Dear Sir/Madam,

We would like to thank you for the opportunity to revise our manuscript. We have spent a great deal of time and effort to prepare this manuscript and perform the requested amendments. Below is a point-by-point reply to the reviewers' comments. We feel that the revisions have greatly improved the quality and appeal of the manuscript, and hope that the revised manuscript will meet with your approval. Any further comments or suggestions would be welcome, and we look forward to hearing from you soon.

Sincerely,

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Reply to Reviewers' Comments:

Reviewer #1:

1. First of all, the focus of the article is not outstanding. For example, the author cited many mechanisms of TCM in the treatment of HCC, but I felt that the explanation was not very clear, and some parts were only a few points. Acupuncture, for example, is just mentioned. Furthermore, the authors listed the role of many traditional Chinese medicines in the regulation of HCC signaling pathways, but did not specify which signaling pathways are better regulated by traditional Chinese medicines. Therefore, I think it is enough to simplify the description of many traditional Chinese medicines in regulating HCC signaling pathway and list a few key points with good regulatory effects.

Reply:

Thank you for the insightful comments. We have added text in the revised manuscript regarding acupuncture's role in HCC.

p. 8, lines 21-38

Acupuncture has been applied to treat liver diseases for centuries. The role of acupuncture in HCC is to regulate the ying and yang, as well as improve body circulation. Recent studies have indicated acupuncture protects against liver injury caused by carbon tetrachloride, and reverses fibrogenesis accompanied with decreasing hyaluronic acid, laminin and procollagen III [21, 22]. Of note, the most commonly chosen acupoints used when treating chronic liver diseases are ST36, LR3, SP6, BL18, GB34, and RN12 [23]. A randomized controlled trial of 90 patients who received acupuncture on ST36, LR3 and SP6 reported a decrease in the liver fibrosis grade after three months of treatment [24]. In addition, low-frequency electroacupuncture (2 Hertz) at ST36, lowered portal pressure by attenuating tumor necrosis factor (TNF)-α, nitric oxide, and 6-keto-prostaglandin F1 alpha (6-keto-PGF1α) overproduction [25]. Furthermore, one study using a rat model reported that moxibustion on BL18 once every 3 days for 10 weeks decreased HCC progression and concurrently increased cluster of differentiation (CD) 3+ and CD 4+ T cell levels and reduced CD 8+ T levels [26]. Meanwhile, a randomized controlled trial by Wei *et al*. studied 72 cases who received acupuncture on RN 8 and RN 12, reporting improved portal circulation [27]. Taken together, these studies indicate that acupuncture may enhance circulation in the liver and portal area and decrease HCC progression.

p. 16, lines 6-10

With regards to the benefits of acupuncture in the amelioration of drug resistance, a limited number of studies have focused directly on the antitumor and synergistic effects associated with acupuncture, electroacupuncture and moxibustion. Although, Yang *et al*. reported that electroacupuncture around a breast cancer tumor increased the local concentration of paclitaxel and decreased the tumor volume [116].

We appreciate your comment and have added text to the revised manuscript to summarize the regulation of key molecular HCC signaling pathways.

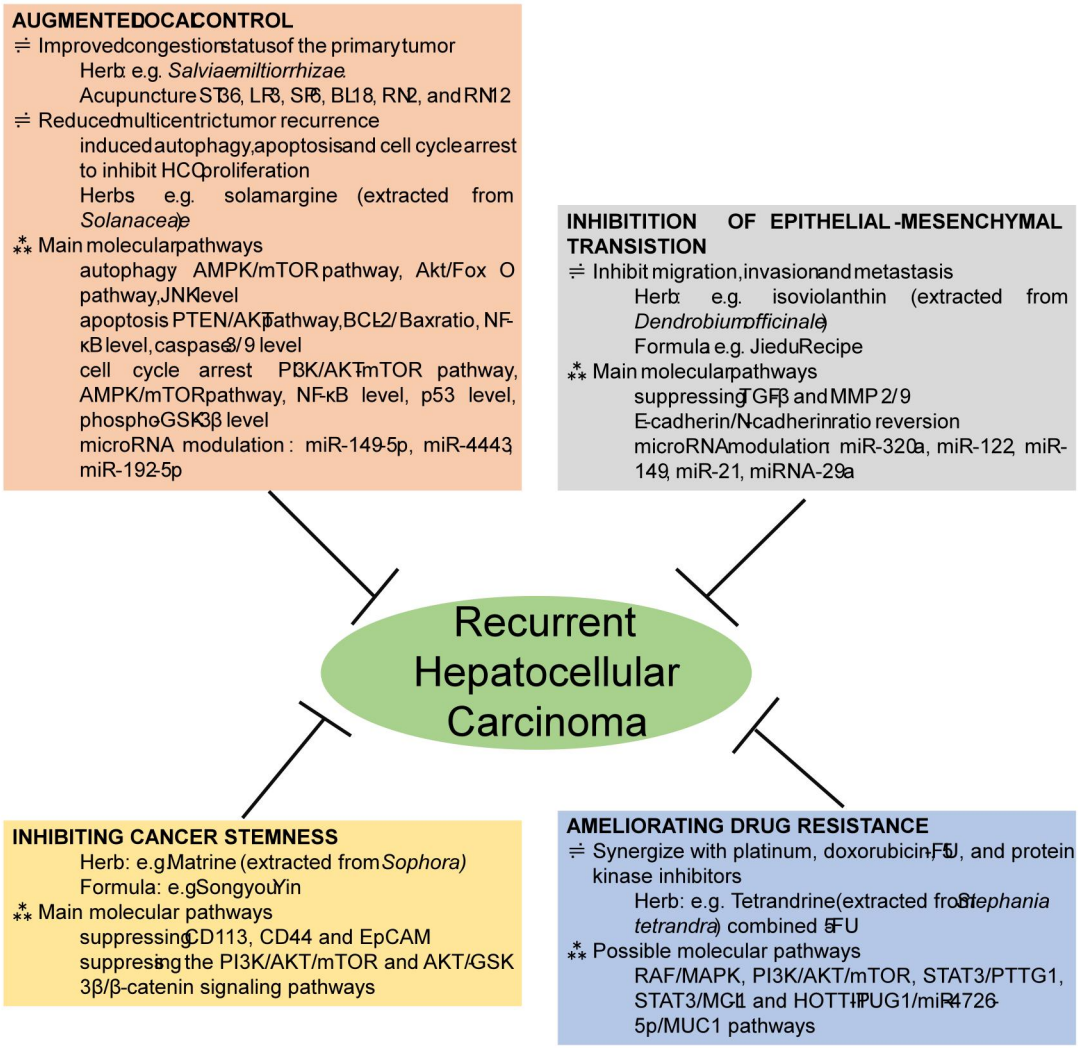
p.10 line 32-36

The molecular mechanisms are related to attenuating nitric oxide production and inhibiting fibrosis progression (via reducing procollagen III), thereby effectively preventing HCC recurrence.

p.10 line 38 to p.11 lines 1-7

With regards to the pathways associated with autophagy, multiple herbs and their derivates have been shown to primarily affect the AMPK/mTOR pathway and the Akt/Fox O pathway, and to regulate the JNK level. Additionally, several herbs and their derivates have been reported to induce apoptosis mainly via regulating the PTEN/AKT pathway, the BCL-2/Bax ratio and the NF-κB level, and by increasing the caspase 3/9 level. As for cell cycle arrest, several herbs and their derivates affect the PI3K/AKT-mTOR pathway, the AMPK/mTOR pathway and the NF-κB level, and act to up-regulate levels of p53 and phospho-GSK-3β.

In addition, we have included a figure summarizing the molecular pathways involved in complementary therapies in recurrent HCC (Figure 1 shown below).



2. The language of the manuscript needs to be revised.

Reply: Thank you for the suggestion. We have invited a native English speaker to ensure the grammar and vocabulary are correct in the revised manuscript. We hope the English language in the revised version meets with your approval.

3. The diagrams in the manuscript are not standard and need to be adjusted.

Reply: We appreciate the insight. We have modified the diagrams in the revised manuscript based on the guidelines for this journal. In addition, we have included abbreviations for each Table and Figure to improve the clarity for readers.

