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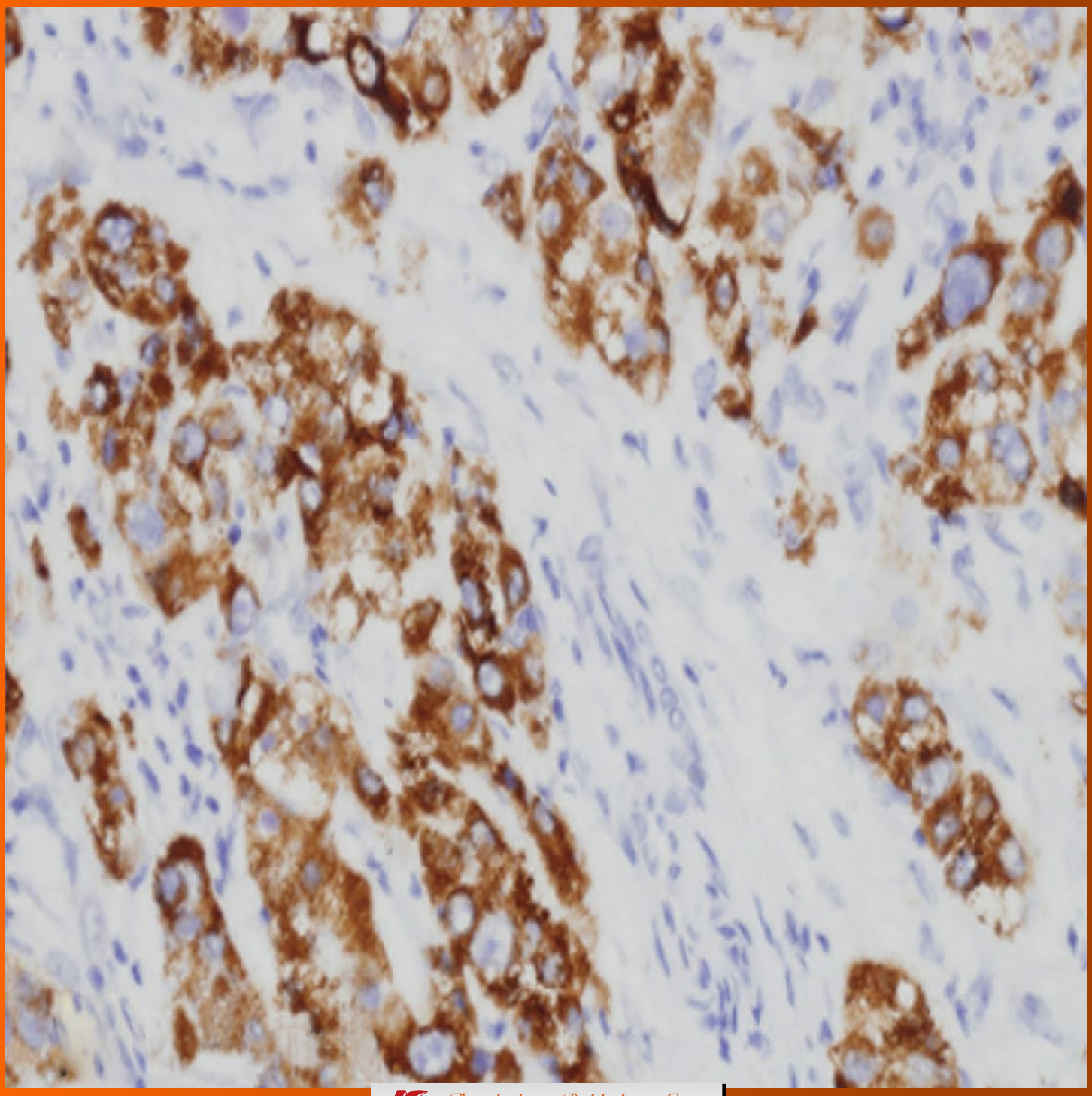

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REVIEW

Terpenoids as potential chemopreventive and therapeutic agents in liver cancer

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Abstract

Despite significant advances in medicine, liver cancer, predominantly hepatocellular carcinoma remains a major cause of death in the United States as well as the rest of the world. As limited treatment options are currently available to patients with liver cancer, novel preventive control and effective therapeutic approaches are considered to be reasonable and decisive measures to combat this disease. Several naturally occurring dietary and non-dietary phytochemicals have shown enormous potential in the prevention and treatment of several cancers, especially those of the gastrointestinal tract. Terpenoids, the largest group of phytochemicals, traditionally used for medicinal purposes in India and China, are currently being explored as anticancer agents in clinical trials. Terpenoids (also called "isoprenoids") are secondary metabolites occurring in most organisms, particularly plants. More than 40 000 individual terpenoids are known to exist in nature with new compounds being discovered every year. A large number of terpenoids exhibit cytotoxicity against a variety of tumor cells and cancer preventive as well as anticancer efficacy in preclinical animal models. This review critically examines the potential role of naturally occurring terpenoids, from diverse origins, in the chemoprevention and treatment of liver tumors. Both *in vitro* and *in vivo* effects of these agents and related cellular and molecular mechanisms are highlighted. Potential challenges and future directions involved in the advancement of these promising natural compounds in the chemoprevention and therapy of human liver cancer are also discussed.

Key words: Terpenoids; Liver cancer cells; Hepatocellular carcinoma; Hepatocarcinogenesis; Chemoprevention; Treatment; Apoptosis; Cell cycle

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common form of primary hepatic carcinoma[1] and a pressing sociomedical problem in several countries, particularly in Asia and sub-Saharan Africa[2]. HCC is currently the fifth most common cancer and third leading cause of cancer-related deaths in the world[3]. HCC has a poor prognosis with the number of deaths almost equal to the number

of cases being diagnosed annually (about 600 000) and the 5-year survival rate is below 9%[4]. In the United States, the incidence of HCC has been steadily rising with a 70% increase registered in the last 25 years[5]. The American Cancer Society[6], estimated that in 2010 alone, more than 24 000 new cases and nearly 19 000 deaths occurred in the United States due to liver cancer (including biliary cancers).

Major risk factors for HCC are well known and are dependent on the geographic area. In Europe, the United States, and Japan, the main risk factors are liver cirrhosis, Hepatitis B virus (HBV) and Hepatitis C virus (HCV), alcohol, and tobacco; in contrast, in Africa and Asia, the etiological factors include HBV and HCV, tobacco use, and aflatoxin exposure[7,8]. Treatment for HCC has been conventionally divided into curative and palliative. Curative treatments, such as resection, liver transplantation and percutaneous ablation, induce complete responses in a high proportion of patients and are expected to improve survival. Palliative treatments are not aimed to cure, but in some cases can obtain good response rates and even improve survival[9]. In the west, curative treatments are applied to 30%-40% of patients in referral centers[10], whereas in Japan 60%-90% of patients benefit because of widespread implementation of surveillance and a broad application of treatments[11,12]. There is no firm evidence to establish the optimum first-line treatment for patients who have a single small HCC and well-preserved liver function[9]. Resection and transplantation achieve the best outcomes in well-selected candidates (5-year survival 60%-70%)[13-16], and compete as the first option from an intention-to-treat perspective[17]. Percutaneous treatments provide good results (5-year survival 40%-50%)[11,18], but have not been able to achieve response rates and outcomes comparable to surgical treatments[11,12]. Liver transplantation has been suggested as the best treatment for patients with one tumor and decompensated cirrhosis or multicentric small tumors[19].

HCC prognosis remains dismal despite many treatment options. Overall, the cure rate among patients who undergo resection is not very high and for those patients who are not eligible for surgery or percutaneous procedures, only chemoembolization appears to improve survival. The need thus arises to test novel agents in large-scale randomized trials; these include intraarterial injection of radiolabeled microsphere and new systemic drugs, such as tyrosine kinase inhibitors, antivascular endothelial growth factor (VEGF) antibody, and antiepithelial growth factor receptor[7]. HCC is also widely considered to be a chemotherapy-resistant disease[20]. Sorafenib, the only drug approved by the United States Food and Drug Administration for the treatment of advanced HCC, increases the median survival time by less than 3 mo[21]. However, this drug does not defer the symptomatic progression of the disease, costs about $5400 per month for treatment[22], and exhibits severe adverse effects, including a significant risk of bleeding[23]. These drawbacks necessitate the search for novel preventive and therapeutic approaches for this disease. Chemoprevention has emerged as an ideal approach whereby the occurrence and progression of the disease can be prevented, slowed, or reversed by the administration of one or more naturally occurring and/or synthetic compounds[24-26].

Phytochemicals, including those obtained from fruits, vegetables, nuts and spices, have drawn a considerable amount of attention due to their ability to selectively kill tumor cells and suppress carcinogenesis in preclinical animal models[27-33]. A large number of these plant-derived substances have been shown to significantly prevent or delay cancer development in several high risk populations[34-36]. Mounting evidence, based on *in vitro* experiments and studies involving animal models as well as humans, support potential chemopreventive and therapeutic effects of diverse phytochemicals in liver cancer[37-41]. This review delves into the current use of terpenoids, the largest families of plant-derived natural products, for either chemoprevention or therapy of hepatic cancer, by examining an extensive number of studies conducted both *in vitro* and *in vivo*.

TERPENOIDS

Terpenoids composed of "isoprenoid" units constitute one of the largest group of natural products accounting for more than 40 000 individual compounds, with several new compounds being discovered every year[42-44]. Most of the terpenoids are of plant origin; however, they are also synthesized by other organisms, such as bacteria and yeast as part of primary or secondary metabolism. Terpenoids are synthesized from two five-carbon building blocks, i.e., the isoprenoid units. Based on the number of building blocks, terpenoids are classified into several classes, such as monoterpenes (e.g., carvone, geraniol, *d*-limonene, and perillyl alcohol), diterpenes (e.g. retinol and *trans*-retinoic acid), triterpenes [e.g., betulinic acid (BA), lupeol, oleanic acid, and ursolic acid (UA)], and tetraterpenes (e.g., α-carotene, β-carotene, lutein, and lycopene)[45].

The diverse array of terpenoid structures and functions has provoked increased interest in their commercial use. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties[45-48]. In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties[49].

Epidemiological and experimental studies suggest that monoterpenes may be helpful in the prevention and therapy of several cancers, including mammary, skin, lung, forestomach, colon, pancreatic and prostate carcinomas[34,50-55]. Triterpenoids are the metabolites of isopentenyl phosphate oligomers and constitute the largest group of phytochemicals with more than 20 000 known compounds available in nature[56]. A large number of tri-

terpenoids have been shown to suppress the growth of a variety of cancer cells without exerting any toxicity in normal cells[57-59]. Numerous preclinical efficacy studies have provided extensive evidence that both naturally occurring and synthetic derivatives of triterpenoids possess chemopreventive and therapeutic effects against colon, breast, prostate and skin cancer[56,60-64]. These triterpenoids and their derivatives act at various stages of tumor development, inhibit initiation and promotion of carcinogenesis, induce tumor cell differentiation and apoptosis, and suppress tumor angiogenesis, invasion and metastasis through regulation of various transcription and growth factors as well as intracellular signaling mechanisms[56,64-66]. Currently, several phase I / II clinical trials have been initiated to evaluate the chemopreventive as well as the anticancer efficacy of a number of triterpenoids[56,67]. Although several excellent articles provide an overview of the cancer preventive and antitumor potential of terpenoids against various cancers, the use of these phytoconstituents for either chemoprevention or therapy of liver cancer has not previously been discussed exclusively.

TERPENOIDS AND LIVER CANCER

The following sections of this review showcase the *in vitro* and *in vivo* studies undertaken by a number of researchers around the world exploring the chemopreventive as well as chemotherapeutic potential of terpenoids in liver cancer.

In vitro studies

There are a number of *in vitro* studies that demonstrate the cytotoxic effects of various terpenoids against proliferation, growth and invasion of a variety of liver cancer cell lines (Table 1).

Monoterpenes: Geraniol, an acyclic dietary monoterpene, represents the only monoterpene that has been studied *in vitro* against liver cancer cells. Geraniol was shown to inhibit the growth of HepG2 human hepatic carcinoma cells by decreasing 3-hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the major rate-limiting enzyme in cholesterol biosynthesis in mammals[68].

