Dongchongxiacao (<em>Cordyceps sinensis</em> s(Berk.) Sacc.) and Huanglian (<em>Coptis chinensis</em> Franch., <em>Coptis deltoidea</em> C. Y. Cheng et P.K. Hsiao, or <em>Coptis teeta</em> Wall.)</td>
<td>5.4 g/60 kg/d</td>
<td>Reversing ADR</td>
<td>↑WBCs</td>
<td>Clinical study</td>
<td>[121]</td>
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Table 2 (continuation)  Chinese medicinal formulas for anti-liver cancer

<table>
<thead>
<tr>
<th>Formula</th>
<th>Content/Chinese name (Latin name)</th>
<th>Minimum dose/duration</th>
<th>Targets</th>
<th>Mechanisms</th>
<th>HCC cell lines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shugan Jianpi Fang</strong></td>
<td>Chaihu (Bupleurum chinense DC.), Bayuezha (Akebia quinata (Houtt.) Decne.), Baishao (Paeonia lactiflora Pall.), Nüzhénzi (Ligustrum lucidum W.T. Aiton.), Danggui (Angelica sinensis (Oliv.) Diels), Ezhu (Curcuma phaeocaulis Valet. on), Danshen (Codonopsis pilosula (Franch.) Nannf.), Baizhu (Atractyloides macrocephala Koidz.), Fuling (Poria cocos (Schw.) Wolf.), Huangqi (Astragalus membranaceus Bunge.), Jineijin (Chicken’s Gizzard-membrane) and Zhigancao (processed Glycyrrhiza uralensis Fisch.)</td>
<td>100 mL/7 week</td>
<td>Regulating immune function</td>
<td>↑NK cells; ↑CD3, CD4 and CD4/CD8</td>
<td>Clinical study</td>
<td>[122]</td>
</tr>
<tr>
<td><strong>Danggui Buxue Tang</strong></td>
<td>Danggui (Angelica sinensis (Oliv.) Diels) and Huangqi (Astragalus membranaceus Bunge.)</td>
<td>0.5 mg/mL/48 h</td>
<td>Reducing ADR</td>
<td>↑EPO mRNA</td>
<td>Hep3B</td>
<td>[133]</td>
</tr>
<tr>
<td><strong>Erbie San</strong></td>
<td>Bieja (shell of Trionyx sinensis Wiegmann) and Tubie (Eupolyphaga Seu Steleophaga)</td>
<td>1.25 g/kg/21 d</td>
<td>Anti-metastasis</td>
<td>↓VEGF; ↑endstatin</td>
<td>Walker-256</td>
<td>[134]</td>
</tr>
<tr>
<td><strong>Qingre Jiedu, Huoxue Huayu and Fuzheng Gaben</strong></td>
<td>Cinobufotalin, ginsenosides Rg3, Panax notoginseng saponins (PNS), and lentinan</td>
<td>9.195 mg/kg/10 d</td>
<td>Reducing ADR</td>
<td>↑WBCs</td>
<td>H22 (in vivo)</td>
<td>[135]</td>
</tr>
<tr>
<td><strong>Jianpi Jiedu Fang</strong></td>
<td>Danshen (Radix Codonopsis), Fuling (Poria), Baizhu (Rhizoma Atractyloids Macrocephala), Banzhilian (Herba Scutellariae Barbatae), Ezhu (Rhizoma Curcumae Phaeocaulis), Chaihu (Radix Bupleuri Chinensis) and Gancao (Radix Glycyrrhiza); ethyl acetate fraction</td>
<td>37.5 g/kg/70 d</td>
<td>Improving survival</td>
<td>↓PTEN; ↑PI3K; ↓FAK</td>
<td>Bel-7402-bearing mice</td>
<td>[136]</td>
</tr>
<tr>
<td><strong>Jiedu Xiaozheng Yin</strong></td>
<td></td>
<td>0.06 g/kg/21 d</td>
<td>Inhibiting tumor growth; G0/G1 cell cycle arrest</td>
<td>↓Cyclin D; ↓cyclin E</td>
<td>HepG2 tumor-bearing mice</td>
<td>[137]</td>
</tr>
</tbody>
</table>

↑: increase or activate; ↓: decrease or inhibit. ADR: adriamycin; G-CSF: granulocyte colony-stimulating factor; FAK: focal adhesion kinase; IkB: inhibitor of nuclear factor-κB; KIM-1: kidney injury molecule-1; NF-κB: nuclear factor-κB; NK: natural killer; PI3K: phosphoinositide 3-kinase; PTEN: phosphatase and tensin homolog; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor; WBCs: white blood cells.
malonaldehyde (MDA) level, increased the total superoxide dismutase (T-SOD) and manganese superoxide dismutase (MnSOD) level and total antioxidant capacity (TAC) in the liver. These findings indicated that ANTIOXIN could reduce the hepatic ROS level by enhancing the activity of antioxidant enzymes.