Reviewer #2:

1. grammatical issue

Reply: Thank you for the comment. We have invited a native English speaker to revise the manuscript to ensure any perceived grammatical issues have been resolved.

2. word editing, font, line spaces

Reply: We appreciate your insight and have carefully checked the English language and formatting to improve the readability and visual appeal of the revised manuscript.

3. it look like somewhat carelessness work

Reply: We appreciate your comment and apologize for the potential lack of appeal with regards to the manuscript. The authors involved have spent much time and effort in the preparation of this review article. We truly believe that this article can provide readers with insight and clarity with regards to the functions and possible mechanisms involved in TCM and its integration with Western medicine in recurrent HCC.

Roles of conventional and complementary therapies in recurrent hepatocellular carcinoma

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Running title:
Complementary therapies in recurrent HCC

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer and the fourth leading cause of cancer-related deaths in the world. HCC has a reported recurrence rate of 70-80% after 5 years of follow-up. Controlling tumor recurrence is the most critical factor associated with HCC mortality. Conventional salvage therapies for recurrent HCC include re-hepatectomy or liver transplantation, transcatheter arterial chemoembolization (TACE), Y-90, target therapy, and immunotherapy; however, these conventional treatment modalities have yet to achieve consistently favorable outcomes. Meanwhile, previous studies have demonstrated that conventional therapies in combination with traditional Chinese medicine, acupuncture, moxibustion or dietary supplements could notably benefit patients with HCC recurrence by strengthening and augmenting the overall management strategy. However, systemic reviews related to the interactions between complementary therapies and conventional therapy in recurrent HCC are limited. In this review, we discuss the molecular mechanisms underlying the functions of complementary therapies for recurrent HCC, which include augmenting the local control to improve the congestion status of primary tumors and reducing multicentric tumor occurrence via inducing autophagy, apoptosis or cell cycle arrest. Traditional Chinese medicine and its derivatives may play important roles in helping to control HCC recurrence by inhibiting epithelial-mesenchymal transition (EMT), migration, invasion, and metastasis, inhibiting cancer stem cells, and ameliorating drug resistance.

Keywords: Recurrence; Hepatocellular carcinoma; Complementary therapy; Traditional Chinese Medicine; Cancer stemness; Drug resistance

Core tip:

Studies have reported a recurrence rate for hepatocellular carcinoma (HCC) of 70-80% after 5 years of follow-up. The primary concern associated with HCC mortality is controlling tumor recurrence. Conventional salvage therapies, including liver transplantation, transcatheter arterial chemoembolization, target therapy, and immunotherapy achieve inconsistent outcomes. Therefore, complementary therapies as an adjuvant treatment modality may act to strengthen and augment conventional therapies. We herein discuss the molecular mechanisms underlying complementary therapies and the interactions with conventional therapy in recurrent HCC related to augmenting the local control, inhibiting epithelial-mesenchymal transition, migration, invasion, metastasis and cancer stem cells, and by ameliorating drug resistance.

1 ABBREVIATIONS

5-FU	5-fluorouracil
6-keto-PGF1α	6-keto-prostaglandin F1 alpha
Akt	Protein kinase B
ALDH	Aldehyde dehydrogenases
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
APC	Adenomatous polyposis coli protein
Bax	BCL2-Associated X Protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra large
BCRP	Breast cancer resistant protein
CD	Cluster of differentiation
CDK	Cyclin-dependent kinases
c-Met	Mesenchymal-epithelial transition factor
COX-2	Cyclooxygenase-2
CSC	Cancer stem cell
DNA	Deoxyribonucleic Acid
EGFR	Epidermal growth factor receptor
EHMT2	Euchromatic histone lysine methyltransferase 2
EMT	Epithelial-mesenchymal transition
EpCAM	Epithelial cell adhesion molecule
EPHB4	EPH Receptor B4
ERK	Extracellular signal-regulated kinase
ETV1	ETS translocation variant 1
EZH2	Enhancer of zeste homolog 2
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FoxO	Forkhead box O
GAS5	Growth arrest-specific transcript 5
GSK	Glycogen synthase kinase
HCC	Hepatocellular carcinoma
HGF	Hepatocyte growth factor
HIF	Hypoxia-Inducible Factor
HOTTIP	HOXA distal transcript antisense RNA
IL	Interleukin
IRE1	Inositol-requiring enzyme 1
IRF3	Interferon regulatory factor 3

JNK	c-Jun N-terminal kinase
LC-3 A/B II	Microtubule-associated protein-1 light chain-3 A/1B II
LCSLC	Liver cancer stem-like cell
LINC01134	Long intergenic non-protein-coding RNA 1134
lncRNA	Long non-coding RNA
LSD1	Lysine-specific demethylase 1
MAPK	Mitogen-activated protein kinase
MCL-1	Myeloid cell leukemia 1
miR	microRNA
MMP	Matrix metalloproteinase
MRP1	Multidrug Resistance Protein 1
Mst1	Mammalian sterile 20-like kinase 1
MTA1	Metastatic tumor antigen 1
mTOR	Mammalian target of rapamycin
MYPT1	Myosin phosphatase target subunit 1
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-κB	Nuclear factor kappa B
NOD	Nucleotide-binding oligomerization domain
Notch1	Neurogenic locus notch homolog protein 1
PDGFR	Platelet-derived growth factor receptors
PI3K	Phosphatidylinositol 3-kinase
PINK1	PTEN-induced kinase 1
PP2Acα	α isoform of the catalytic subunit of protein phosphatase 2A
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PTTG1	Pituitary tumor transforming gene 1
RAF	Rapidly accelerated fibrosarcoma
RFA	Radiofrequency ablation
RNF51	RING finger protein 51
ROS	Reactive oxygen species
SD rat	Sprague-Dawley rat
SHP	Src homology 2 domain-containing protein tyrosine phosphatases
Skp2	S-phase kinase-associated protein 2
SMAD	Suppressor of Mothers against Decapentaplegic
STAT3	Signal transducer and activator of transcription 3
TACE	Transcatheter arterial chemoembolization
TBK1	Tank-binding kinase 1
TBX3	T-Box Transcription Factor 3

TCM	Traditional Chinese medicine
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TUG1	Taurine upregulated 1
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WWOX	WW domain-containing oxidoreductase
YAP	Yes-associated protein
ZO	Zonula Occludens Protein
β-TrCP	β-transducin repeat-containing protein

INTRODUCTION

Worldwide, hepatocellular carcinoma (HCC) is the fifth most common type of cancer and the fourth leading cause of cancer-related deaths. The highest prevalence rates of HCC are reported in East Asia, while the incidence rates are approximately 6.7/100,000 among the age-adjusted population, 2.6% in nonalcoholic steatohepatitis (NASH) cirrhosis patients, and 0.13 % in nonalcoholic fatty liver disease (NAFLD) patients [1, 2]. The annual incidence rate increased by 2% to 3% between 2007 and 2016, while HCC notably has the second poorest 5-year survival rate of all cancer types (18%) [3]. Early-stage treatments for HCC include resection, liver transplantation, and radiofrequency ablation (RFA), while transcatheter arterial chemoembolization (TACE), chemotherapy, molecular target therapy, immunotherapy with immune checkpoint inhibitors may commonly be applied in later stages. Even in resected HCC, the recurrence rate remains over 10% after 1 year, and 70-80% after 5 years [4]. Thus, controlling tumor recurrence is a primary concern to reduce HCC mortality rates.

Salvage therapies for recurrent HCC include re-hepatectomy or liver transplantation, TACE, Y-90, target therapy, and immunotherapy. Meanwhile, studies have reported that complementary therapies such as traditional Chinese medicine (TCM), acupuncture, and dietary supplements have demonstrated notable anti-tumor effects [5, 6]. These complementary therapies affect multiple biological mechanisms such as promoting tumor cell apoptosis, autophagy, cell cycle arrest, anti-metastasis, anti-angiogenesis, anti-proliferation, anti-epithelial-mesenchymal transition (EMT), and control of cancer stem cell proliferation [5, 6]. In addition, TCM has been noted to prevent drug resistance and act to facilitate conventional therapies in cases of recurrence.