Diterpenes: Andrographolide, a potential antiinflammatory diterpenoid lactone isolated from the traditional medicinal plant *Andrographis paniculata*, demonstrated inhibitory effects against the growth of hepatoma-derived Hep3B cells. Apoptosis as well as activation of the c-Jun-N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) pathways were shown to play an important role in the cytotoxic effect exerted by andrographolide[69].

Excisanin A, a diterpenoid compound purified from *Isodon macrocalyxin D*, was tested on human Hep3B liver cancer cells with positive results indicating growth inhibition mediated by apoptosis through inhibition of the AKT signaling pathway[70].

Gnidimacrin, a daphnane-type diterpenoid, was successfully shown to inhibit the growth of protein kinase C βII gene-transfected human hepatoma HLE cells through G_2 phase arrest, not only by the suppression of cdc2 activity, but also by the subsequent transcriptional suppression of cdc2 itself. There was also an increase in $p21^{WAF1/CIP1}$, a potent and universal inhibitor of the cyclin-dependent kinase-1 (cdk1)[71].

Oridonin, a diterpenoid isolated from *Rabdosia rubescences*, promoted cytotoxic activities against HepG2 cells through an increase in the apoptotic cell death process and reactive oxygen species (ROS) generation. Increase in the tumor suppressor gene *p53*, apoptotic proteins, such as caspase-3, and -9, cytochrome *c* (cyt. *c*), and p38 protein expression with decrease in mitochondrial membrane potential ($\Delta\Psi m$) were involved in the cytotoxic effects of this compound[72].

Triterpenes: Actein is an active component from the herb black cohosh [*Actaea racemosa* L. syn. *Cimicifuga racemosa* (L) Nutt]. Recent studies indicate that black cohosh may have chemopreventive and chemotherapeutic potential[73,74]. Actein was found to inhibit the growth of p53-positive HepG2 cells with an IC_{50} value of 27 μg/mL[75].

Ardisiacrispin (A+B), a triterpenoid saponin mixture in the fixed proportion 2:1 of ardisiacrispin A and ardisiacrispin B, is derived from *Ardisia crenata*. This mixture exerted cytotoxic activity against Bel-7402 liver cancer cells through pro-apoptotic, anti-proliferative, and microtubule disruptive activities[76].

Astragaloside IV is the major active triterpenoid in *Radix astragali*, a dietary supplement widely used in traditional Chinese medicine to prevent and treat various cancers[77]. This compound decreased the colonogenic survival and inhibited anchorage-independent growth of HepG2 cells possibly by decreasing the expression of oncogene Vav3.1 and increasing the stress proteins, namely glucose-regulated protein BiP/GRP78, heat shock protein (HSP) 70, HSPA1A, and HSPA8[78].

Asiatic acid, a pentacyclic triterpene isolated from the Brahmi plant, *Centella asiatica*, was successfully tested for its apoptotic effects in HepG2 cells. This triterpene decreased the viablility of HepG2 cells mediated by an increase in intracellular calcium levels, which led to the increase in expression of the tumor suppressor gene *p53*[79].

BA, another pentacyclic triterpene, was significantly effective in inducing cytotoxicity in hepatoblastoma cell lines, namely HUH6, HepT1 and HepT3. Apoptosis through proteolytic cleavage of caspase-3 was found to be the primary mechanism involved in the antihepatoblastoma effects of this natural compound. BA also inhibited the phosphatidylinositol 3-kinase/AKT pathway and target genes of hedgehog signaling, e.g. protein patched homolog 1, insulin growth factor 2, and glioma-associated oncogene homolog 1[80]. Deregulation of the hedgehog signaling pathway plays a fundamental role in an increasing number of malignancies[81,82]. However, BA failed to induce apoptosis in HepG2 cells (known to lack

Table 1 ***In vitro*** **effects of natural terpenoids on various liver cancer cells**

Terpenoids	Compounds	Cellular effects	Mechanisms	Conc.	Ref.
Monoterpenes	Geraniol	Inhibited the growth of HepG2 cells	↓HMG-CoA reductase	50-400 μmol/L	Polo *et al*[68]
Diterpenes	Andrographolide	Inhibited the growth of Hep3B cells	↑apoptosis; ↑ MAPKs; ↑pJNK; ↑ERK1/2	3-100 μmol/L	Ji *et al*[69]
	Excisanin A	Decreased viability of Hep3B cells	↑apoptosis; ↓pAKT	1-32 μmol/L	Deng *et al*[70]
	Gnidimacrin	Demonstrated tumor inhibition in PKC βII-transfected HLE cells	⊥G2 ; ↓cdc2; ↑p21WAF1/CIP1	0.00001-0.1 μg/mL	Yoshida *et al*[71]
	Oridonin	Promoted cytotoxicity in HepG2 cells	↑apoptosis; ↑ROS; ↑p53; ↑p38; ↓ΔΨm; ↑cyt. *c*; ↑caspase-3, -9	10-50 μmol/L	Huang *et al*[72]
Triterpenes	Actein	Inhibited the growth of p53-positive HepG2 cells		27 μg/mL [IC_{50}]	Einbond *et al*[75]
	Ardisiacrispin (A+B)	Exhibited cytotoxicity against Bel-7402 cells	↑apoptosis; ↓proliferation; microtubule disassembly	1-10 μg/mL	Li *et al*[76]
	Astragaloside IV	Decreased colonogenic survival and inhibited anchorage-independent growth of HepG2 cells	↓Vav3.1; ↑HSP70; ↑HSPA1A; ↑HSPA8; ↑BiP/GRP78	150-200 μg/mL	Qi *et al*[78]
	Asiatic acid	Decreased viability of HepG2 cells	↑apoptosis; ↑Ca^{2+}; ↑p53	10-100 μmol/L	Lee *et al*[79]
	Betulinic acid	Displayed cytotoxicity against HUH6, HepT1 and HepT3 cells	↑apoptosis; ↑caspase 3; ↓PI3K/AKT; ↓GLI1; ↓PTCH1; ↓IGF2	1-50 μg/mL	Eichenmüller *et al*[80]
		Failed to induce apoptosis against HepG2 cells	↑survivin; ↑Bcl-2	0.1-50 μg/mL	Eichenmüller *et al*[80]
	Cucurbitacin B	Decreased the viability of HepG2 cells	↑apoptosis; ↓Bcl-2; ↓pSTAT3	10 nmol/L-10 μmol/L	Zhang *et al*[83]
		Exhibited growth inhibitory effects on Bel-7402 cells	↑apoptosis; ⊥S; ↓cyclin D1; ↓cdc2; ↓c-Raf; ↑ERK 1/2	0.01-1000 μmol/L	Chan *et al*[84]
	Cucurbitacin D	Suppressed the growth of Hep3b cells	↑apoptosis; ↑caspase-3; ↑pJNK	0-10 μmol/L	Takahashi *et al*[85]
	Cucurbitacin D, I	Displayed cytotoxicity against Bel-7402 cells		< 1 μmol/L [IC_{50}]	Meng *et al*[86]
	Echinocystic acid	Exhibited antiproliferative effect against HepG2 cells	↑apoptosis; ↓Bcl-2; ↑caspase-3, -9, -8; ↑PARP cleavage; ↓ΔΨm; ↓cyt. *c*; ↑p38; ↑JNK	15-100 μmol/L	Tong *et al*[87]
	Escin	Demonstrated inhibitory effects on cell viability of HepG2 cells	↑apoptosis; ⊥G_1/S; ↑AIF; ↑cyt. *c*; ↑bax; ↓Bcl-2; ↓cyclin-E/cdk2; ↓pRb; ↓E2F	10-60 μg/mL	Zhou *et al*[88]
	Ganoderic acid	Demonstrated an inhibition in the growth of BEL 7402 cells	⊥G_1/S	50-500 mg/mL	Yang[89]
	Ganoderiol F	Displayed antiproliferative effects in HepG2 , Huh7 and Hep3B cells	⊥G_1; ↓DNA synthesis; ↓topo Ⅰ and Ⅱ; ↑p16; ↓p21; ↑pERK2	0.1-60 μmol/L	Chang *et al*[90]
	Ginsenoside-Rg1	Accelerated the growth of SK-Hep-1 cells	↑cyclin E; ↑cdk2	2.5-100 μmol/L	Lee *et al*[91]
	Ginsenoside-Rg5	Suppressed the growth of SK-Hep-1 cells	⊥G_1/S; ↓cyclin E; ↓cdk2; ↑p21WAF1/CIP1; ↓cdc25A	0.1-25 μmol/L	Lee *et al*[92]
	Ginsenoside Rh2	Inhibited DNA synthesis in SK-Hep-1 cells	⊥G_1/S; ↓cyclin E; ↑p27kip1; ↓cdc25A	0.25-100 μmol/L	Lee *et al*[93]
		Induced cell-death in SK-Hep-1 cells	↑apoptosis; ↑caspase-3; ↑PARP	2-12 mg/mL	Park *et al*[94]
	Ginsenoside Rk1	Inhibited the growth of HepG2 cells	↑apoptosis; ↑caspase-3, -8; ↓FADD; ↓telomerase activity	12.5-100 μmol/L	Kim *et al*[95]
		Exhibited antiproliferative effects on HepG2 cells	↑autophagy; ⊥G_1	0-100 μmol/L	Ko *et al*[96]
	Ginsenoside Rs3	Displayed suppressive effects against the growth of SK-Hep-1 cells	↑apoptosis; ↓cyclin E, A; ↑p53; ↑p21WAF1/CIP1; ↓cdk2	0.1-25 μmol/L	Kim *et al*[97]
	Gypenosides	Exhibited cytotoxicity and decreased viability of Huh7, Hep3B and HA22T cells	↑apoptosis; ↑Bax; ↑Bak; ↑Bcl-X_L; ↓Bcl-2; ↓Bad; ↑cyt. *c*; ↑caspase-3, -9, -8	0.1-400 mg/mL	Wang *et al*[98]
	IH-901	Conferred antiproliferative effects against HepG2 cells	↑apoptosis; ↑caspase-3, -8, -9; ↑PARP; ↑cyt. *c*	10-60 μmol/L	Oh *et al*[99]
		Inhibited the growth of SMMC7721 cells	↑apoptosis; ⊥G_0/G_1; ↑Bax; ↑p53; ↑cyt. *c*; ↓pro-caspase-3, -9	5-100 μmol/L	Ming *et al*[100]
	Kalopana-xsaponins A, I	Showed significant cytotoxicity against HepG2 and R-HepG2 cells	↑apoptosis	8.9-18.1 μmol/L	Tian *et al*[101]
	Keto- and acetyl-keto-boswellic acids	Exhibited antiproliferative effects in HepG2 cells	↑apoptosis; ⊥G_1; ↑caspase-3, -8, -9	25-200 μmol/L	Liu *et al*[102]
	Lucidenic acid A,B,C, N	Displayed antiproliferative and anti-invasive effects against HepG2 cells	↓MMP-9; ↓ERK1/2; ↓NF-κB; ↓AP-1; ↓c-Jun; ↓c-Fos	10-100 μmol/L	Weng *et al*[103,104]
	Lupeol	Exhibited growth inhibitory effects against SMMC7721 cells	↑apoptosis; ↑caspase-3, -8; ↓DR3; ↑FADD	6.25-200 μmol/L	Zhang *et al*[105]