({-}Epicatechin-3-gallate (isolated from green tea) induced Nrf2 and UPD-glucuronosyl-transferase (UGT) expression, which prevented oncogenesis and metastasis of liver cancer[185]. Non-polar fractions of Zihuayeju (Chrysanthemum zawadskii Herbich, CZ) and extract of licorice root (Gancao, Glycyrrhiza uralensis Fisch., LE) induced ARE-luciferase activity in HepG2 C8 cells. Furthermore, CZ and LE initiated the mRNA transcription of the endogenous Nrf2-target gene, NAD(P)H dehydrogenase (quinone) 1 (NQO-1). The mRNA expression of phase II UGT 1A1 was also increased by LE and CZ. Results from this study indicated that LE and CZ may provide some chemoprevention against HCC[41]. In contrast, prolonged over-expression of Nrf2 was found not only to induce oncogenesis and tumor growth, but also to enhance the drug resistance of cancer cells by increasing drug transporters and GSH[186].

4 In vivo anti-HCC effects and mechanisms of CMs

Inhibition of HCC cell growth may result from a wide variety of actions of CMs, including their roles in induction of cell death, immune regulation, prevention of metastasis, reduction of inflammation and anti-viral activity. Fang et al[89] found that MAP30 protein (isolated from Kugua, Momordica charantia Linn.) could induce cell cycle arrest at S-phase and apoptosis in HepG2. An in vivo study showed that MAP30 induced apoptosis and necrosis in male nude HepG2-bearing mice. Sun et al[88] assessed the antiproliferative effects of Kushen (Sophora flavescensAiton) flavonoids (KS-Fs, including kurarinone, flavescens, 2′-methoxy-kurarinone and others) in vivo. The inhibition rates of tumor were 43.40%, 66.45% and 78.98%, respectively when H22 hepatoma mice were treated with KS-Fs at the concentrations of 20, 100 and 500 mg/kg[89].

To observe the anti-metastasis effects of ardipusilloside I (from Jiujielong, Ardisia pusilla A. DC.), Lou et al[69] studied in vivo orthotopic implantation of HCC in athymic BALB/c nu/nu mice. Tumor cells invading the lung were suppressed when mice were treated with ardipusilloside I at the dose of 100 mg/(kg·d), suggesting that ardipusilloside I might be a potential compound to inhibit metastasis of HCC[43]. Erbie San is a composite formula powder containing two animal parts, Biejia (shell of Trionyx sinensis Wiegmann) and Tubiechong (Eupolyphaga Seu Steleophaga). Erbie San inhibited the angiogenesis of tumors in Walker-256-bearing rats through the inhibition of VEGF and induction of endostatin[134].

Advanced chronic hepatitis viral infection (HBV and HCV) is believed to be a major factor leading to liver cirrhosis[187,188], resulting in HCC characterized by replacement of normal liver tissue by fibrotic tissue[189–192]. Oxymatrine, emodin and astragalus polysaccharide (APS) have been shown to inhibit HBV and HCV in transgenic mice[26,76]. Salvinanolic acid B (isolated from Danshen, Salvia miltiorrhiza Bunge), reduced hepatic stellate cell (HSC) proliferation and HSC collagen production by inhibiting platelet-derived growth factor, the mitogen-activated protein kinase pathway, and TGF-{\( \beta \)}-Smad signaling[125]. Together these actions may reduce risk of fibrosis-related carcigenosis.

The anti-HCC effects of CMs in vivo may also be due to their regulation of the immune system. Xiao Chai Hu Tang decreased oncogenesis in mice by boosting up immunological system in vivo[127,128]. Luo et al[100] reported that As_{2}O_{3} (Pishuang) could synergize with B7H3-mediated immunotherapy to eradicate HCC by generating cytotoxic T cells, and selectively enhancing interferon-\( \gamma \) expression.