However, systemic reviews related to complementary therapies in recurrent HCC are limited. The aim of this review is to introduce and discuss the molecular mechanisms underlying the effects of complementary therapies in recurrent HCC.

AUGMENTED LOCAL CONTROL

Patients graded as Child-Pugh class A or B, presenting with three or fewer tumors of <3cm are commonly recommended local control methods such as hepatectomy and RFA [7]. Although hepatic resection has a lower reported rate of recurrence as compared to RFA or TACE, the recurrence rate remains relatively high (70-80%) [4]. This could be due to incomplete treatment of the primary tumor related to poor tumor location or surgical factors. Studies have indicated two critical mechanisms which come into play after local control.

Improved congestion status of the primary tumor

The first mechanism involves the congestion status, including increased intratumoral pressure or portal hypertension causing microrupture and tunnel seeding in the operation process, thereby increasing the risk of metastasis [8]. Patients presenting with a hepatic venous pressure gradient over 10 mmHg have a 5-year survival rate of approximately 50%, while that rate increases to approximately 70% in patients with a hepatic venous pressure gradient less than 10 mmHg [9]. In this regard, TCM characterizes high hepatic venous pressure as "blood stasis", while liver and spleen "stiffness" have been associated with late HCC recurrence [10]. Thus, herbs promoting blood circulation could act to improve portal hypertension, thereby reducing the risk of HCC recurrence. *Salviae miltiorrhizae* (Danshen) is noted for effectively treating angina pectoris and ischemic stroke. Recent studies have demonstrated that *Salviae miltiorrhizae* or its derivates lower portal hypertension by inhibiting nitric oxide production, the RhoA signaling pathway and downstream myosin phosphatase target subunit 1 (MYPT1) phosphorylation [11-14]. Furthermore, the reported anti-cancer effects exhibited by *Salviae miltiorrhizae* in liver cancer [15] likewise act to decrease HCC recurrence [16, 17]. Wan *et al*. demonstrated that tetrandrine (1ml/0.1kg) gavage in a Sprague-Dawley (SD) rat model could inhibit nitric oxide production and ameliorate cirrhosis and portal hypertension [18], whereby HCC recurrence risks may be reduced. Additionally, *Aconiti Lateralis Radix Praeparata* and *Fructus Aurantii* used for 14 consecutive days reduced portal pressure in an SD rat model [19, 20].

Acupuncture has been applied to treat liver diseases for centuries. The role of acupuncture in HCC is to regulate the ying and yang, as well as improve body circulation. Recent studies have indicated acupuncture protects against liver injury caused by carbon tetrachloride, and reverses fibrogenesis accompanied with decreasing hyaluronic acid, laminin and procollagen III [21, 22]. Of note, the most commonly chosen acupoints used when treating chronic liver diseases are ST36, LR3, SP6, BL18, GB34, and RN12 [23]. A randomized controlled trial of 90 patients who received acupuncture on ST36, LR3 and SP6 reported a decrease in the liver fibrosis grade after three months of treatment [24]. In addition, low-frequency electroacupuncture (2 Hertz) at ST36, lowered portal pressure by attenuating tumor necrosis factor (TNF)-α, nitric oxide, and 6-keto-prostaglandin F1 alpha (6-keto-PGF1α) overproduction [25]. Furthermore, one study using a rat model reported that moxibustion on BL18 once every 3 days for 10 weeks decreased HCC progression and concurrently increased cluster of differentiation (CD) 3+ and CD 4+ T cell levels and reduced CD 8+ T levels [26]. Meanwhile, a randomized controlled trial by Wei *et al*. studied 72 cases who received acupuncture on RN 8 and RN 12,

reporting improved portal circulation [27]. Taken together, these studies indicate that acupuncture may enhance circulation in the liver and portal area and decrease HCC progression.

Reduced multicentric tumor recurrence

The second mechanism involves multicentric tumor occurrence in the liver which could lead to recurrence. To this end, TCM offers multiple compounds presenting anti-tumor effects, such as flavonoids [28], phenylpropanoids, quinones, and alkaloids [5]. Alkaloids act to induce autophagy and apoptosis to inhibit HCC proliferation [5]. Piperidine alkaloids have been reported to induce mitochondrial fission and to regulate the mammalian sterile 20-like kinase 1(Mst1)-c-Jun N-terminal kinase (JNK) pathway, the extracellular signal-regulated kinase (ERK) signaling pathway and the phosphatase and tensin homolog deleted on chromosome 10 (PTEN)/ protein kinase B (AKT) pathway [5]. In addition, isoquinoline alkaloids have been shown to affect the AKT pathway, the AKT/Forkhead box O (FoxO) 3a/S-phase kinase-associated protein 2 (Skp2) axis, the phosphatidylinositol 3-kinase (PI3K)/AKT mammalian target of rapamycin (mTOR) pathway, and the Wnt/β-catenin-mediated pathway, thereby hindering HCC cell growth [5]. Further studies have indicated that terpenoid alkaloids, including perillyl alcohol, geraniol and paclitaxel are effective antitumor agents [29]. In a study involving Hep G2 and BEL-7402 cell lines, terpenoid alkaloids regulated AKT, p53, caspase-3, mitogen-activated protein kinase (MAPK) and Ras which induced apoptosis and cell cycle arrest, and effectively inhibited proliferation [5]. Meanwhile, indole alkaloids have been noted to influence the nucleotide-binding oligomerization domain (NOD)1 pathway, the AKT pathway, and the WW domain-containing oxidoreductase (WWOX)-dependent pathway to induce apoptosis and cell cycle arrest, and thereby inhibit HCC proliferation [5]. In terms of TCM, herbs containing steroidal alkaloids include *Solanaceae*, *Apocynaceae,* and *Liliaceae* [30]. Steroidal alkaloids may induce necroptosis, apoptosis, and cell cycle arrest to inhibit cell proliferation. Yin *et al.* reported that the compound solamargine induced autophagy and apoptosis by affecting the microRNA(miR)-192-5p/CYR61/Akt signaling pathways [31]. Additionally, in a study involving a HepG2 cell line, quinoline alkaloids were reported to induce necroptosis and apoptosis [5]. Of further note, flavonoids have been shown to offer notable anti-HCC effects. Wogonin, one type of flavonoid, acts to induce apoptosis and cell cycle arrest by activating the MOB1-LATS1 signal pathway and over-expressing phospho-glycogen synthase kinase (GSK) 3beta Tyr216 [32, 33]. Additionally, wogonin has been reported to inhibit HCC proliferation by affecting nuclear factor kappa B (NF-κB)/B-cell lymphoma 2(Bcl-2), epidermal growth factor