	25-Metho-xyhispidol A	Showed antiproliferative effects against SK-Hep1 cells	↑apoptosis; ⊥G_0/G_1; ↓cyclin D1; ↓CDK4; ↓c-myc; ↓pRb; ↑p21	0.8-100 μmol/L	Hong *et al*[106]
	Oleanolic acid	Decreased the viability of HepG2, Hep3B, Huh7 and HA22T cells	↑apoptosis; ↓ΔΨm; ↑caspase-3, -8; ↓Na^+-K^+- ATPase; ↓ICAM-1; ↓VEGF	2-8 μmol/L	Yan *et al*[107]
		Reduced the viability of Huh7 cells	↑apoptosis; ↓ΔΨm; ↑Bax; ↓Bcl2; ↑cyt. *c*; ↑ caspase-3, -9; ↓NF-κB; ↓XIAP	20-100 μmol/L	Shyu *et al*[108]
	Ursolic acid	Inhibited the proliferation of HepG2 and R-HepG2 cells	↑apoptosis; ⊥G_1/G_0; ↓COX-2; ↑HSP105	3.125-100 μmol/L	Tian *et al*[109]
		Suppressed the proliferation of HepG2 cells	↑apoptosis; ↑p53; ↓Bcl-2; ↓survivin; ↑caspase-3; ↓PI3K/Akt	5-80 μmol/L	Tang *et al*[110]
		Decreased the viability of HepG2, Hep3B, Huh7 and HA22T cells	↑apoptosis; ↓ΔΨm; ↑caspase-3, -8; ↓Na^+-K^+- ATPase; ↓ICAM-1; ↓VEGF	2-8 μmol/L	Yan *et al*[107]
		Reduced the viability of Huh7 cells	↑apoptosis; ↓ΔΨm; ↑Bax; ↓Bcl2; ↑cyt. *c*; ↑ caspase-3, -9; ↓NF-κB; ↓XIAP	20-100 μmol/L	Shyu *et al*[108]
	Waltonitone	Conferred inhibitory effects on the growth of BEL-7402 cells	↑apoptosis; ↑caspase-3, -8, -9; ↑Bax; ↑cyt. *c*; ↑Fas; ↑FasL; ↑apaf-1	0.4-100 μmol/L	Zhang *et al*[111]
	Miscellaneous	Induced reduction in the viability of HepG2 and Hep3B cells		9.4-71.1 μmol/L [IC_{50}]	Wang *et al*[112]
		Exhibited cytotoxicity against HepG2 cells	↑apoptosis; ↑p53; ⊥G_1/S	0.5-80 μmol/L	Huang *et al*[113]
Tetraterpenes	Astaxanthin, β-Carotene	Suppressed the invasive characteristic of AH109A cells	Antioxidant mechanisms	2.5-20 μmol/L	Kozuki *et al*[114]
		Inhibited the migration of SK-Hep-1 cells		1-20 μmol/L	Huang *et al*[115]
		Delayed the proliferation and improved the differentiation of oval cells obtained from neoplastic liver	↑albumin; ↑fibrinogen; ↑ hapatoglobin	5 μmol/L	Wójcik *et al*[116]
		Exerted genotoxic and cytotoxic effects in HepG2 cells	↑apoptosis; ↑necrosis; ↑TBARS	4-8 μmol/L	Yurtcu *et al*[117]
	Fucoxanthin	Inhibited the growth of HepG2 cells	⊥G_0/G_1; ↓cyclin D1, D3; ↓cdk4; ↓pRb	10-50 μmol/L	Das *et al*[118]
		Exhibited antiproliferative effect against SK-Hep-1 cells	↑apoptosis; ⊥G_0-G_1; ↑GJIC; ↑Cx43; ↑Cx32; ↓pJNK; ↓pERK; ↑$[Ca]^{++}$	1-20 μmol/L	Liu *et al*[119]
	Lycopene	Suppressed the invasive property of AH109A cells		0.1-20 μmol/L	Kozuki *et al*[114]
		Induced an inhibitory effect on the growth of Hep3b cells	⊥G_0/G_1; DNA damage	0.1-50 μmol/L	Park *et al*[120]
		Displayed antimigration and anti-invasive activity in SK-Hep-1 cells	↑nm23-H1	1-20 μmol/L	Huang *et al*[115]
		Inhibited adhesion, invasion and migration of SK-Hep-1 cells	↓MMP-9, -2	0.1-50 μmol/L	Hwang *et al*[121]
		Inhibited SK-Hep-1 cell invasion	↓MMP-9; ↓NF-κB; ↑IκBα; ↓Sp1; ↓IGF-1R; ↓ROS	1-10 μmol/L	Huang *et al*[122]
Sesquiterpenes	α-Bisabolbol	Induced cytoxicity in HepG2 cells	↑apoptosis; ↑caspase-8, -9; ↑cyt. *c*; ↑Bax; ↑Bak; ↓Bcl-2; ↑p53; ↑NF-κB; ↑Fas	0.1-20 μmol/L	Chen *et al*[123]
	Dehydro-costuslactone	Inhibited the proliferation of HepG2 and PLC/PRF/5 cells	↑apoptosis; ↑Bax; ↑Bak; ↓Bcl-2; ↓Bcl-X_L; ↑caspase-4, -9; ↑AIF; ↑Endo G; ↑ER stress; ↓CHOP/GADD153; ↑Bip; ↑MAPK; ↑pJNK; ↑ERK1/2; ↑p38	1-40 μmol/L	Hsu *et al*[124]
	Furanodiene	Inhibited cell growth of HepG2 cells	↑apoptosis; ⊥G2/M; altered ΔΨm; ↑cyt. *c*; ↑caspase-3; ↑pp38; ↓pERK1/2	0.1-1000 μmol/L	Xiao *et al*[125]
	HOBS1, HOBS2	Exhibited antiproliferative effects and induced redifferentiation in SMMC-7721 cells	⊥G_1; ↓AFP; ↓γ-GT; ↓TAT	0.1-30 μg/mL	Miao *et al*[126]
	Zerumbone	Showed antiproliferative activity against HepG2 cells	↑apoptosis; ↓Bcl-2; ↑Bax	3.45 μg/mL [IC_{50}]	Sakinah *et al*[127]

AFP: α-fetoprotein; AIF: Apoptosis-inducing factor; AP-1: Activator protein-1; apapf-1: Apoptotic protease activating factor 1; Cd: Cadmium; COX-2: Cyclooxygenase-2; Cx: Connexin; DR: Death receptor; cyt. *c*: Cytochrome *c*; GR: Glucocorticoid receptor; IC_{50}: Inhibitory concentration 50%; ER: Endoplasmic reticulum; ERα: Estrogen receptor α; ERK: Extracellular signal-regulated kinase; FADD: Fas-associated death domain; GLI1: Glioma-associated oncogene protein 1; GJIC: Gap junctional intercellular communication; γ-GT: γ glutamyl transferase; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; HSP, heat shock protein; ICAM-1: Intercellular adhesion molecule-1; IκB: Inhibitory κB; IKKα/β: IκB kinase α/β; IL-1β: Interleukin 1β; IL-6: Interlukin-6; IGF2: Insulin growth factor 2; IGF-1R: Insulin growth factor-1 receptor; JAK1: Janus-activated kinase 1; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MD: Mitochondrial dehydrogenase; MMP: Matrix metalloproteinase; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor-κB; NO: Nitric oxide; PARP: Polypeptide poly(ADP-ribose) polymerase; PGE_2: Prostaglandin E_2; PhIP: 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine; PI3K: Phosphatidylinositol 3-kinase; PKC: Protein kinase C; PMA: Phorbol 12-myristate; Pop: Population; PTCH1: Protein patched homolog 1; Rb: Retinoblastoma protein; ROS: Reactive oxygen species; Sp1: Stimulatory protein-1; SULT-1: Sulfotransferase-1; STAT3: Signal transducer and activator of transcription 3; TAT: Tyrosine-α-ketoglutarate transaminase; TPA: 12-*O*-tetradecanoylphorbol-13-acetate; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; uPA: Urinary plasminogen activator; VEGF: Vascular endothelial growth factor; ΔΨm: Mitochondrial membrane potential; XIAP: X-linked inhibitor of apoptotic protein.

activation of the hedgehog signaling pathway) but increased the protein levels of survivin and anti-apoptotic Bcl-2[80].

Cucurbitacins, a group of oxygenated triterpenes, are characterized by the presence of the tetracyclic cucurbitane skeleton. Cucurbitacin B, one of the most abundant forms of cucurbitacins, was found to effectively decrease the viability of HepG2 cells through apoptosis with a simultaneous decrease in the levels of the pro-apoptotic protein Bcl-2. Suppression of transcription factor signal transducer and activator of transcription-3 (STAT3) phosphorylation was associated with the cell killing effect of this natural compound[83]. Chan *et al*[84] were successful in achieving similar growth inhibitory effects of cucurbitacin B in Bel-7402 cells. Treatment of Bel-7402 cells with cucurbitacin B also induced S phase arrest and apoptosis associated with down regulation of cyclin D1 and cdc-2. Additional studies indicated cucurbitacin B mediated the inhibition of c-Raf activation without any alteration of the STAT3 phosphorylation. Cucurbitacin D, isolated from *Trichosanthes kirilowii*, was shown to suppress the growth of Hep3b cells by apoptosis through caspase-3 and phosphorylation of JNK protein[85]. Meng *et al*[86] isolated cucurbitacins D and I from *Elaeocarpus hainanensis* and reported strong cytoxic effects of these compounds against Bel-7402 cells.

Echinocystic acid, a triterpene present in various herbs, is used for medicinal purposes in many Asian countries. This compound was shown to exhibit antiproliferative effects against HepG2 cells through typical apoptosis mechanisms, characterized by DNA fragmentation, activation of caspase-3, -8 and -9, polypeptide poly(ADP-ribose) polymerase (PARP) cleavage, truncation of Bid, reduction of Bcl-2, loss of $\Delta\Psi$m, cyt. *c* release and activation of JNK and p38 kinase[87].

Escin, a mixture of triterpene saponins extracted from *Aesculus wilsonii* Rehd., displayed its potency against HepG2 cell viability through disruption of the G_1/S phase of cell cycle progression and caspase-independent cell death *via* apoptosis-inducing factor/cyt. *c* translocation from the mitochondria to the nucleus[88].

Ganoderic acid, produced by the submerged culture of *Ganoderma lucidum*, was effective in inhibiting the growth of Bel-7402 cells by blocking the transition of cells from G_1 to S phase[89]. Ganoderiol F, a tetracyclic triterpene isolated from *Ganoderiol amboinense*, induced antiproliferative effects through senescence in HepG2, Huh7 and Hep3b. Ganoderiol F treatment in HepG2 and Huh7 cells resulted in inhibition of DNA synthesis and arrest of cell cycle progression in G_1 phase. The inhibition of DNA synthesis in HepG2 cells was attributed to the suppression of topoisomerases I and II[90].

The triterpenoid ginsenoside-Rg1 failed to confer cytotoxicity against SK-Hep-1 cells and instead stimulated its growth through an increase in cyclin E and cdk2 protein expression[91]. Ginsenoside-Rg5, a new diol-containing ginsenoside, is isolated from red ginseng. This triterpenoid saponin was found to suppress the growth of SK-Hep-1 cells through cell cycle arrest in the G_1/S phase associated with downregulation of cdk2 activity. This was caused by selective induction of $p21^{WAF1/CIP1}$ with a concurrent decrease in cyclin E, cdk2 and cdc25A[92].

Ginsenoside-Rh2 inhibited DNA synthesis in SK-Hep-1 cells through cell cycle arrest in the G_1/S phase by selectively inducing $p27^{kip1}$ expression consequently downregulating cyclin E-dependent kinase activity[93]. A similar study conducted by Park *et al*[94] described an inhibitory effect of ginsenoside-Rh2 on the growth of SK-Hep-1 cells by apoptosis through Bcl-2-insensitive activation of caspase-3 followed by proteolytic cleavage of PARP.

Ginsenoside Rk1, obtained from heat-processed *Panax ginseng* C.A. MEYER, suppressed the growth of HepG2 cells associated with a significant inhibition of telomerase activity 48 h following the treatment. Moreover, this compound induced apoptosis through activation of caspase-8 and -3 with a decrease in Fas-associated death domain expression[95]. Interestingly, when HepG2 cells were incubated with ginsenoside Rk1 for 24 h, cell growth inhibition, G_1 cell cycle arrest and autophagy were observed[96].

Kim *et al*[97] reported the growth suppressive effects of ginsenoside Rs3 in SK-Hep-1 cells by apoptosis, cell cycle arrest in the G_1/S phase and selective elevation of the protein levels of p53 and $p21^{WAF1/CIP1}$ with a concurrent decrease in the activities of cyclin E- and A-associated kinases.

Gypenosides, triterpenoid saponins present in the extract from *Gynostemma pentaphyllum* Makino, were found to exhibit cytoxicity against HUH7, Hep3B and HA22T cancer cell lines through the intrinsic cell death pathway mediated by alteration of apoptosis-associated proteins, such as Bax, Bak, Bcl-X_L, Bcl-2, Bad, cyt. *c*, caspase-3, -9 and -8[98].