5 Clinical application of CMs for treatment of HCC

5.1 CMs as prevention and therapy for HCC patients

Application of CMs to the treatment of HCC is very common in Asian countries. Successful cases have been reported from the use of either single compounds or fractions isolated from CMs. Patients with HCC survived with quality of life. In the 1970s, physicians used Pishuang to treat acute promyelocytic leukemia (APL) in China. Today, Pishuang is marketed in Mainland China as a pharmaceutical product for APL and advanced primary liver cancer[195]. On the other hand, CMs delay the onset of HCC in patients with progressive liver diseases. Lu et al[86] conducted a clinical trial with 216 chronic hepatitis B patients, of whom 108 received oxymatrine capsules (OM, isolated from Kushen or Kudouzi), 36 received OM injections and 72 received placebo treatment. The complete response rates in the OM capsule group and the OM injection group were 24.51% and 33.33%, respectively. Over a long-term patient follow-up study, Veldt et al[157] evaluated the effect of glycyrrhizin (isolated from Gancao, Glycyrrhiza uralensis) on the incidence of HCC in 1 093 patients with chronic hepatitis C who had not responded to treatment with interferon. The results showed that the hazard ratio was 0.39 (95% CI 0.21–0.72; \( P < 0.01 \)). The data from this study indicated that patients with chronic hepatitis C and stage 3 or 4 fibrosis, which had not responded to treatment with interferon, had a reduced incidence of HCC when receiving glycyrrhizin therapy. It is speculated that this treatment may have acted by improving the normalization of alanine aminotransferase (ALT) levels[197]. Liao et al[198] conducted a cross-sectional study, and reported that
among 6,358 patients with liver cancer, 1,240 (19.5%) of the study population was also taking CMs. Additionally, the clinical use of CMs is most commonly in the form of composite formula prescribed by a CM practitioner, based on the Bianzheng (syndrome differentiation) theory of TCM. Kuo and Hung presented a case study in which a 60-year-old male with cirrhosis, presenting liver cancer, was treated with a CM prescription containing 17 herbs. The level of his AST was reduced and the AFP level descended to a normal level.

5.2 CMs in combination with conventional medicine

Fuzheng Xiaozheng capsules (made of 6 CMs) have been used to treat the side effects of conventional drugs on the immune system, with a total effective rate of 82.8%.[121] Shugan Jianpi Fang (made of 12 CM herbs) was found to enhance the immune function after TACE.[122] Shu et al.[200] summarized the results of 26 clinical studies with 2,079 patients and concluded that CMs combined with chemotherapy could improve the response of HCC patients to chemotherapy. In contrast, Liu et al.[201] reported that CM treatment might make minimal contributions to the treatment outcomes of combination therapy. Generally, CMs combined with conventional therapy are ranked as adjuvant or CAM, however, the efficacy and contributions of CMs to these cases still need further investigation.[135,202] Reports of this complementary use of CMs are summarized in Table 2.

5.3 CMs for improving quality of life in liver cancer patients

Beyond their direct action on HCC tumors, CMs are considered to relieve the side effects and improve the quality of life in HCC patients. The herbal formula QHF, which is composed of three composite formulas, Qingre Jiedu, Huoxue Huayu and Fuzheng Guben, was shown to reduce cisplatin (CDDP)-induced leukopenia, spleen, thymus atrophy and other toxic reactions when taken in combination with CDDP.[133] Yadanzi (Brucea javanica L. Merr.) injection[203] and Chansu (venom of Bufo gargarizans Cantor) injection[204,205] have been marketed in China. Kanglaite is the first Chinese herbal formula (fractions of Coix lachryma-jobi L.) that approved into a phase II trial by the China Food and Drug Administration.[206,207]

CMs are also used for relieving cancer-associated pain in patients. Wang et al.[111] conducted a single-blinded controlled trial to evaluate the efficacy of a Dingqi analgesic patch on moderate to severe pain induced by liver cancer. CM formulas were found to significantly reduce pain without causing obvious side effects.[111] CMs commonly used for alleviating pain include but are not limited to Yanhusuo (Corydalis yanhusuo W. T. Wang ex Z. Y. Su et C. Y. Wu), Chuanxiong (Ligusticum chuanxiong Hort), Ruxiang (Oil of Boswellia serrata Roxb. ex Colebr.), Moyao (oil of Commiphora myrrha (Nees) Engl.), Xuchangqing (Cynanchum paniculatum (Bunge) Kitag.), Weilingxian (Clematis chinensis Osbeck.), Wutou (Aconitum carmichaeli Debeaux), Caowu (Aconitum kusnezoffii Rehb.), Baishao (Paeonia lactiflora Pall.), Honghua (Carthamus tinctorius L.) and Duhuo (Angelica pubescens Maxim.).[208,209]

6 Discussion

CMs have been used for preventing and treating human cancers in Asian societies for centuries. As an important component of traditional medicines and ethnomedicinal systems in the world, the efficacy and safety of CM have been evaluated individually for practitioners of CM. However, in the context of evidence-based medicine, determination of efficacy requires critical evaluation with modern medical research approaches. In recent years, a lot of effort has been made to evaluate the potential of CMs for the prevention and treatment of HCC. Actions of CMs on HCC from experimental and clinical studies are shown in Figure 1.