receptor (EGFR) and the EGFR downstream ERK/AKT signal pathway [5]. Furthermore, baicalein has been reported to inhibit cancer progression by inducing autophagy, apoptosis and cell cycle arrest in HCC cell lines [34]. Similarly, the long non-coding RNAs (lncRNAs)-hsa-miR-4443-AKT1 pathway responds positively to baicalein treatment [35]. Studies have further revealed that silibinin induces autophagy through the adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway and induces apoptosis by up-regulated p21/cyclin-dependent kinases (CDK) 4 and p27/CDK4 complexes, and down-regulated Rb-phosphorylation and E2F1/DP1 complex [36, 37]. Lee *et al*. demonstrated that luteolin causes ER stress in p53-wild type HepG2 cells and Hep3B cells [38]. It has further been shown that luteolin induces apoptosis and cell cycle arrest by transforming the growth factor (TGF)-β1, p53, and Fas/Fas-ligand signaling pathway and increasing the BCL2-associated X protein (Bax)/Bcl-XL ratio [39, 40]. Similarly, studies have reported that kaempferol induces autophagy by activating the AMPK signaling pathway [41]. Moreover, the combination of luteolin and kaempferol has been shown to increase caspase-3 and reactive oxygen species (ROS) reactions and induce apoptosis in a rat model [42]. Additionally, quercetin has been noted to inhibit cell proliferation by decreasing ROS and downregulating the PI3K pathway and induce apoptosis and autophagy by modulation of the PI3K/Akt/mTOR, Wnt/-catenin and mitogen-activated protein kinase (MAPK)/ERK1/2 pathways [43, 44]. Additionally, studies have reported that phenylpropanoid (chlorogenic acid and 16-O-caffeoyl-16-hydroxylhexadecanoic acid) and quinone (thymoquinone and juglanthraquinone C) induce apoptosis in HCC cell lines [45-48]. Separate studies have demonstrated that 4-acetylantrocamol LT3 induces autophagy by activation of the AMPK pathway [49], while aloin and andrographolide induce apoptosis [50, 51], and plantamajoside and *sanguisorba Officinalis* L. decrease proliferation in HCC cell lines [52, 53]. Curcumin also offers antioxidant, apoptotic, and anti-inflammatory effects, and is thus applied in the treatment of HCC [54]. Meanwhile, several herbs associated with TCM have been reported to inhibit HCC proliferation by targeting miRNAs, these include *Coptidis rhizoma* (miR21 and miR23a), berberine (miR-23a), ginsenoside (miR-491), camptothecin (miR-122), and matrine (miR-21) [55]. Yang *et al*. reported on a randomized control trial of 291 patients who received the Fuzheng Jiedu Xiaoji formula and consequently exhibited a reduced mortality rate by the effective inhibition of liver cancer cell proliferation and migration via modulated AKT/CyclinD1/p21/p27 pathways [56]. The compounds associated with reducing multicentric tumor occurrence are shown in Table 1.

In summary, TCM and acupuncture treatments have been reported to augment the local control by improving the congestion status of the primary tumor. The

molecular mechanisms are related to attenuating nitric oxide production and inhibiting fibrosis progression (via reducing procollagen III), thereby effectively preventing HCC recurrence. Meanwhile, various anti-tumor compounds have been reported to reduce multicentric tumor occurrence by inducing autophagy, apoptosis and cell cycle arrest to inhibit HCC proliferation. With regards to the pathways associated with autophagy, multiple herbs and their derivates have been shown to primarily affect the AMPK/mTOR pathway and the Akt/Fox O pathway, and to regulate the JNK level. Additionally, several herbs and their derivates have been reported to induce apoptosis mainly via regulating the PTEN/AKT pathway, the BCL-2/Bax ratio and the NF-κB level, and by increasing the caspase 3/9 level. As for cell cycle arrest, several herbs and their derivates affect the PI3K/AKT-mTOR pathway, the AMPK/mTOR pathway and the NF-κB level, and act to up-regulate levels of p53 and phospho-GSK-3β. (Figure 1)

INHIBITITION OF EPITHELIAL-MESENCHYMAL TRANSISTION, MIGRATION, INVASION, AND METASTASIS

The liver has a sinus structure, abundant blood flow, an immunosuppressive microenvironment, and is involved in regulating blood circulation and the lymphatic system [57]. The migration, invasion, and metastasis associated with HCC recurrence significantly influence mortality rates. HCC is prone to metastasis to the lungs (47%), lymph nodes (45%), bones (37%), and adrenal glands (12%). The prognosis of HCC patients presenting with extrahepatic metastasis is poor [58], and likewise linked to a poor survival rate. Of note, anti-metastasis drugs, including sorafenib, lenvatinib, and a combination of protocols (e.g. sintilimab plus bevacizumab) have not demonstrated favorable outcomes in metastatic HCC patients [59, 60].

Research indicates that the progression of cancer metastasis involves a series of steps [61]. First, EMT occurs in the early stages of tumor-cell metastasis, which allows epithelial phenotypic cells to convert into mesenchymal-like cells [62]. EMT studies have observed the involvement of epithelial proteins (E-cadherin, claudins, occludins, and α-catenin) as well as mesenchymal phenotypic proteins (N-cadherin, β-catenin, and vimentin). There are various pathways, including Wnt/β-catenin, mesenchymal-epithelial transition factor (c-Met)/hepatocyte growth factor (HGF)/Snail, neurogenic locus notch homolog protein 1 (Notch-1)/NF-κB, TGF-β/suppressor of mothers against decapentaplegic (SMAD), and basic fibroblast growth factor (FGF)-related signaling which play roles in EMT [62]. A coordinated sequence of invasion and metastasis subsequently occurs, which involves parenchymal, nonparenchymal and immune cells related to cytokines, histone methyltransferase/demethylase (e.g. enhancer of zeste homolog 2 (EZH2), SETDB1

(KMT1E) and euchromatic histone lysine methyltransferase 2 (G9a, EHMT2)), and non-coding RNAs [63, 64].

Multiple TCM herbs and their derivatives have been shown to possess inhibitory effects against EMT as related to HCC. *Scutellariae baicalensis*, and its derivative baicalin, have recognized hepatoprotective effects, acting to modulate the TGF-β/SMAD, MAPK and NF-κB pathways, and inhibit matrix metalloproteinase (MMP)-1 to hinder EMT in HCC [65]. In a study involving Huh7 and MHCC97-H cell lines, astragaloside IV modulated the Akt/GSK-3β/β-catenin pathway and inhibited EMT [66]. Additionally, camptothecin, a topoisomerase inhibitor, has been shown to inhibit EMT by upregulating the expressions of zonula occludens protein (ZO)-1, E-cadherin, and claudin-1 [57]. Isoviolanthin has been shown to inhibit TGF-β1, associated with the downregulation of the TGF-β/SMAD and PI3K/Akt/mTOR signaling pathways, which resulted in the inhibition of EMT [67]. 18β-Glycyrrhetinic Acid, an ingredient of *Glycyrrhiza glabra L.* root (licorice), has been found to inhibit EMT and metastasis by suppressing the Src (Sarcoma) homology 2 domain phosphatase (SHP)1&SHP2/signal transducer and activator of transcription 3 (STAT3)/Snail pathway [68]. One study revealed that *Echinacea purpurea* regulated the PI3K/Akt signaling pathway to inhibit EMT [69]. Separately, tetrandrine impeded the Wnt/β-catenin signaling pathway and decreased metastatic tumor antigen 1 (MTA1) expression in Huh7 and Hep3B cell lines, leading to the inhibition of EMT, invasion, and migration [70]. Other studies have reported that scorpion and myricetin regulated the epithelial/mesenchymal proteins ratio and inhibited EMT [71, 72]. More recently, miRNA and lncRNA have been associated with impacting the EMT process and drug resistance in HCC. Hydroxygenkwanin (upregulation in miR320a) [73], oleanolic acid (upregulation in miR-122) [74], aloin (regulation in circ_0011385/miR-149-5p/WT1 axis) [50], and puerarin (regulation in miR-21/PTEN/EMT axis) [75] inhibited EMT, invasion and migration in HCC cell lines. Both *in vivo* and *in vitro* studies by Chen *et al*. demonstrated that corylin, a flavonoid compound extracted from *Psoralea corylifolia L.*, upregulated lncRNA growth arrest-specific transcript 5 (GAS5) to inhibit EMT and decrease tumor size [76].