IH-901, a novel metabolite of the ginseng saponin, exhibited cytotoxicity against HepG2 cells with simultaneous induction of apoptosis *via* mitochondrial-mediated pathway, which resulted in the activation of caspase-9 and subsequent mitochondrial pathway activation. Caspase-8 was shown to cleave Bid which subsequently relocated to the mitochondria and amplified the mitochondrial pathway activation[99]. IH-901 was also tested against SMCC7721 cells with antiproliferative and apoptotic effects *via* the mitochondrial-mediated pathway, which resulted in activation of caspase-9 and subsequently caspase-3 through release of cyt. *c*[100].

Kalopanaxsaponins A and I are two oleanane triterpene saponins isolated from the seeds of *Nigella glandulifera*. Through apoptotic mechanisms, these triterpenes displayed significant cytotoxicity against HepG2 as well as drug-resistant HepG2 (R-HepG2) cells[101].

Boswellic acids, natural compounds isolated from the gum resin of *Bosewellia serrata*, have recently drawn attention because of their potential as chemopreventive and therapeutic agents. Keto- and acetyl-keto-boswellic acids exhibited antiproliferative effects against HepG2 cells,

with apoptosis accompanied by activation of caspase-3, -8 and -9 being the mechanisms by which the boswellic acids exerted their liver cancer cell killing effects[102].

Lucidenic acids A, B, C and N were isolated from *Ganoderma lucidum*, a well-known mushroom with pharmacological effects. These triterpenoid components exhibited antiproliferative and anti-invasive effects against HepG2 cells as evidenced by lucidenic acid-mediated inhibition of phorbol-12-myristate-13-acetate-induced matrix metalloproteinase-9 (MMP-9) activity[103]. Subsequent studies from the same group demonstrated that the anti-invasive effects of lucidenic acid B might be effected through inhibiting the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 and downregulating MMP-9 expression by suppressing activator protein-1- and nuclear factor κB (NF-κB)-DNA binding activities[104].

Lupeol, a novel dietary triterpene found in several fruits and vegetables as well as in a number of medicinal plants, greatly inhibited the growth of SMMC7721 cells with apoptotic death through activation of caspase-3 mediated by the downregulation of death receptor 3[105].

25-Methoxyhispidol A, a novel triterpenoid isolated from the fruit of *Poncirus trifolata,* was successfully shown to display antiproliferative effects against SK-Hep-1 cells. Apoptosis and G_0/G_1 phase arrest were suggested as the mechanisms of action. This correlated well with the down-regulation of cyclin D1, CDK4, c-myc, and retinoblastoma protein expressions, along with the upregulation of p21[106].

The pentacyclic triterpene oleanolic acid (OA) proved its beneficial chemotherapeutic properties against HCC by inducing significant decrease in viability of various liver cancer cell lines, namely HepG2, Hep3B, Huh7 and HA22T. The observed elevation in caspase activities and DNA fragmentation suggested apoptosis to be a key mechanism in OA action. Concentration-dependent inhibition of intercellular adhesion molecule 1 (ICAM-1) levels along with that of VEGF, a pro-angiogenic protein, provided insight into specific targets of the anti-cancer effects of OA against liver cancer[107]. A similar study also reported the antiproliferative effects of OA in Huh7 cells through apoptosis associated with alteration in Bcl-2 family proteins and downregulation of NF-κB and the X-linked inhibitor of apoptotic protein (XIAP)[108].

UA, isolated from *Aralia decaisneana*, displayed anti-hepatoma activity against HepG2 and R-HepG2 cells through apoptosis and G_0/G_1 cell cycle arrest. UA-mediated downregulation of cyclooxygenase-2 and upregulation of HSP105 provided additional mechanisms[109]. UA was also found to exert antiproliferative effects against HepG2 cells through activation of apoptosis accompanied by a significant decrease in Bcl-2 and survivin expression[110]. Two recent studies confirmed the anti-tumor effects of UA using HepG2 as well as additional cell lines, such as Hep3B, Huh7 and HA22T. Ancillary studies revealed mitochondrial-mediated apoptosis and inhibition of ICAM, VEGF, NF-κB and XIAP as underlying molecular mechanisms of UA action[107,108].

Waltonitone, a new pentacyclic triterpene isolated from *Gentian waltonii* Burkill, was found to inhibit the growth of BEL-7402 human hepatic carcinoma cells through apoptosis *via* the intrinsic and extrinsic cell death pathways. Waltonitone was found to upregulate the mRNA expressions of caspase-3, -8, -9, Bax, apoptotic protease activating factor 1 (apaf-1), Fas and FasL in BEL-7402 cells[111].

Two new triterpenoid saponins along with five other known triterpene compounds were extracted from *Androsace umbellate* and tested for their anti-tumor efficacies in HepG2 and Hep3B cells. The new triterpenoid saponins, namely 3-*O*-{β-D-glucopyranosyl-(1→2)-β-D-glucopyranosul-(1→4)-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosl}-3-β-hydroxy13β,28-epoxy-16-oxo-oleanan-30-al and 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L- arabinopyranosl-3β-hydroxy-13β,28-epoxy-16-oxo-oleanan-30-al, as well as the known triterpene compounds [3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoslcyclamiretin A, primulanin, saxifragifolin B, C and D were found to exert cytotoxity in the aforementioned liver cancer cell lines although the mechanisms of such effects were not elucidated[112]. A new triterpene compound, 20(*R*), 22(ζ), 24(*S*)-dammar-25(26)-ene-3β, 6α, 12β, 20, 22, 24-hexanol, and three known triterpenoids, β-D-glucopyranoside,(3β,12β)-12,20-dihydroxydammar-24-en-3-yl,6-acetate, 20(*R*)ginsenoside Rg_3, and 20(*R*)-ginsenoside Rh_2, isolated from the leaves of *Panax ginseng,* exhibited various degrees of cytoxicity against HepG2 cells through p53-mediated cell cycle arrest and apoptosis *via* activation of the caspase signaling pathway[113].

Tetraterpenes: The effect of carotenoids, including astaxanthin and β-carotene, on the invasion of AH109A rat ascites hepatoma cells was investigated by co-culturing the hepatoma cells with rat mesentery-derived mesothelial cells. Both carotenoids inhibited AH109A invasion through antioxidant mechanisms[114]. Huang *et al*[115] showed that β-carotene effectively inhibited cell migration in SK-Hep-1 cell lines. The effects of astaxanthin and β-carotene on the proliferation and differentiation of isolated rat oval cells were investigated. Oval cells were isolated from the livers of rats subjected to partial hepatectomy or chronic diethylnitrosamine (DENA) treatment. Both compounds decreased the proliferation and intensified the differentiation of oval cells with increased expression of albumin, hapatoglobin and fibrinogen[116]. Recently, Yurtcu *et al*[117] reported the genotoxic and cytotoxic effects of β-carotene in HepG2 cells as evidenced from increased apoptosis and necrosis. A concurrent increased in thiobarbituric acid-reactive substances indicated elevated oxidative damage of β-carotene-exposed cells.

Fucoxanthin, an oxygenated carotenoid present in several types of edible seaweed, such as *Laminaria japonica*, *Undaria pinnatifida* and *Hijikia fusiformis*, was found to suppress the growth of HepG2 cells with inhibition of cell cycle arrest in the G_0/G_1 phase being the typical mechanism of action. Other mechanisms observed were

decrease in the cyclin proteins D1, D3 and cdk4 with the inhibition of the phosphorylation of the retinoblastoma[118]. A separate study by Liu *et al*[119] described similar antiproliferative effects of fucoxanthin against SK-Hep-1 cells. Apoptosis was induced in these cells as well as cell cycle phase arrest at G_0/G_1. An upregulation of the connexin genes *43 (Cx43)* and *32 (Cx32)* provided evidence for the enhancement of gap junctional intercellular communication resulting in an increase in intracellular calcium concentrations. A decrease in the phosphorylation of ERK and JNK was also associated with fucoxanthin action.

Lycopene, the major carotenoid present in tomatoes and tomato-derived products, was found to decrease the invasive properties of AH109A rat ascites cells[114]. Park *et al*[120] demonstrated the inhibitory effect exerted by lycopene in Hep3B cells through mechanisms involving cell cycle arrest in the G_0/G_1 phase and DNA damage. This tetraterpenoid also effectively displayed antimigration and anti-invasive properties against highly invasive SK-Hep-1 cells which were associated with the increase of the metastasis suppressor gene, nm23-H1[115]. The study by Hwang and Lee[121] concluded with similar results displaying lycopene's inhibitory effects on adhesion, invasion and migration of SK-Hep-1 cells. Additionally, these investigators showed a decrease in the activities of MMP-2 and MMP-9. Additional studies by Huang *et al*[122] demonstrated that lycopene strongly inhibited the invasion of SK-Hep-1 cells and this effect was mediated by inhibition of NF-κB and stimulatory protein-1 binding activity leading to a decrease in the secretion of MMP-9. Lycopene also decreased the level of ROS and insulin-like growth factor-1 (IGF-1R), albeit to a lesser extent[122].

Sesquiterpenes: α-Bisabolbol, a sesquiterpene, was found to induce cytotoxicity in HepG2 cells. Apoptosis, through both the intrinsic and extrinsic pathway, was identified as the mechanism of action exhibited by this natural compound. Associated proteins of the apoptotic pathways, namely caspase-3, -8, Bax, Bak, cyt *c*, p53 and Fas were found to be increased with a concomitant decrease in the Bcl-2[123].

Dehydrocostuslactone (DHE), sesquiterpene lactone, exhibits its antitumor activity against different human hepatoma cell lines, namely HepG2 and PLC/PRF/5, through a wide variety of mechanisms. The anti-proliferative effects exerted by DHE in these cell lines involved apoptotic death of liver cancer cells through the mitochondrial pathway mediated through increases in the pro-apoptotic proteins, decreases in the anti-apoptotic proteins, increases of caspase-3, -4, apoptosis-inducing factor and endonuclease G. DHE also triggered endoplasmic reticulum stress, as evidenced by changes in cytosol calcium levels, double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase phosphorylation, inositol-requiring protein 1 and CHOP/GADD153 upregulation, X-box transcription factor-1 mRNA splicing and caspase-4 activation[124].

Furanodiene, a sesquiterpene compound isolated from the essential oil of the Chinese medicinal plant *Curcuma wenyujin*, was found to inhibit the growth of HepG2 cells by causing cell cycle arrest at G_2/M phase and inducing apoptosis related to mitochondrial transmembrane depolarization, release of cyt. *c*, activation of caspase-3 and cleavage of PARP. These effects were associated with the activation of p38 and inactivation of ERK1/2 MAPK signaling cascades[125].

Antiproliferative effects along with redifferentiation were induced in SMMC-7721 human hepatoma cells by two highly oxygenated bisabolane-type sesquiterpenes, namely HOBS1 and HOBS2, isolated from *Cremanthodium discoideum*. These sequiterpenes arrested cell growth in the G_1 phase of the cell cycle, increased tyrosine-α-ketoglutarate transaminase activity, decreased α-fetoprotein (AFP) level and γ-glutamyl transferase activity[126].

Zerumbone (ZER), a cytotoxic component isolated from the wild ginger, *Zingiber zerumbet* Smith, was shown to induce significant antiproliferative activity against HepG2 cells through mechanisms involving an elevation of the apoptotic process with increase in the level of pro-apoptotic protein Bax and decrease of anti-apoptotic protein Bcl-2 without involving p53[127].

In vivo studies

Although the various aforementioned *in vitro* studies demonstrated the antiproliferative and cytotoxic effects of various terpenoids against a broad spectrum of liver cancer cells, surprisingly very few compounds from these studies have been further investigated for similar effects *in vivo*. Nevertheless, a large number of other natural terpenoids have been tested *in vivo* to show positive chemopreventive and antitumor potential against several animal models of liver cancer, with a few studies describing the lack of any beneficial effect produced by these compounds (Table 2). Most studies have been conducted in animals with chemically-induced liver tumors while other studies were conducted using xenografted animal models of liver cancer to test the potential beneficial effects of these natural terpenoid compounds.