Determining cellular mechanisms of CM action is challenging, as cellular signaling in cancer is quite complicated and there may be some cross-talk among pathways. For instance, ROS exhibits a dual role in cancer therapy as described above. On one hand, ROS participate in oncogenesis and the metastasis of cancer. The level of ROS in cancer cells is higher than in normal cells and ROS may be associated with MDR through interactions with P-gp and multidrug resistance-associated protein (MRP) within cancer cells.[210] Studies have further shown that MDR cancer cells have reduced levels of ROS. Additionally, CM treatment combined with drugs elevating ROS might potentiate drug-resistant cancer cells.[177] On the other hand, drugs inducing ROS production could lead to death of cancer cells. This paradoxical role of ROS requires investigators to carefully consider the pharmacological behavior of CMs when a treatment could act on ROS. One way to address this issue may be to develop targeted treatments that increase the generation of mitochondrial ROS, and regulate the genes that maintain the balance of ROS in MDR cancer cells. Another approach may be to introduce ROS directly into tumors by using nanoparticle vehicles.[177] This practice needs more research before it can be considered for CM-targeted therapy in clinical settings. Another interesting example of CM therapy is found in the effects of CMs on TNF-α signaling. Since carcinogenesis in live rats at least partially results from TNF-α-induced inflammation, a promising observation in CM research is that compounds and extracts from CMs like curcumin could block TNF-α[55,211]. This property of CMs may be useful in both preventing the occurrence of HCC and reducing the side effect of conventional therapy. However, a contrasting observation was also reported. Triptolide was found to inhibit TNF-α in T-cells[21] but induce TNF-α-mediated apoptotic cell

[www.jcimjournal.com/jim]
death in HCC\textsuperscript{[135]}. This paradoxical action may also occur in a composite formula that comprises several herbs. For instance, Xiao-chaihu Tang had a net effect of increasing TNF-\(\alpha\) activity although some of its components exhibit opposite effects on HCC\textsuperscript{[128]}. This indicates that the action of specific CMs may depend on drug targets, or on the interactions among different components of a composite CM.

Clinically, it is believed that TCM has three advantages in preventing and treating tumors: by preventing tumorigenesis, by attenuating toxicity and enhancing the treatment effects, and by reducing tumor recurrence and metastasis\textsuperscript{[212]}. We have done meta-analysis for 67 trials (\(n = 5211\)) and found CMs taken in conjunction with TACE have beneficial effects on tumor response, quality of life and reduction of TACE toxicity compared with TACE alone. However, poor research methods limit the extrapolation of these findings to broader applications\textsuperscript{[213]}. In the present review, we mainly focus on pharmacological actions and mechanisms of CMs in HCC, but many of these studies are still superficial. New cancer hallmarks and omics technologies provide new avenues for CM research. Therefore it is anticipated that there will be an expansion of the exploration of the uses of CMs in cancer treatments, including HCC\textsuperscript{[214]}. We introduced miRNAs into our studies and got a whole picture about miRNAs alteration in HCC after treatment of coptis and its principal compound berberine. The results showed that miRNA-23a was up-regulated by coptis and berberine in HCC\textsuperscript{[115]}. We further investigated the involvement of miRNA-23a in p53-mediated inhibition of HCC by berberine; this study found that berberine could induce the expression of miR-23a and enhance the expression of p53 in HCC cells treated with berberine\textsuperscript{[215]}. We also found that berberine inhibited the growth and development of lung metastases in HCC by suppressing Id-1 expression\textsuperscript{[216]}. One of the mechanisms for the prevention of metastasis by berberine and coptis extract in HCC may be the suppression of vascular endothelial growth factor via inactivation of eukaryotic elongation factor 2\textsuperscript{[217]}.  

7 Conclusions

The actions of CMs on HCC may include tumor growth inhibition, anti-metastatic activities, anti-inflammation, reversal of MDR and induction/reduction of oxidative stress. Multiple molecules were found to contribute in the above actions. Many studies showed that CMs may have the potential to both prevent HCC occurrence and retard HCC progression with several molecular targets involved. More pre-clinical and clinical evaluations that incorporate novel and advanced technologies in evidence-based medicine, pharmacology, immunology and OMICS approaches are needed.

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9 Competing interests

All authors declare that they have no competing interests.

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