Researchers have revealed that TCM offers multiple herbs and formulas found to inhibit migration and invasion and may therefore be applied to prevent HCC recurrence. Both kaempferol and dulcitol have been noted to decrease MMP to impede migration [77, 78]. *Sanguisorba officinalis* has been shown to modulate the PI3K/AKT, NF-κB and MAPK signaling pathways to inhibit HepG2 cell migration and invasion [52]. *Zanthoxylum avicennae* augemented PP2Acα, GSK-3β, adenomatous polyposis coli protein (APC) and β-transducin repeat-containing protein

(β-TrCP) levels, and diminished β-catenin, p-GSK-3β, TBX 3 and interleukin (IL)-8 proteins to prevent metastasis [79]. A study by Feng *et al*. reported that bufalin upregulated tank-binding kinase 1 (TBK1) and the interferon regulatory factor 3 (IRF3) and NF-κB pathways to hinder migration and invasion [80]. In terms of specific TCM formulas linked to EMT inhibition, QHF (consisting of HuaChanSu, 20(R) ginseng saponin Rg3, notoginseng total saponin, and lentinan) activated p38/JNK/MAPK pathway and inactivated ERK pathway to inhibit migration and invasion in a study using HepG2 cells [81]. The main ingredients in QHF, including cinobufotalin, ginsenoside Rg3, panax notoginsenosides, and lentinan, act to downregulate the HGF/c-Met signaling pathway to prevent metastasis and invasion [82]. In addition, the Biejiajian pill and Jiedu recipe have separately been found to prevent EMT by suppressing the Akt/GSK-3β/Snail signaling cascade and modulating E-cadherin/N-cadherin ratio, respectively [83, 84]. In a study involving multiple HCC cell lines, the Xiaoai Jiedu recipe regulated the miRNA-29a/transcription 3 axis and decreased metastasis [85]. The TCM compounds and formulas associated with reducing HCC recurrence via inhibition of EMT, migration, invasion and metastasis are summarized in Table 2.

Collectively, multiple investigations have demonstrated that TCM and its derivative compounds act to prevent HCC recurrence by inhibiting EMT, migration, invasion and metastasis. The possible mechanisms involved in this prevention include suppressing TGF-β and MMP 2/9, E-cadherin/N-cadherin ratio reversion, and microRNA modulation (Figure 1).

INHIBITING CANCER STEMNESS

Cancer stem cells (CSCs) may be characterized as possessing features of self-renewal, differentiation potential, and colony-forming. Research indicates that CSCs may be a major cause of tumorigenesis, metastasis and antitumor agent resistance, and are thus a primary culprit in tumor relapse after therapy. Dai *et al*. suggested that HCC CSCs create an immunosuppressive microenvironment through both intrinsic and extrinsic mechanisms to escape immune surveillance [86]. In HCC cell lines, CSCs are primarily identified as CD133 [87]; while other surface markers include epithelial cell adhesion molecule (EpCAM), CD44, CD13, CD90, CD24, CD47, oval cell marker OV6, K19, c-kit, breast cancer resistant protein (BCRP), and aldehyde dehydrogenases (ALDH) [88]. Meanwhile, signaling pathways including the Wnt/β-catenin, AKT/GSK-3β/β-catenin, ERK/Snail, AKT/PKB, AKT/mTOR, and TGF-β pathways have been recognized in CSC formation [88].

With regards to TCM treatments associated with CSC hindrance, investigations have revealed several notable findings. *Antrodia cinnamomea*, a fungus species, has

well-documented anti-HCC effects, and has been found to hinder CSC by decreasing vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1α levels [89]. Pterostilbene, a compound isolated from blueberries, decreased CD133+, c-Myc, and cyclooxygenase-2 (COX-2) while concurrently increasing E-cadherin in CD133+ Mahlavu cells [90]. In addition, 8-bromo-7-methoxychrysin decreased expressions of CD133, CD44 and IL-6, and inhibited self-renewal of SMMC-7721- and MHCC97H-derived liver cancer stem-like cells (LCSLCs) [91]. Curcumin decreased expressions of several CSC markers (c-KIT, EpCAM, CD133, RING finger protein 51 (RNF51), and NANOG) and inhibited the oncogenic NF-κB signaling pathway [92]. Sophocarpine decreased expressions of CD133, CD90, and EpCAM as well as TGF-β to inhibit both EMT and CSC [93]. Matrine, extracted from *Sophora flavescens*, reduced the EpCAM+/CD133+ in HCC cells by inactivating the PI3K/AKT/mTOR and AKT/GSK-3β/β-catenin signaling pathways [94]. In addition, *Brucea javanica* has been found to decrease expressions of CD133, NANOG and EpCAM, subsequently inducing apoptosis and suppressing CSCs [95]. 2-Ethoxystypandrone, extracted from *Polygonum cuspidatum*, blocked STAT3 activation to decrease cancer stemness [96]. Meanwhile, the formula BRM270 decreased CD113+ cells and inhibited liver CSCs both *in vivo* and *in vitro* [97]. Songyou Yin (consisting of *Salvia miltiorrhiza, Astragalus membranaceus, Lycium barbarum, Crataegus pinnatifida,* and *Trionyx sinensis*) prevented CSCs by decreasing the expressions of CSC markers including CD90, CD24 and EpCAM, and increased chemosensitivity to oxaliplatin [98]. Moreover, differentiation therapy has revealed further opportunities for controlling CSCs. The combination of *Astragalus membranaceus* and *Salvia miltiorrhiza* extract has been found to increase the differentiation of HCC cells by modulating TGF-β/TβR and Imp7/8 protein expression [99]. Additionally, Rui-Chuan *et al*. reported that isoverbascoside induced SMMC-7721 differentiation, thereby acting as a potential anti-tumor target [100]. The compounds and formulas applied for preventing HCC recurrence via inhibition of cancer stemness are presented in Table 3.

In summary, CSC plays an important role in HCC recurrence. In this regard, TCM and its derivative compounds could suppress CSC markers, particularly in CD113, CD44 and EpCAM, reduce TGF-β which promotes CSC properties, and suppress the PI3K/AKT/mTOR and AKT/GSK-3β/β-catenin signaling pathways (Figure 1).

AMELIORATING DRUG RESISTANCE

In the advanced stages of HCC and in patients presenting with recurrence, molecular target therapy has become a viable alternative treatment. Target therapy

agents such as sorafenib (targeting VEGFR and platelet-derived growth factor receptors (PDGFR)), ramucirumab (targeting VEGFR), regorafenib (targeting VEGFR), gefitinib (targeting EGFR), erlotinib (targeting EGFR), lenvatinib (targeting VEGFR, PDGFR, fibroblast growth factor receptor (FGFR)), and everolimus (targeting mTOR) are commonly prescribed. However, patients having received target therapy have not exhibited significant beneficial effects in terms of overall survival, while drug resistance has further limited the anticancer effect. Previous studies have shown that inflammation and fibrosis have caused sorafenib-resistance and HCC progression. TNF-α and IL-6 are key cytokines which promote intrahepatic HCC progression via STAT3 activation [101]. The combination of two or three drugs which impact multiple targets may improve treatment to control the complex cancer metabolic system, whereby TCM may serve as a multi-target adjuvant therapy in preventing HCC recurrence.

Investigations have revealed that cisplatin and oxaliplatin, platinum-based chemotherapeutic agents, cause cytotoxic effects through deoxyribonucleic acid (DNA) damage. The resistance to oxaliplatin in HCC has been associated with the lysine-specific demethylase 1 (LSD1)/long intergenic non-protein-coding RNA 1134 (LINC01134)/SP1/p62 axis or the miR-129-5p/ETS translocation variant 1 (ETV1) axis [102, 103]. It has been reported that *trametes robiniophila* extract repressed the expression of Yes-associated protein (YAP) and apoptosis-related proteins (Bcl-2) to sensitize the oxaliplatin effect [104]. In as separate study, falcarindiol sensitized the cisplatin anti-Huh7 and LM3 effects by downregulation of the STAT3/pituitary tumor transforming gene 1 (PTTG1) pathway expression [105]. As applied in advanced or recurrent HCC, doxorubicin has been shown to intercalate the DNA, stabilize the topoisomerase II complex and halt the DNA replication process. In addition, dihydroartemisinin has been found to decrease P-gp expression through downregulating the p53 (R248Q)-ERK1/2-NF-κB signaling pathway to augment anticancer effects in mutant p53 (R248Q)-expressing Hep3B cells (doxorubicin resistant cell line) [106]. Of note, it has been reported that *Solanum nigrum* enhanced cisplatin and doxorubicin's anti-HCC effect through apoptosis and autophagy by cleavage of caspase-7 and accumulation of microtubule-associated protein-1 light chain-3 A/1B II (LC-3 A/B II) [107]. Meanwhile, 5-fluorouracil (5-FU) is a thymidylate synthase inhibitor which interferes with DNA replication and leads to cytotoxicity. As reported, H1 (a derivative of tetrandrine, molecular formula: $C_{27}H_{40}N_2O_6Br$) and bufalin increased 5-FU sensitivity in 5-FU-resistant HCC cells (BEL-7402 /5FU) [108, 109]. Additionally, bufalin induced apoptosis by increasing in the Bax/Bcl-xL ratio, inhibited drug efflux pump activity via downregulation of multidrug resistance protein 1 (MRP1) and reduced the expression of thymidylate