Monoterpenes: Auraptene, a citrus antioxidant, was found to be effective in suppressing the DENA-induced hepatocarcinogenesis in male F344 rats upon dietary supplementation. A dose range of 100-500 ppm over a period of 7 wk inhibited the development of DENA-induced transforming growth factor (TGF)-α-positive foci and placental glutathione *S*-transferase (GSTp)-positive foci through mechanisms that include increase in apoptosis and decrease in cell proliferation[128].

Atleast two studies on the isoprenoid geraniol have contributed to its chemopreventive effects against hepatocarcinogenesis. Ong *et al*[129] found that oral geraniol treatment (250 mg/kg) for 8 wk in Wistar rats, initiated with DENA and promoted by 2-acetylaminofluorene (2-AAF), decreased both hepatic remodeling and persistent GSTp-positive preneoplastic lesions. A recent

Table 2 ***In vivo*** **effects of natural terpenoids on development and growth of liver cancer**

Terpenoids	Compounds	Effects	Mechanisms	Dose/duration	Route	Ref.
Monoter-penoids	Auraptene	Suppressed DENA-induced TGF-α foci and carcinoma in male F344 rats	↑apoptosis; ↓cell proliferation	100-500 ppm; 7 wk	Diet	Sakata *et al*[128]
	Geraniol	Decreased GSTp foci and nodules during DENA-initiated and 2-AAF-promoted hepatocarcinogenesis in male Wistar rats	↓cell proliferation; ↑apoptosis	25 mg/100 g; 8 wk	po	Ong *et al*[129]
		Inhibited liver carcinogenesis with decrease in mean number of GSTp foci and nodule induced by DENA and promoted by PB in male Wistar rats	↑apoptosis; ↓RhoA activation	25 mg/100 g; 5 wk	po	Cardozo *et al*[130]
	d-Limonene	Decreased the number of GSTp foci in DENA-initiated and Glu-P-1-promoted hepatocarcinogenesis in male F344 rats		0.5%; 6 wk	Diet	Hirose *et al*[131]
		Exhibited chemopreventive effects against DENA/PB hepatocarcinogenesis in male AKR mice	↓c-jun; ↓c-myc	5%; 80 d	Diet	Giri *et al*[132]; Parija *et al*[133]
		Reduced the incidence, number and size of GSTp foci and neoplastic nodules induced by NNM in male Sprague-Dawley rats	↑apoptosis; ↓cell proliferation	1%, 2%; 7 wk	Diet	Kaji *et al*[134]
		Suppressed hepatic preneoplasia and tumor growth induced by DENA in male WR rats	↑GJIC	3 mL/kg per day; 5 times/wk; 5.5 mo	po	Bodake *et al*[135]
	Perillyl alcohol	Inhibited liver tumor growth induced by DENA in male F344 rats	↑ apoptosis; ↑TGFβ; ↑M6P/IGF Ⅱ R; ↑TGFβ type Ⅰ, Ⅱ, Ⅲ R	1% w/w for 1 wk and 2% w/w for 18 wk	Diet	Mills *et al*[136]
Diter-penoids	Andro-grapholide	Suppressed the formation of BHC- induced nodules in Swiss male albino mice	↑GSH; ↑GR; ↑GPX; ↑SOD; ↑CAT; ↓GGT; ↓GST	5-10 mg/kg per day; 1-8 mo	po	Trivedi *et al*[137]
		Reversed histomorphological and ultrastructural changes during BHC-induced hepato-carcinogenesis male Swiss mice	↑G-6-Pase; ↑ATPase; ↑SDH; ↓SGPT; ↓SGOT; ↓ALP; ↓OCT; ↓ACP	5,7 and 10 mg/kg; 1-8 mo	Diet	Trivedi *et al*[138]
	Epoxy clerodane diterpene	Reduced incidence, multiplicity and size of nodules induced by DENA in male Wistar rats	↓SGOT; ↓SGPT; ↓LDH; ↑SOD; ↑CAT; ↓GST; ↓GGT; ↑GSH; ↑GPX	10 mg/kg per day; 8 wk	po	Dhanasekaran *et al*[139]
	Excisanin A	Suppressed tumor growth in BALB/c nude mice implanted with Hep3B cells	↓apoptosis; ↓pAKT	10-20 mg/kg; 12 d	ip	Deng *et al*[70]
	Gerany-lgeraniol	Reduced the incidence, number and size of visible nodules and GSTp foci in DENA/2-AAF hepatocarcinogenesis in male Wistar rats	↓cell proliferation; ↓DNA strand break; ↓plasma cholesterol; ↓NF-κB p65	8-16 mg/kg per day; 7 wk	po	de Moura Espíndola *et al*[140]
Triter-penoids	Bacoside A	Delayed the development and growth of neoplastic nodules induced by DENA in male Wistar albino rats	↓lipid peroxidation; ↑Vit A; ↑Vit E; ↑GSH; ↓ROS; ↓AFP; ↓CEA; ↓5′-nucleotidase; ↓ALT; ↓AST; ↓LDH; ↓ALP; ↓GGT; ↑SOD; ↑CAT; ↑GPX; ↑GR; ↓MMP-2; ↓MMP-9	15 mg/kg; 16 wk	po	Janani *et al*[141,142]
	Cucurbitacin B	Decreased HepG2 tumor volume and inhibited tumor growth in mice xenograft model		26-110 μg/kg per day; 12 d	po	Zhang *et al*[83]
		Inhibited tumor growth in BEL-7402 xenografted mice model		0.1-3 mg/kg; twice per day for 26 d	po	Chan *et al*[84]
	Escin	Demonstrated inhibitory effects on tumor growth in H22 tumor- bearing female Kunming mice		1.4-2.8 mg/kg; 7 d	ip	Zhou *et al*[88]
	Ginseng extract	Inhibited AFB_1-initiated and FB-promoted hepatocarcinogenesis in female SD rats	↓ALT; ↓AST; ↑albumin; ↓TG; ↓cholesterol; ↓LDL; ↑HDL; ↓AFP, ↓CEA, ↓MDA, ↑TAC; ↓fibrosis	150 mg/kg; 1-12 wk	po	Abdel-Wahhab *et al*[145]
	Glycyrrhizin	Decreased the incidence of nodules and HCC induced by DENA in BALB/c mice	↑albumin; ↑AST	2 mg/animal; 3 d/wk for 12-32 wk	im	Shiota *et al*[146]
		Reduced the size, volume and number of preneoplastic liver lesions initiated by DENA and promoted with 2-AAF in male SD rats		0.1% 25 mg/kg; 1 wk	po	Wan *et al*[147]
	EC-2, EC-4	Failed to show modifying effects against DENA-induced hepato-carcinogenesis in male F-344 rats		1 mg/kg; 5 times a week for 2-8 wk	po	Karim *et al*[148]
	Squalene	Failed to exhibit chemoprevention against DENA-initiated and 2-AAF- promoted hepatocarcinogenesis in male Wistar rats	↑ plasma cholesterol	1-1.5 g/kg; 8 wk	po	Scolastici *et al*[149]
	Ursolic acid	Inhibited the growth of H22 hepatoma implanted in male CD-1 mice		15-30 mg/kg; 10 d	ip	Tian *et al*[109]
		Suppressed DENA-initiated and PB- promoted hepatocarcinogenesis in male Wistar rats	↓MDA; ↓PC; ↓membrane damage; ↑Na^+K^+ ATPase; ↑Mg^{2+} ATPase; ↑Ca^{2+} ATPase	20 mg/kg per day; 6 wk	po	Gayathri *et al*[150]

	Waltonitone	Delayed the growth of tumors in athymic nude BALB/c nu/nu mice implanted with BEL-7402 cells	↑cleaved caspase-3, -9; ↓pro-caspase-9	20-50 mg/kg; Once in 2 d for 15 d	iv	Zhang *et al*[111]
Tetraterpenoids	α-Carotene	Decreased the number of hepatomas during spontaneous hepatocarcinogenesis in male C3H/He mice		0.005%-0.05%; 40 wk	dw	Murakoshi *et al*[151]
		Reduced the number of liver tumors in multi-organ carcinogenesis in male and female B6C3F$_1$ mice		0.4 mg/mouse; three times a week for 24 wk	po	Tsuda *et al*[152]
	β-Carotene	Failed to have an effect on the number and size of preneoplastic liver foci induced by DENA/2-AAF/PB in male Wistar rats		300 mg/kg diet or 10 mg/kg bw (3 times/wk); 3 wk	Diet; ip	Astorg *et al*[153]
		Failed to exhibit any effect on 2-NP- or DENA-initiated hepato- carcinogenesis in male Wistar rats		300 mg/kg diet; 3 wk	Diet	Astorg *et al*[154]
		Did not modify DENA-initiated and PCB-promoted hepatocarcinogene-sis in female Sprague-Dawley rats		0.5%; 12 wk	Diet	Tharappel *et al*[155]
		Inhibited the development of preneoplastic foci and nodules initiated with DENA and promoted by 2-AAF in male Wistar rats		70 mg/kg; alternate d for 2-8 wk	po	Moreno *et al*[156]
		Reduced the generation of nodules and GGT foci induced by DENA/2-AAF in male Wistar rats		70 mg/kg; 8 wk	po	Moreno *et al*[157]
		Decreased the incidence and total number of GGT foci and nodules in DENA-initiated and 2-AAF-promoted hepatocarcinogenesis in male Wistar rats	Remodeling of lesions	70 mg/kg; 5 wk	po	Rizzi *et al*[158]
		Attenuated the development of GSTp foci in the resistant hepatocyte model in male Wistar rats	↓oval cell reaction	70 mg/kg; alternate d for 8 wk	po	Dagli *et al*[160]
		Suppressed progression of hepatocarcinogenesis induced by DENA in male Wistar rats	↓cell proliferation	70 mg/kg; 8 wk	po	Moreno *et al*[161]
		Exerted chemoprevention of initiation phage of DENA-induced hepatocarcinogenesis in male Wistar rats	↓cell proliferation; ↓DNA damage; remodeling of lesions	70 mg/kg; 8 wk	po	Ambrogi *et al*[162]
		Conferred a weak chemopreventive effect against DMH-initiated hepatocarcinogenesis in male Wistar rats		70 mg/kg; 9 wk	po	Sampaio *et al*[163]
		Decreased the incidence, number and size of nodules induced by 2-AAF in male Sprague-Dawley rats	↑cyt. b5; ↑CYP; ↑NADPH cyt. *c* reductase; ↑AHH; ↓UDPGT; ↓GST	100 mg/kg diet; 4-20 wk	Diet	Sarkar *et al*[164]
		Suppressed 3′-Met-DAB-induced nodulogenesis in male Sprague-Dawley rats	↓GSH; ↓GGT; ↓GST; ↓GPX; ↓GR; ↑Vit A	120 mg/kg diet; 4-20 wk	Diet	Sarkar *et al*[165]
		Decreased DENA-initiated and PB-promoted nodule incidence and area in male Sprague-Dawley rats	↓RBC protein damage; ↓lipid peroxidation; ↑G-6-Pase; ↓SOD; ↓CAT	120 mg/kg diet; 4-20 wk	Diet	Sarkar *et al*[166]
		Suppressed DENA-induced hepatic chromosomal aberrations in male Sprague-Dawley rats	↓frequency of chromosomal aberrations; ↓DNA chain breaks	120 mg/kg per day; 15-45 d	Diet	Sarkar *et al*[167]
		Reduced the incidence, total number, multiplicity and size of nodules in DENA/PB hepato-carcinogenesis in male Sprague-Dawley rats	↓GSH; ↓GGT; ↓GPX; ↑CYP; ↑GST	500 mg/kg; 4-20 wk	po	Bishayee *et al*[168]
		Decreased the number and size of GSTp foci in IQ-initiated and PB- promoted hepatocarcinogenesis in male Fischer rats		0.02%; 8 d	Diet	Tsuda *et al*[169]
		Inhibited the development of GSTp foci and HCC induced by EE in female Wistar rats		250 mg/kg diet; 1-12 mo	Diet	Ogawa *et al*[170]
		Reduced the development of GSTp foci in Glu-P-1-induced hepato-carcinogenesis in male F344 rats		0.1%; 6 wk	Diet	Hirose *et al*[131]
		Inhibited carcinogen-DNA adduct formation induced by IQ in male F344 rats	↓CYP1A1; ↓CYP1A2	0.02%; 8 d	Diet	Uehara *et al*[171]
		Reduced the number of liver tumors in multi-organ carcinogenesis in male B6C3F$_1$ mice		0.4 mg/mouse; three times a week for 24 wk	po	Tsuda *et al*[152]
		Reduced AFB$_1$-induced hepato-carcinogenesis in male Wistar rats		300 ppm; 3 wk	Diet	Gradelet *et al*[172]
		Decreased the number and volume of GSTp foci initiated by AFB$_1$ in male Wistar rats		300 mg/kg diet; 3 wk	Diet	Gradelet *et al*[173]
		Inhibited liver tumor formation induced by DMBA in both male and female toads		0.05 mg/toad; twice a week for 12 wk	sc	Sadek *et al*[174]
		Suppressed the formation of GSTp foci and nodules initiated with DENA and promoted by PB in male Sprague-Dawley rats	↓GSH; ↓GST; ↑O_2 consumption	120 mg/kg; 16 wk	Diet	Chattopadhyay *et al*[175]
		Reversed histomorphological changes during DENA-induced hepatocarcinogenesis in male Sprague-Dawley rats	↓DNA SSB; ↓K; ↑Ca; ↓Mn; ↓Fe; ↑Cu; ↑Zn; ↓Se	120 mg/kg; 16 wk	Diet	Chattopadhyay *et al*[176]