synthase [108]. Furthermore, H1 downregulated the STAT3/ myeloid cell leukemia 1 (MCL-1) pathway to sensitize 5-FU treatment [109]. Sorafenib is a protein kinase inhibitor which acts against VEGFR and PDGFR, and rapidly accelerates fibrosarcoma (RAF) kinases. In separate studies, artesunate and tetrandrine increased the effectiveness of sorafenib on HCC apoptosis by inhibiting the PI3K/AKT/mTOR pathway [110, 111]. Artesunate has also been shown to inhibit the RAF/MAPK pathway [110]. Meanwhile, Zhai *et al*. reported that bufalin reversed sorafenib resistance via the inositol-requiring enzyme 1 (IRE1) pathway in HepG2 and Huh7 cell lines [112]. Furthermore, solamargine has been shown to provide a synergistic anticancer effect with sorafenib by regulating HOXA distal transcript antisense RNA (HOTTIP) – the taurine upregulated 1 (TUG1)/miR-4726-5p/mucin 1 signaling pathway [113]. The combination of 8-bromo-7-methoxychrysin and sorafenib has been reported to decrease expressions of HIF-1α and the EMT regulator Twist1 to inhibit CSC [114]. To be applied in cases of HCC recurrence or in advanced cases, icaritin has been found to enhance the effects of doxorubicin and lenvatinib in Hepa1-6 and Huh7 cells [115]. The compounds involved in reversing drug resistance are listed in Table 4.

With regards to the benefits of acupuncture in the amelioration of drug resistance, a limited number of studies have focused directly on the antitumor and synergistic effects associated with acupuncture, electroacupuncture and moxibustion. Although, Yang *et al*. reported that electroacupuncture around a breast cancer tumor increased the local concentration of paclitaxel and decreased the tumor volume [116].

Drug resistance indeed limits the therapeutic effectiveness of drug treatments for recurrent HCC. However, investigations have demonstrated that the combination of two or three drugs impacting multiple targets may offer promising anti-HCC treatment strategies. As such, TCM has been found to provide a wide range of synergistic effects associated with platinum, doxorubicin, 5-FU, and protein kinase inhibitors. The mechanisms underlying these effects are associated with the RAF/MAPK, PI3K/AKT/mTOR, STAT3/PTTG1, STAT3/MCL-1 and HOTTIP-TUG1/miR-4726-5p/MUC1 pathways (Figure 1).

FOOTNOTES

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Table 1 Compounds for reducing multicentric tumor occurrence by inducing autophagy, apoptosis and cell cycle arrest in HCC

Compound or Chinese herbal medicine	Cell line	Molecular mechanism	Effect	Ref.
Piperidine alkaloids	HepG2, Hep3B	Modulate Mst1-JNK pathway, ERK pathway, PINK1/Parkin axis and PTEN/AKT pathway	↑autophagy, apoptosis, mitochondrial fission ↓ proliferation	Liu *et al*[5]
Isoquinoline alkaloids	SMMC-7721, HCCLM9, Huh7, HepG2	Modulate AKT pathway, AKT/FoxO3a/Skp2 axis, PI3K/AKT-mTOR pathway, Wnt/β-catenin-mediated pathway and anthranilic acid metabolic pathway	↑autophagy, apoptosis, cell cycle arrest ↓proliferation	Liu *et al* [5]
Indole alkaloids	HepG2, SMMC-7721, Hepa1-6, BEL-7404, Hep3B, Huh7	Modulate NOD1 pathway, AKT pathway and WWOX -dependent pathway	↑apoptosis, cell cycle arrest ↓proliferation	Liu *et al* [5]
Terpenoids alkaloids	HLE, L-02, BEL-7402, HepG2	Modulate AKT, p53, caspase-3, MAPK, AFP, Ras	↑apoptosis, cell cycle arrest ↓proliferation	Liu *et al* [5]
Steroidal alkaloids	HepG2, SMMC-7721, Hep3B	↑ gene expression of human TNFR I	↑necroptosis, apoptosis, cell	Liu *et al* [5]

			cycle arrest ↓proliferation	
Quinoline alkaloids	HepG2, L-02, QGY-7703	Modulate MMP-9, PCNE, ANT3 and VEGF	↑necroptosis, apoptosis	Liu *et al* [5]
Solamargine	HepG2, Huh7	Modulate miR-192-5p/CYR61/Akt pathway	↑autophagy, apoptosis ↓proliferation	Yin *et al* [31]
Wogonin	HepG2, BEL-7402	Modulate NF-κB/Bcl-2, EGFR and EGFR/ERK/AKT pathway	↓proliferation	Liu *et al* [5]
	SMMC-7721, HCCLM3	↑ MOB1-LATS1 pathway ↓YAP, WW domain–containing transcription regulator 1, and expression of Claspin	↑ apoptosis, cell cycle arrest	Wu *et al* [32]
	MHCC97-L, HepG2	↑phospho-GSK-3β Tyr216 ↓ Cyclin D1	↑cell cycle arrest ↓proliferation	Hong *et al* [33]
Baicalein	Human HCC tissues	Modulate lncRNAs-hsa-miR-4443-AKT1 pathway	↓proliferation	Zhao *et al* [35]
Silibinin	HepG2, Hep3B	Modulate AMPK pathway	↑ autophagy ↓ glycolysis	Yang *et al* [36]
	Huh7, HepG2, Hep3B,	↑p21/CDK4 and p27/CDK4	↑ apoptosis,	Lah *et al* [37]

	PLC/PRF/5 human hepatoma cells	complexes ↑caspase-3 and -9 ↓ Rb-phosphorylation and E2F1/DP1 complex	↓proliferation	
luteolin	p53-wild type HepG2 cells, Hep3B	↑ Endoplasmic reticulum stress	↑autophagy, apoptosis	Lee *et al* [38]
	HepG2	Modulate TGF-β1, p53, Fas/Fas ligand pathway	↑apoptosis, cell cycle arrest	Yee *et al* [39]
	HepG2, SK-Hep-1, PLC/PRF/5, Hep3B, HA22T/VGH	↑ Bax/ Bcl-XL ratio ↑ caspase-3	↑ cell cycle arrest	Chang *et al* [40]
Kaempferol	HepG2, Huh7, BEL-7402, SMMC	↑AMPK pathway ↑melanoma antigen 6, AMPK ubiquitin ligase, AMPKα1	↑autophagy	Han *et al* [41]
Luteolin and Kaempferol	diethylnitrosamine (DEN) and 2-acetylaminofluorene (2-AAF) induced rat model	↑caspase-3 and ROS reaction	↑ apoptosis	Seydi *et al* [42]
Quercetin	HepG2	↑ p53, BAX ↓ ROS, PI3K, COX-2, PKC	↓proliferation	Maurya *et al* [43]
	--	Modulate PI3K/Akt/mTOR,	↑apoptosis and	Reyes-Farias *et al* [44]

		Wnt/β-catenin, MAPK/ERK1/2 pathway	autophagy	
Chlorogenic acid	Hep-G2, Huh7	↑ BH3-only protein Bcl-2 binding component 3 ↓ noncanonical NF-κB pathway	↑apoptosis	Jiang *et al* [45]
16-O-caffeoyl-16-hydroxylhexadecanoic acid	HepG2, BEL-7402	Modulate mitochondria-mediated pathway and ROS-mediated endoplasmic reticulum stress	↑apoptosis	Huang *et al* [46]
Thymoquinone	thioacetamide (TAA)-induced HCC, Sprague Dawley rats	↓ oxidative stress, ↓ TGF-β1	↑apoptosis	Helmy *et al* [47]
Juglanthraquinone C	HepG2, BEL-7402	↑ Akt/Fox O pathway ↑ intracellular ROS level	↑apoptosis	Hou *et al* [48]
4-acetylantrocamol LT3	HepG2	↑ AMPK pathway	↑autophagy	Chen *et al* [49]
Aloin	--	Modulate circ_0011385/miR-149-5p/WT1 axis	↑apoptosis and autophagy	Fu *et al* [50]
Andrographolide	Hep G2	↓ EphB4	↑ apoptosis	Duan *et al* [51]
Sanguisorba Officinalis L.	HepG2 cells	Modulate EGFR, PI3K/AKT, NF-κB and MAPK pathways	↓proliferation	Jiang *et al* [52]
Plantamajoside	Huh7, PLC/PRF 5,	↓ NF-κB and Cox-2	↓proleferation	Luo *et al* [53]

	THLE-2			

Akt: Protein kinase B; AMPK: AMP-activated protein kinase; Bax: BCL2-Associated X Protein; Bcl-2: B-cell lymphoma 2; CDK: Cyclin-dependent kinases; COX-2: Cyclooxygenase-2 ; EGFR: Epidermal growth factor receptor; EPHB4: EPH Receptor B4; ERK: Extracellular signal-regulated kinase; FoxO: Forkhead box O; GSK: Glycogen synthase kinase; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MMP: Matrix metalloproteinase; Mst1: Mammalian sterile 20-like kinase 1; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B; NOD: Nucleotide-binding oligomerization domain; PI3K: Phosphatidylinositol 3-kinase; PINK1: PTEN-induced kinase 1; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; ROS: Reactive oxygen species; Skp2: S-phase kinase-associated protein 2; TGF: Transforming growth factor; TNFR: Tumor necrosis factor receptor; VEGF: Vascular endothelial growth factor; WWOX: WW domain-containing oxidoreductase; YAP: Yes-associated protein.

Table 2 Compounds and formulas for reducing HCC recurrence via inhibition of EMT, migration, invasion and metastasis

Compound or Chinese herbal medicine or formula	Cell line/Animal/Human	Molecular mechanism	Effect and outcome	Ref.
Astragaloside IV	Huh7, MHCC97-H	Modulate Akt/GSK-3β/β-catenin pathway ↑ E-cadherin ↓ N-cadherin, vimentin, α-Smooth Muscle Actin, Slug	↓ EMT, invasion, migration	Qin *et al* [66]
Camptothecin	Huh7	Modulate ZO-1, E-cadherin, claudin-1	↓ EMT, metastasis	Liu *et al* [57]
Isoviolanthin	HepG2, BEL-7402	↓TGF-β1 ↓TGF-β/SMAD and PI3K/Akt/mTOR pathway ↓MMP-2 and -9	↓ EMT	Xing *et al* [67]
18β-Glycyrrhetinic Acid	BEL-7402, LM3	Modulate SHP1&SHP2/STAT3/Snail pathway ↓ phosphorylation of STAT3 ↑SHP1 and SHP2	↓EMT and metastasis	Jie *et al* [68]
Echinacea purpurea	Hepa1-6, HepG2, L-02	Modulate PI3K/Akt pathway	↓EMT	Xu *et al* [69]
Tetrandrine	Huh7, Hep3B	↓ Wnt/β-catenin pathway ↓metastatic tumor antigen 1	↓ EMT, invasion, migration	Zhang *et al* [70]

Scorpion	Hepa1-6/ Sprague-Dawley rats (6-week, male, 0.63 g/200 g, every day for 4 weeks)	↑ E-cadherin ↓ N-cadherin	↓ EMT, migration, invasion	Yan *et al* [71]
Myricetin	MHCC97-H	↑ E-cadherin expression ↓ N-cadherin	↓ migration, invasion	Ma *et al* [72]
Hydroxygenkwanin	HepG2 and Huh7/ nude mice (6-week, male, 1 mg/kg for 3 times per week)	↑miR-320a, ↓forkhead box protein M1	↓ EMT, invasion, migration ↓tumor size	Chou *et al* [73]
Oleanolic acid	HepG2, SK-Hep-1	↑ miR-122, E-cadherin ↓ β-catenin, N-cadherin, vimentin	↓EMT, migration, invasion	He *et al* [74]
Aloin	--	Modulate circ_0011385/miR-149-5p/WT1 axis	↓ invasion	Fu *et al* [50]
Puerarin	BEL-7402, Huh7, L-02	↑ PTEN Modulate miR-21/PTEN/EMT axis	↓EMT, migration, invasion	Fu *et al* [75]
Corylin	Hep G2, Huh7/ nude mice (BALB/cAnN-Foxnln u/CrlNarl, 6-week, male, 60 mg/kg, 3 times per week)	↑GAS5	↓ EMT ↓tumor size	Chen *et al* [76]
Kaempferol	Huh7, SK-Hep-1	↓MMP-9 and Akt pathway	↓ migration	Ju *et al* [77]
Dulcitol	HepG2	↓ MMP-2, uPA, MMP-9 ↑ E-cadherin	↓migration and invasion	Lin *et al* [78]

Sanguisorba officinalis	HepG2	Modulate EGFR, PI3K/AKT, NF-κB and MAPK pathway	↓migration, invasion	Jiang *et al* [52]
Zanthoxylum avicennae	HA22T	↑ PP2Acα, GSK-3β, APC, β-TrCP/HOS ↓β-catenin, p-GSK-3β, TBX 3, IL-8 ↓nuclear and cytosolic β-catenin	↓metastasis	Wu *et al* [79]
Bufalin	MHCC97-H	↑TBK1, IRF3 and NF-κB pathway	↓migration, invasion	Feng *et al* [80]
QHF (consisting of HuaChanSu, 20(R)ginseng saponin Rg3, notoginseng total saponin and lentinan)	HepG2	↑ p38, JNK, MAPK pathway ↓ ERK pathway	↓migration, invasion	Chen *et al* [81]
QHF (consisting of cinobufotalin, ginsenoside Rg3, panax notoginsenosides, lentinan)	HCCLM3, HepG2/ SPF BALB/c mice (20g, male, 0.2ml/mice, once every other day for four weeks)	↓ p-c-Met protein ↓ HGF/c-Met pathway	↓ metastasis, invasion	Yuan *et al* [82]
Biejiajian pill	MHCC-97H, SMMC-7721/ BALB/c nude mice (4-5 weeks, female, 1.1 g/kg, daily for 4 weeks)	↓ Akt/GSK-3β/Snail pathway	↓EMT, metastasis	Sun *et al* [83]
Jiedu Recipe	SMMC-7721, Huh7	↑ E-cadherin ↓ p-Smad2/3, Smad2/3 ↓ TGF-β1, vimentin, N-cadherin, MMP2/9.	↓ EMT, invasion, migration	Liang *et al* [84]

Xiaoai Jiedu Recipe	Male nude mice (BALB/c (nu/nu), 4–5 weeks, male, 10 g/kg, 4 consecutive days) 40 HCC patients and 40 volunteer controls	Modulate miRNA-29a signal transducer ↑Transcription 3 Axis	↓metastasis	Shi *et al* [85]

Akt: Protein kinase B; EMT: Epithelial-mesenchymal transition; ERK: Extracellular signal-regulated kinase; FGFR: Fibroblast growth factor receptor; GAS5: Growth arrest-specific transcript 5; GSK: Glycogen synthase kinase; HCC: Hepatocellular carcinoma; HGF: Hepatocyte growth factor; IL: Interleukin; IRF3: Interferon regulatory factor 3; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MMP: Matrix metalloproteinase; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B; PI3K: Phosphatidylinositol 3-kinase ; PP2Acα: α isoform of the catalytic subunit of protein phosphatase 2A; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; SHP: Src homology 2 domain-containing protein tyrosine phosphatases; SMAD: Suppressor of Mothers against Decapentaplegic; STAT3: Signal transducer and activator of transcription 3; TBK1: Tank-binding kinase 1; TBX3: T-Box Transcription Factor 3; TGF: Transforming growth factor; ZO: Zonula Occludens Protein.