	Carotenoids (astaxanthin, β-apo-8′-carotenal, cantha-xanthin)	Did not modify DENA/2-AAF/PB hepatocarcinogenesis in male Wistar rats		300 mg/kg diet; 3 wk	Diet	Astorg *et al*[153]
		Failed to exhibit any effect on 2-NP- or DENA-initiated liver carcinogenesis in male Wistar rats		300 mg/kg diet; 3 wk	Diet	Astorg *et al*[154]
		Attenuated the number and size of GSTp foci induced by AFB_1/2-AAF in male Wistar rats	↓DNA single strand breaks	300 ppm; 3 wk	Diet	Gradelet *et al*[172]
		Reduced initiation of liver carcinogenesis by AFB_1 in male Wistar rats	↓AFB_1-DNA binding; ↓DNA single strand breaks; ↑AFB_1 metabolism	300 mg/kg diet; 3 wk	Diet	Gradelet *et al*[173]
	β-Ionone	Reduced the incidence, number and size of GSTp foci and nodules in the initial phase of DENA/2-AAF hepatocarcinogenesis in male Wistar rats	↓cell proliferation; ↓plasma cholesterol; ↓DNA damage	80160 mg/kg; 7 wk	po	de Moura Espíndola *et al*[140]
		Inhibited GSTp foci and nodules during promotional phase of DENA/2-AAF hepatocarcino-genesis in male Wistar rats	↓cell proliferation; ↓HMGCoA reductase	160 mg/kg; 5 wk	po	Cardozo *et al*[130]
	Lutein	Reduced the number and size of GSTp foci initiated by DENA and promoted with 2-AAF in male Wistar rats	↓DNA strand breaks	70 mg/kg per day; 8 wk	po	Toledo *et al*[181]
		Reduced the size of nodules during the promotional phase of DENA/2-AAF hepatocarcinogenesis in male Wistar rats	↓DNA damage	70 mg/kg; 2-6 wk	po	Moreno *et al*[182]
	Lycopene	Failed to modify AFB_1-induced hepatocarcinogenesis in male Wistar rats		300 ppm; 3 wk	Diet	Gradelet *et al*[172]
		Exhibited no effect on the initiation of hepatocarcinogenesis induced by AFB_1 in male Wistar rats		300 mg/kg diet; 3 wk	Diet	Gradelet *et al*[173]
		Failed to influence the risk of developing spon-taneous HCC in male Long-Evans Cinnamon rats		0.005% w/w; 70 wk	Diet	Watanabe *et al*[183]
		Decreased the volume of GGT and GSTp foci in DENA-initiated and 2-AAF-promoted liver carcinogenesis in male Wistar rats	↓CYP2E1	300 mg/kg diet; 3 wk	Diet	Astorg *et al*[154]
		Attenuated the number and size of GSTp foci initiated by DENA and promoted with 2-AAF in male Wistar rats	↓DNA strand breaks	70 mg/kg per day; 8 wk	po	Toledo *et al*[181]
		Decreased the number of GSTp foci in DENA-initiated and PCB-promoted hepatocarcinogenesis in female Sprague-Dawley rats		1%; 12 wk	Diet	Tharappel *et al*[155]
		Inhibited DENA-initiated and NASH-promoted GSTp foci development in male Sprague-Dawley rats	↓PCNA; ↓cyclinD1; ↓lipid peroxidation; ↑Nrf2; ↑HO-1; ↓pERK; ↓NF-κB p65	15 mg/kg per day; 6 wk	Diet	Wang *et al*[184]
	Tomato extract	Reduced the number of GSTp foci initiated by DENA and promoted by NASH in male Sprague-Dawley rats	↓PCNA; ↓cyclinD1; ↓TNF-α; ↓IL-1β; ↓IL-12; ↓lipid peroxidation; ↓CYP2E1; ↓pERK; ↓NF-κB	250 mg/kg per day; 6 wk	Diet	Wang *et al*[184]
Sesquiter-penoids	Dehydro-costus-lactone	Inhibited tumor growth in nude mouse implanted with PLC/PRF/5 cells	↑apoptosis; ↑pPERK; ↑IRE-1	10 mg/kg per day; 45 d	ip	Hsu *et al*[124]
	Farnesol	Suppressed the development of GSTp foci and nodules initiated with DENA and promoted by 2-AAF in male Wistar rats	↓cell proliferation; ↓DNA damage; ↓plasma cholesterol; ↓HMG-CoA reductase	250 mg/kg; 8 wk	po	Ong *et al*[129]
	Zerumbone	Reduced DENA/2-AAF- induced hepatocarcinogenesis in Sprague- Dawley rats	↓ALP; ↓ALT; ↓AST; ↓AFP; ↓lipid peroxidation; ↓GSH; ↓PCNA; ↑Bax; ↓Bcl-2	15, 30 or 60 mg/kg; twice a week for 11 wk	ip	Taha *et al*[186]

2-AAF: 2-acetylaminofluorene; AFB-1: Aflatoxin B1; AFP: α-fetoprotein; AHH: Aryl hydrocarbon hydroxylase; AHF: Altered hepatic foci; ALA-S: δ-aminolevulinate synthetase; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; BHC: Benzene hexachloride; CA: Chromosomal aberrations; CAT: Catalase; CEA: Carcinoembryonic antigen; CYP: Cytochrome P-450; CYP2E1: Cytochrome P-450 2E1; DAB: 1,4,-dimethyl aminoazobenzene; DENA: Diethylnitrosamine; DMH: 1,2-dimethylhydrazine; dw: Drinking water; EAF: Enzyme altered foci; EC-2: 3,4-Seco-8βH-ferna-4-(23),9(11)-dien-3-oic acid; EC-4: 3,4-Seco-oleana-4-(23),18-dien-3-oic acid; EE: Ethynlyestradiol; ERK: Extracellular signal-regulated kinase; FB: Fumonisin; GGT: γ-glutamyl transpeptidase; GJIC: Gap-junctional intercellular communication; Glu-P-1: 2-amino-6-methyldipyrido[1,2-α:3′,2′-d] imidazole; G-6-Pase: Glucose-6-phosphatase; GPX: Glutathione peroxidase; GR: Glutathione reductase; GSTp: Glutathione S-transferase, placental form; GSH: Reduced glutathione; HCC: Hepatocellular carcinoma; HDL: High-density lipoprotein; HO-1: Heme oxygenase-1; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; HTHQ: 1-O-hexyl-2,3,5-trimethylhydroquinone; IL: Interleukin; ip: Intraperitoneal; po: Per os; im: Injectio intramuscularis; sc: Subcutaneous route; IQ: 2-amino-3-methylimidazo[4,5-f]quinoline; JNK: c-Jun N-terminal kinase; LDH: Lactate dehydrogenase; LDL: Low-density lipoprotein; MAPK: Mitogen-activated protein kinase; MDA: Malondialdehyde; M6P/IGF Ⅱ-R: Mannose 6-phosphate/insulin-like growth factor Ⅱ receptor; 3′-Met-DAB: 3′-methyl-4-dimethlyaminoazobenzene; MMP: Matrix metalloproteinase; MPE: Mango pulp extract; NASH: Nonalcoholic steatohepatitis; NF-κB: Nuclear factor κB; NMDA: N-nitrosodimethylamine; NNM: Nitrosomorpholine; NMU: N-nitrosomethyl urea; 2-NP: 2-nitropropane; Nrf2: NF-E2-related factor 2; OCT: Ornithine carbamoyltransferase; PB: Phenobarbital; PC: Protein carbonyl; PCB: 3,3′,4,4′-tetrachlorobiphenyl; PCNA: Proliferating cell nuclear antigen; RBC: Red blood cell; ROS: Reactive oxygen species; sc: Subcutaneous; SD: Sprague-Dawley; SDH: Succinate dehydrogenase; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamyl oxalate transaminase; SOD: Superoxide dismutase; SSB: Single stranded breaks; SULT-1: Sulfotransferase-1; TAC: Total antioxidant capacity; TAT: Tyrosine ketoglutarate; TG: Triglyceride; TGF: Transforming growth factor; TNF: Tumor necrosis factor; Vit: Vitamin; YY1: Ying Yang 1 protein.

and similar study by Cardozo *et al*[130] observed the same chemopreventive benefits of geraniol in male Wistar rats initiated with DENA promoted by phenobarbital (PB). Geraniol was found to suppress liver carcinogenesis by inhibiting the promotion phase through induction of apoptosis and reduction of hepatic membrane protein RhoA.

A number of *in vivo* studies have been performed with *d*-limonene showing its efficacy against HCC. From earlier studies, it has been shown that 0.5% *d*-limonene in diet has been responsible for a 32% inhibition of the GSTp foci in DENA-initiated and 2-amino-6-methyldipyrido [1,2-α:3',2'-*d*] imidazole (Glu-P-1)-promoted hepatocarcinogenesis in male F-344 rats[131]. Later studies demonstrated that *d*-limonene's chemopreventive efficacy arises from its capability to block alterations in the level of oncogene expression both at the RNA and protein levels brought about by DENA alone or along with PB in the liver of AKR mice. Inhibition of the overexpression of c-myc and c-jun proteins, the characteristics of DENA hepatocarcinogenesis, is one mechanism by which *d*-limonene exhibits its anticarcinogenic effect[132]. According to a subsequent study, a potential role of *d*-limonene might involve modulation of the ying yang 1 protein which was found to be correlated with c-myc in DENA-induced hepatocarcinogenesis[133]. Kaji *et al*[134] confirmed *d*-limonene's antitumor effect in Sprague Dawley rat hepatocarcinogenesis induced by N-nitrosomorpholine. *d*-Limonene was found to reduce the incidence, number as well as the size of GSTp foci without the involvement of $p21^{RAS}$ plasma membrane association. In 2002, the chemopreventive effect of orange oil (containing 90%-95% *d*-limonene) was tested and proved by Bodake *et al*[135]. Oral administration of orange oil for more than 5 mo following DENA treatment in male Wistar rats significantly suppressed the growth of liver tumors with the restoration of the normal phenotype and upregulation of gap junctional complexes.

Significant inhibition of liver tumors through a marked increase in the frequency of apoptosis was achieved by perillyl alcohol upon treatment of F344 rats challenged with DENA. In addition, the elevated expressions of mannose 6-phosphate/IGF receptor Ⅱ and TGF-β type Ⅰ, Ⅱ and Ⅲ receptors were also demonstrated as underlying mechanisms of perillyl alcohol's chemopreventive activity[136].

Diterpenes: The antihepatocarcinogenic effect exerted by the antioxidant andrographolide extracted from *Andrographis paniculata* (Kalmegh) was demonstrated in male Swiss-albino mice challenged with benzene hexachloride (BHC). The oral administration of andrographolide resulted in suppression of BHC-induced hepatic nodules. Spectrometric analysis showed significant increases in glutathione (GSH) and antioxidant enzyme activities of glutathione reductase (GR), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) with concurrent decreases in γ-glutamyl transpeptidase (GGT) and GST[137]. A follow-up study confirmed the potent anti-tumor activity possessed by andrographolide. This study indicated a reversal of the histomorphological and ultrastructural changes induced by BHC, along with improved glycogenesis in the liver due to increased activities of glucose-6-phosphate and phosphorylase. Regenerative effects elicited by andrographolide were the result of decreased activities of serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, alkaline phosphatase (AP), ornithine carbomoyl transferase and acid phosphatase, all of which are markers of liver damage[138].