Table 3 Compounds and formulas for reducing HCC recurrence via inhibition of cancer stemness

Compound or Chinese herbal medicine or formula	Cell line/Animal	Molecular mechanism	Effect	Ref.
Antrodia cinnamomea	HA22T/VGH	↓ VEGF and HIF-1α	↓ CSC	Liu *et al* [89]
Pterostilbene	CD133+ Mahlavu cells	↓CD133+, c-Myc, COX-2 ↑ E-cadherin	↓tumor sphere formation, ↓stemness gene expression ↓ invasion and migration ↑ apoptosis	Lee *et al* [90]
8-bromo-7-methoxychrysin	SMMC-7721, MHCC97H-derived LCSLCs	↓CD133, CD44 ↓IL-6	↓ CSC	Wen *et al* [91]
Curcumin	PLC/PRF5, WRL68, Huh7, KMCH, AFP-negative primary HCC cell line	↓ CD117, EpCAM, CD133, RNF51, NANOG ↓NF-κB	↓CSC	Marquardt *et al* [92]
Sophocarpine	HCC-LM3, MHCC-97H BALB/c nude mice (4-week, male, 0.4-6g/kg, twice a week for 4 weeks)	↓TGF-β ↓CD133, CD90 and EpCAM	↓ EMT ↓ CSC	Zhang *et al* [93]
Matrine	Hep3B, Huh7 BALB/c nude mice (-,-,10 mg/kg, daily for 3 weeks)	↓ EpCAM+/CD133+ cell number ↓ PI3K/AKT/mTOR pathway, AKT/GSK-3β/β-catenin pathway	↓ sphere formation ↓stem cell markers ↑ mature hepatocyte markers	Liu *et al* [94]
Brucea javanica	HepG2 (HB-8065, wild-type p53),	↓CD133, NANOG, EpCAM	↑ apoptosis	Chen *et al* [95]

	Hep3B (HB-8064, p53-null)		↓ stem-like cells	
2-Ethoxystypandrone	Hep3B, HepG2, Huh7, Li-7, SK-Hep-1	↓ STAT3 activation	↓proliferation, ↑apoptosis ↓CSC	Li *et al* [96]
BRM270	HepG2(CD133+), SNU-398 CRJORI:CD-1-5WM (6-week, male, 5 mg/kg/day, daily for 12 weeks)	↓ CyclinD1/Bcl2 mediated c-Jun apoptotic pathway ↓ CD113	↓proliferation ↑apoptosis ↓CSC	Kumar *et al* [97]
Songyou Yin (consisted by *Salvia miltiorrhiza, Astragalus membranaceus, Lycium barbarum, Crataegus pinnatifida* and *Trionyx sinensis)*	MHCC97-H, Hep3B	↓ CD90, BCRP, ALDH, CD44, EpCAM, vimentin, MMP-9 ↑ E-cadherin	↑oxaliplatin chemosensitivity ↓motility, invasion, and colony formation ↓CSC	Jia *et al* [98]

Akt: Protein kinase B; ALDH: Aldehyde dehydrogenases; Bcl-2: B-cell lymphoma 2; BCRP: Breast cancer resistant protein; CD: Cluster of differentiation; COX-2: Cyclooxygenase-2; EpCAM: Epithelial cell adhesion molecule; GSK: Glycogen synthase kinase; HIF: Hypoxia-Inducible Factor; IL: Interleukin; MMP: Matrix metalloproteinase; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B; PI3K: Phosphatidylinositol 3-kinase; RNF51: RING finger protein 51; STAT3: Signal transducer and activator of transcription 3; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor.

Table 4 Compounds for reversing drug resistance

Compound or Chinese herbal medicine	Conventional drug	Cell line	Molecular mechanism	Ref.
Trametes robiniophila Murr.	Oxaliplatin	BEL-7404, SMMC-7721	↓YAP and apoptosis related proteins	Tao *et al* [104]
Falcarindiol	Cisplatin	Huh7, LM3	↓STAT3/PTTG1 pathway	Hong *et al* [105]
Dihydroartemisinin	Doxorubicin	mutant p53 (R248Q)-expressing Hep3B	Modulate p53 (R248Q)-ERK1/2-NF-κB pathway ↓ P-gp expression	Yang *et al* [106]
Solanum nigrum	cisplatin and doxorubicin	Hep3B, HepJ5	↑cleavage of caspase-7 ↑LC-3 A/B II ↑apoptosis, autophagy	Wang *et al* [107]
Bufalin	5-FU	BEL-7402/5-FU	↑ apoptosis arrested the cell cycle at the G_0/G_1 phase ↑Bax/Bcl-xL ratio ↓ MRP1, thymidylate synthase (inhibit drug efflux pump activity)	Gu *et al* [108]
H1 (a derivative of tetrandrine, molecular formula: $C_{27}H_{40}N_2O_6Br$)	5-fluorouracil (5-FU)	BEL-7402 /5FU	↓STAT3/MCL-1 pathway ↑PUMA expression	Li *et al* [109]
Artesunate	sorafenib	SK-hep1, SMMC-7721	↑apoptosis ↓ RAF/MAPK pathway ↓PI3K/AKT/mTOR pathway	Jing *et al* [110]
Tetrandrine	sorafenib	SMMC-7721, PLC/PRF/5	↓PI3K/AKT/mTOR pathway ↓proliferation	Niu *et al* [111]

			↑apoptosis	
Bufalin	sorafenib	HepG2, Huh7	↓p-Akt Modulate IRE1 pathway	Zhai *et al* [112]
Solamargine	sorafenib	HepG2, Huh7	↓ lncRNA HOTTIP and TUG1 Modulate HOTTIP-TUG1/miR-4726-5p/ mucin 1 pathway	Tang *et al* [113]
8-bromo-7-methoxychrysin	sorafenib	SMMC-7721	↓ migration and invasion ↓N-cadherin ↑E-cadherin ↑apoptosis in LCSLCs ↓ HIF-1α and EMT regulator Twist1	Zou *et al* [114]
Icaritin	Doxorubicin and lenvatinib	Hepa1-6, Huh7	↑mitophagy and apoptosis ↑immunogenic cell death	Yu *et al* [115]

Akt: Protein kinase B; Bax: BCL2-Associated X Protein; Bcl-xL: B-cell lymphoma extra large; EMT: Epithelial-mesenchymal transition; ERK: Extracellular signal-regulated kinase; HIF: Hypoxia-Inducible Factor; HOTTIP: HOXA distal transcript antisense RNA; IRE1: Inositol-requiring enzyme 1; LC-3 A/B II: Microtubule-associated protein-1 light chain-3 A/1B II; LCSLC: Liver cancer stem-like cell; MCL-1: Myeloid cell leukemia 1; MRP1: Multidrug Resistance Protein 1; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B; PI3K: Phosphatidylinositol 3-kinase; PTTG1: Pituitary tumor transforming gene 1; RAF : Rapidly accelerated fibrosarcoma; STAT3: Signal transducer and activator of transcription 3; TUG1: Taurine upregulated 1; YAP : Yes-associated protein.

Figure 1 Summary of molecular pathways involved in complementary therapies in recurrent HCC

Akt: Protein kinase B; AMPK: AMP-activated protein kinase; Bax: BCL2-Associated X Protein; Bcl-2: B-cell lymphoma 2; CD: Cluster of differentiation; EpCAM: Epithelial cell adhesion molecule; ERK: Extracellular signal-regulated kinase; FoxO: Forkhead box O; GSK: Glycogen synthase kinase; HCC: Hepatocellular carcinoma; HOTTIP: HOXA distal transcript antisense RNA; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MCL-1: Myeloid cell leukemia 1; MMP: Matrix metalloproteinase; mTOR: Mammalian target of rapamycin; MUC1: Mucin 1; NF-κB: Nuclear factor kappa B; PI3K: Phosphatidylinositol 3-kinase; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; PTTG1: Pituitary tumor transforming gene 1; RAF: Rapidly accelerated fibrosarcoma; STAT3: Signal transducer and activator of transcription 3; TGF: Transforming growth factor; TUG1:Taurine upregulated 1.