A recent investigation by Dhanasekaran *et al*[139] on the epoxy clerodane diterpene extracted from *Tinospora cordifolia* proposed increases in the hepatic antioxidant status of Wistar rats administered with DENA to induce HCC. Treatment for 8 wk at a dose of 8 mg/kg per day resulted in reduced incidence, multiplicity and size of hepatic nodules. The treatment was instrumental in increasing the level of detoxifying enzymes and bringing down the elevated levels of serum transaminase and hepatic marker enzymes to near normal.

Excisanin A, a diterpenoid compound extracted from *Isodon MacrocalyxinD*, was found to be an effective and potent tumor growth inhibitor in BALB/c nude mice implanted with Hep3B cells. A daily intraperitoneal (ip) injection of 10-20 mg/kg of excisanin A in this xenografted mouse model and the subsequent analysis of the tumor samples after sacrifice established the inhibition of the AKT signaling pathway in tumor cells. Upon performing the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay on frozen liver sections, TUNEL-positive cells were identified, indicating apoptotic activity in the tumor cells[70].

de Moura Espíndola *et al*[140] studied the anticarcinogenic effects of geranylgeraniol and showed the potential usefulness of this diterpenoid as a chemopreventive agent in hepatocarcinogenesis. Geranylgeraniol treatment administration to male Wistar rats subjected to DENA/2-AAF regimen resulted in significant reduction of nodule incidence and size. The antitumor activity of geranylgeraniol was closely interlinked to decreases in cell proliferation and DNA damage. Decreases in the transcription factor NF-κB and plasma cholesterol levels were also interesting hallmarks of the chemopreventive activity exhibited by geranylgeraniol in the initiation phase of hepatocarcinogenesis.

Triterpenes: Bacoside A, a triterpenoid saponin isolated from *Bacopa monniera* Linn., was studied for its chemopreventive potential against rat liver carcinogenesis induced by DENA. Treatment with bacoside A demonstrated the delayed development and growth of neoplastic nodules. The levels of AFP, carcinoembryonic antigen (CEA), aspartate transaminase (AST), alanine transaminase (ALT), ROS, lipid peroxides, GSH antioxidant enzymes, SOD and CAT, vitamins A and E and MMP-2 and -9 were decreased in the bacoside A-treated rats[141,142].

Two groups of researchers have investigated the chemopreventive effects of cucurbitacin B using xenografted mouse models of liver cancer. Zhang *et al*[83] showed the decrease in tumor volume along with tumor growth inhibition exerted by cucurbitacin B in mice xenografted with HepG2 cells, whereas Chan *et al*[84] demonstrated the inhibitory activity of cucurbitacin B on the growth of xenografted BEL-7402 tumor. Nevertheless, the *in vivo* mechanisms of such antitumor effects of cucurbitacin B were not studied.

Escin, a natural mixture of triterpene saponins, was evaluated by Zhou *et al*[88] to determine its antitumor efficacy against mice xenografted with H22 malignant HCC cells. Therapy with escin at a concentration of 1.4 mg/kg per day or 2.8 mg/kg per day for 7 d was conducted *via* ip injections in these animals. The highest dose of escin therapy was effective in significantly lowering the tumor weights compared to the controls.

Korean ginseng is one of the most widely used medicinal plants, particularly in traditional oriental medicine, and has a wide range of pharmacological and physiological actions[143,144]. These along with other beneficial effects prompted Abdel-Wahhab *et al*[145] to evaluate the chemopreventive effects of ginseng extract (GE) against precancerous lesions in female Sprague-Dawley rats treated with aflatoxin B_1 (AFB_1) and fumonisin. A dose of 150 mg/kg of GE for 12 wk inhibited hepatocarcinogenesis in these rats and decreased the levels of ALT, AST, CEA, malondialdehyde, and AFP with concurrent increases in serum albumin and high density lipoprotein levels compared to the positive controls. This study concluded that GE administration before or after treatment with mycotoxins could be effective in preventing hepatocellular carcinogenesis.

Shiota *et al*[146] earn the credit as the first group of researchers to have used glycyrrhizin as a chemopreventive agent against HCC in an experimental animal study. Their work demonstrated that glycyrrhizin decreased nodule incidence and HCC induced by DENA. Serum albumin and AST, markers of liver function, were found to be normalized following glycyrrhizin treatment. A later study conducted by Wan *et al*[147] evaluated the hepatoprotective and antihepatocarcinogenic effects of glycyrrhizin. This study reported the reduction in size, volume and number of hepatic lesions initiated by DENA and promoted with 2-AAF in male Sprague Dawley rats.

In contrary to all the above studies on triterpenes, the study conducted by Karim *et al*[148] on two fernane-type triterpenes, namely EC-2 and EC-4, did not observe any modifying effects on DENA-induced liver cancer in rats. No significant differences were observed in the number and area/cm^2 of GSTp-positive foci induced by DENA between the treatment and control groups. Neither was there any treatment-related variation in cell proliferation as demonstrated by 5-bromo-2'-deoxyuridine (BrdU) labeling.

Squalene, a triterpenoid present in olive oil, also failed to exhibit chemopreventive activities against DENA/2-AAF rat hepatocarcinogenesis model. Squalene induced an increase in levels of plasma cholesterol in the treated rats leading to hypercholesterolemia[149].

The *in vivo* chemotherapeutic efficacy of UA was first demonstrated by Tian *et al*[109] who used CD-1 mice implanted with H22 hepatoma cell lines. Results showed an inhibition in the growth of hepatoma in UA-treated mice. The drawback on using UA as a potential anti-hepatoma agent, as presented in this study, was the poor water solubility of UA which confines its potential use. A later study evaluated UA's efficacy in DENA-initiated and PB-promoted hepatocarcinogenesis rats. Oral administration of UA at 20 mg/kg per day for 6 wk suppressed hepatocellular carcinogenesis and decreased lipid peroxidation and protein carbonyls by about 52%, along with reversing the membrane damage and elevated glycoprotein levels[150].

Athymic nude BALB/c nu/nu mice implanted with BEL-7402 cells was used to generate the HCC model by Zhang *et al*[111] in order to study the chemopreventive characteristics exhibited by waltonitone, a new ursane-type pentacyclic triterpene isolated from *Gentian waltonii* Burkill. Results of this study showed a delay in tumor growth and inhibition of tumor weight in waltonitone-treated mice with HCC compared to the control. Western blotting analysis of tumor tissue indicated an increase in expression of proapoptotic proteins, such as cleaved caspase-3 and caspase-9, suggesting the involvement of the mitochondrial apoptotic pathway in the chemopreventive effect of waltitonine.

Tetraterpenes: Tetraterpenes represent one of the most widely studied classes of terpenes for the chemopreventive and therapeutic treatment potential for HCC. Murakoshi *et al*[151] described a study evaluating α-carotene's chemopreventive effects against spontaneous liver carcinogenesis. Their study showed that α-carotene, obtained from palm oil, significantly decreased the number of hepatomas. α-carotene's chemopreventive capability was confirmed by Tsuda *et al*[152] when they tested its effects (0.4 mg/animal) in both male and female B6C3F mice injected intraperitoneally with DENA and *N*-methyl-*N*-nitrosourea to produce a multiorgan carcinogenesis model. The results demonstrated that α-carotene reduced the number of liver tumors in both sexes.

Astorg *et al*[153,154] demonstrated in two studies that β-carotene failed to exhibit any chemopreventive activity against a variety of chemically-induced hepatocarcinogenesis models in rats. This includes absence of effects on the number and size of preneoplastic foci in male Wistar rats that were induced with DENA/2-AAF, DENA or 2-nitropropane (2-NP) to develop preneoplastic liver foci. A similar study, conducted by Tharappel *et al*[155] using the DENA hepatocarcinogenesis model in female Wistar rats, showed that β-carotene treatment for 12 wk failed to produce any modifying effect, reiterating the results obtained by the previous investigators. Contrary to these studies, Moreno *et al*[156], have provided substantial evidence, over two decades, on the chemopreventive as well as chemo-

therapeutic efficacies of β-carotene. In one of the early studies, a dose of 70 mg/kg of β-carotene for 2-8 wk in the resistant hepatocyte model significantly reduced the incidence, multiplicity as well as the total number and size of hepatocyte nodules. β-carotene also attenuated the number of foci, average focal area and percentage of liver parenchyma occupied by these foci. These inhibitory effects were primarily exerted in the initiation phase of the hepatocarcinogenic process[156]. In another study, chronic administration of β-carotene throughout the study resulted in a drastic reduction in hepatocyte nodule incidence, total number of nodules and nodule multiplicity as well as the number and size of GGT-positive foci in DENA-initiated and 2-AAF-promoted hepatocarcinogenesis in rats[157]. A similar study described decreases in the incidence and total number of hepatocyte nodules and GGT-positive foci upon treatment with β-carotene during early promotional phase of DENA-initiated 2-AAF-promoted hepatocarcinogenesis in rats. Additionally, this study showed remodeling of persistent hepatic lesions as a mechanism by which β-carotene exhibited its chemopreventive activity[158]. Along with the initiated hepatocytes, another group of cells known as "oval cells", proliferate in the liver during rat and mouse hepatocarcinogenesis[159]. Dagli *et al*[160] showed that β-carotene was responsible for attenuating the development of GSTp foci along with decreasing oval cell reaction in Wistar rats submitted to the resistant hepatocyte model of carcinogenesis. Moreno *et al*[161] reported that β-carotene treatment during progression of chemically-induced liver cancer exhibited a lower of incidence of neoplastic lesions with reduction in hepatic BrdU labeling indexes indicating an inhibitory action of β-carotene on cell proliferation. β-Carotene's chemopreventive activity was further explored during the initial phases of DENA-induced hepatocarcinogenesis in rats, with results showing a decrease in cell proliferation and DNA damage in conjunction with remodeling of GSTp-positive lesions as likely mechanisms of action[162]. In a study with the 1, 2-dimethylhydrazine-induced hepatocarcinogenesis rat model, β-carotene in conjunction with anticancer drug, 5-azacytidine[163]. Conferred only weak chemoprotective effects. Studies by Sarkar and associates[164-167] using three different chemically-induced hepatocarcinogenesis models added extensively to the existing knowledge on mechanism-based chemopreventive properties of β-carotene. A treatment dose of β-carotene at 100-120 mg/kg per day from 4 to 20 wk in all these studies notably decreased nodulogenesis and hepatic chromosomal aberrations in male Sprague Dawley rats. This group of researchers provided extensive evidence of the antioxidant effects of β-carotene as the underlying mechanism of action. Decreases in hepatic DNA strand breaks, frequency of chromosomal aberrations, red blood cell protein damage and lipid peroxidation were observed. There were also decreases in hepatic GSH, GGT, GST, GPX, GR, SOD and CAT. Increased cytochrome b_5, vitamin A and glucose-6-phosphatase (G6Pase) levels also appeared as potential mechanisms by which β-carotene exhibited its chemopreventive activity. Bishayee *et al*[168] reported β-carotene to have a superior chemopreventive action to that of retinoic acid. As described in this study, β-carotene greatly reduced the incidence and multiplicity of nodules in DENA/PB hepatocarcinogenesis in rats with decreased levels of GSH, GGT, GPX and increased levels of cytochrome P-450 (CYP) and GST. Several other studies also demonstrated the chemopreventive efficacy exerted by β-carotene based on reduced number and size of GSTp foci. Interestingly, all these studies showed positive results in several rat hepatocarcinogenesis model induced by IQ, ethynlyestradiol or GLU-P-1[140,169,170]. Inhibition in the formation of IQ-DNA adducts with decreases in CYPA1A2 protein expressions was a characteristic effect of β-carotene treatment in male F344 rats challenged with IQ[171]. Utilizing a multiorgan carcinogenesis model in B6C3F1 mice, Tsuda *et al*[152] were successful in showing a reduction in the number of liver tumors with β-carotene treatment. However, the results of this study were only significant in male B6C31 mice and not in the female mice model. Gradelet *et al*[172,173] investigated the chemopreventive effect of dietary β-carotene during initiation of AFB_1-induced hepatocarcinogenesis in male Wistar rats. They demonstrated a reduction in hepatocarcinogenesis as evidenced from a decrease in number and volume of GSTp foci possibly due to deactivation of AFB_1 metabolism towards detoxification pathways. Results of an interesting study on toad liver carcinogenesis suggested β-carotene inhibition in liver tumor formation induced by 7, 12, dimethylbenz(a)anthracene in female toads[174]. Chattopadhyay *et al*[175,176] conducted two similar studies that involved β-carotene treatment in male Sprague-Dawley rats initiated with DENA. The authors effectively proved β-carotene's chemopreventive capability as indicated by suppression in the formation of GSTp foci along with reversal of hepatic histomorphological changes in DENA-initiated animals[175]. Mechanistic studies revealed that dietary supplementation of β-carotene decreased the levels of hepatic GSH, GST and DNA single strand breaks[175,176]. The latter study also investigated various mineral levels following β-carotene treatment and found that levels of calcium, copper and zinc were increased while levels of manganese, iron, potassium and selenium appeared to have decreased in the blood of experimental animals[176]. Another interesting observation from these studies was that anticarcinogenic micronutrient vanadium[177] was found to augment the effects of β-carotene[175,176].

Studies have been also been performed with various carotenoid triterpenes, such as β-apo-8'carotenal, astaxanthin and canthaxanthin, to determine their possible chemopreventive efficacies against liver cancer. However, only two studies were successful in achieving any beneficial effects exerted by these carotenoids. Astorg *et al*[153,154] conducted two studies with canthaxanthin and astaxanthin, and did not observe any preventive effects exerted by them against chemically-induced hepatocarcinogenesis

in male Wistar rats. A dietary treatment regimen of either carotenoid at 300 mg/kg for 3 wk did not modify the number and size of GGT or GSTp foci in rats subjected to DENA or 2-NP hepatocarcinogenic regimen. On the contrary, two other studies[172,173] demonstrated chemopreventive effects of dietary β-apo-8'carotenal, astaxanthin and canthaxanthin against AFB_1-initiated hepatocarcinogenesis in rats as evidenced by a reduction in the number and size of GSTp-positive preneoplastic foci. An improved detoxification of AFB1 metabolism has been proposed as the basis of the observed protective effects of these carotenoids against rat liver carcinogenesis.

Dietary isoprenic derivatives, including β-ionone, a cyclic isoprenoid present in grapes and wine, represent a promising class of chemopreventive agents[178-180]. β-Ionone was found to inhibit hepatic preneoplastic lesions with a decrease in cell proliferation, inhibition of plasma cholesterol and amelioration of DNA damage during the initial phases of hepatocarcinogenesis initiated with DENA and promoted by 2-AAF in rats[140]. A later study conducted using a similar hepatocarcinogenesis model documented the antihepatocarcinogenic effects of β-ionone during the promotional phase with a concurrent inhibition of cell proliferation and modulation of HMGCoA reductase[130]. These two studies warrant further investigations into the chemopreventive efficacies of β-ionone against liver cancer.

The carotenoid lutein was successfully shown to be an effective chemopreventive agent against DENA-initiated and 2-AAF promoted hepatocarcinogenesis by two studies in Wistar rats. Lutein when administered at 70 mg/kg per day for 8 wk played an important role in reducing the number and size of GSTp foci with a concurrent decrease in DNA damage[181]. Moreno *et al*[182] however showed that lutein presented its inhibitory actions during promotional stage but not during the initiation phase of hepatocarcinogenesis. During the initiation phase lutein did not inhibit nor induce hepatic preneoplastic lesions or DNA damage. However, treatment during the promotional phase inhibited the size of nodules with reduced DNA damage. Accordingly, lutein has been classified as a suppressing agent.

Another carotenoid studied with great expectations for its chemopreventive potential is lycopene. While several laboratories reported potential beneficial effects, others demonstrated the lack of effects exerted by lycopene. Studies conducted by Gradelet *et al*[172,173] showed that dietary lycopene treatment failed to show any modifying effects, either on the initiation or on the promotional phases of hepatocarcinogenesis induced by AFB_1 in male Wistar rats. Similar results were reported by Watanabe *et al*[183] indicating that lycopene failed to produce any chemopreventive effect against spontaneous HCC in Long-Evans Cinnamon rats. Few other studies conducted with different treatment regimens of lycopene arrived at conclusions that were contrary to the aforementioned studies. Astorg *et al*[154] showed significant decreases in GSTp foci including decrease in liver volume occupied by the foci. They also concluded that lycopene's chemopreventive effects were due to its modulating effect on the liver enzyme activating DENA, namely CYP2E1. Three other research groups[155,181,184] conducted their studies using several chemical hepatocarcinogenesis model and observed similar end points as Astorg *et al*[154]. All three research groups observed the chemopreventive effects of lycopene on hepatocellular carcinogenesis induced by DENA in rats as indicated by inhibition of GSTp foci. Wang *et al*[184] confirmed the efficacy of lycopene against nonalcoholic steatohepatitis (NASH)-promoted hepatocarcinogenesis and provided several molecular mechanisms, such as lowering of protein expression of proliferating cell nuclear antigen, cyclin D1, and NF-κB as well as induction in nuclear NF-E2 related factor-2 and heme oxygenase-1 protein expressions. The efficacy of tomato extract (TE) as a chemopreventive agent against DENA-initiated NASH-promoted hepatocarcinogenesis in male rats was also investigated. TE supplemented in the diet at a concentration of 250 mg/kg per day for 6 wk produced similar inhibitory effects to lycopene on the development of GSTp foci. Ancillary studies showed decreases in CYP2E1, inflammatory foci and mRNA expression of proinflammatory cytokines, namely TNF-α, IL-1β and IL-12.

Sesquiterpenes: The chemotherapeutic effects of DHE, a medicinal plant-derived sesquiterpene lactone, have been investigated in male nude mice subcutaneously (sc) injected with PLC/PRF/5 human liver cancer cells. DHE treatment (10 mg/kg per day carried out for 45 d) elicited a significant decrease in tumor volume. Tumor samples analyzed from DHE-treated animals displayed TUNEL-positive cells indicative of apoptosis within the tumor. ER-stress related proteins, namely inositol-requiring protein-1 and phospho-PKR-like ER kinase, were found to be increased in DHE-treated animals[124].

Ong *et al*[129] studied the chemopreventive effects of the sesquiterpene farnesol using Wistar rats submitted to the RH model of hepatocarcinogenesis. Farnesol inhibited the incidence and mean number of visible hepatocyte nodules, as well as the size of total, persistent and remodeling GSTp-positive preneoplastic lesions. Farnesol was also found to inhibit tumor cell proliferation and HMG-CoA reductase activity, thus reducing synthesis of cholesterol and intermediates of the mevalonate pathway, such as farnesyl pyrophosphates[185]. The study also highlighted that the chemopreventive effects exerted by farnesol did not involve the farnesoid X activated receptor.

ZER, a monosesquiterpene, was assessed for its chemopreventive properties against DENA-initiated and 2-AAF-promoted liver carcinogenesis in rats. ZER was administered (ip) for at 15-60 mg/kg body weight for 11 wk. Histopathological observation showed the antihepatocarcinogenic effects of ZER. Serum levels of ALT, AST, AP and AFP were found to be lower in ZER-treated rats. ZER treatment also reduced oxidative stress and proliferation accompanied with apoptosis. An increase in Bax and decrease in Bcl-2 protein expression have

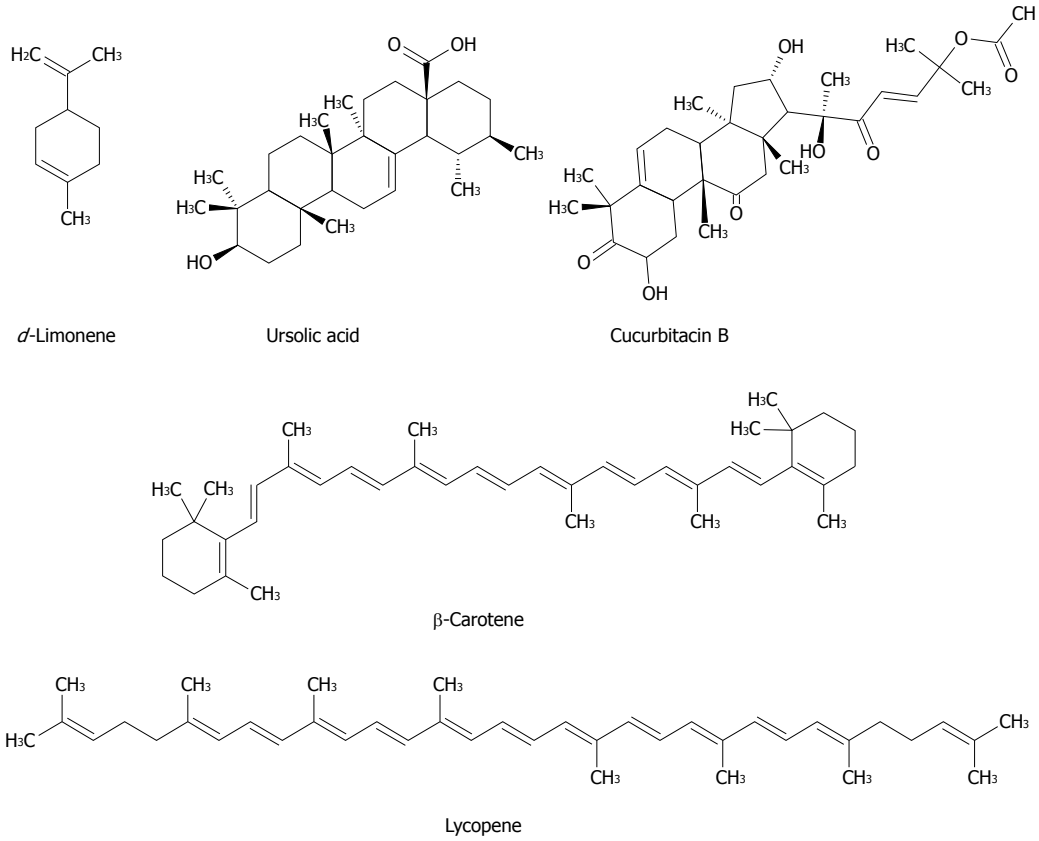

Figure 1 Chemical structure of selected natural terpenoids with potential for use as a chemopreventive and therapeutic drug for liver cancer.

been postulated as the mechanistic basis of proapoptotic properties of ZER during experimental hepatocarcinogenesis[186].

CONCLUSION

Liver cancer remains the third leading cause of cancer mortality throughout the world with few therapeutic options to combat its prevalance. Natural compounds obtained from different sources such as fruits, vegetables, herbs and even fungi, open up a novel and exciting prospective on liver cancer prevention as well as treatment. Among the various secondary metabolites produced by plants and other organisms, terpenoids have emerged as a promising group of phytochemicals. Terpenoids selectively kill liver cancer cells with a pleiotropic mode of action while sparing normal cells. The various studies, conducted *in vitro* and *in vivo* by numerous research groups, describe the use of these phytoconstituents as potential chemopreventive and therapeutic agents in the fight against liver cancer. Based on available data, the terpenoids which may have the greatest therapeutic potential for liver cancer prevention and treatment include *d*-limonene, cucurbitacin B, ursolic acid, β-carotene and lycopene (Figure 1).

Terpenoids have not only been shown to exert biological activities that are a far cry from any single chemotherapeutic drug but have also exhibited little or no toxicity. These phytochemicals have been found to induce a wide spectrum of activities such as reduction in oxidative stress, suppression of inflammation, induction of apoptosis, regulation of cell cycle, inhibition of cell proliferation and also modulation of multiple signal transduction pathways. In essence, these pleiotropic mechanisms could explain their antineoplastic properties against liver cancer.

From the studies discussed here, it is apparent that terpenoids of various classes are unique as they have characteristic mechanisms of action. However, in most of the studies, individual compounds have been tested against *in vitro* or *in vivo* liver cancer models. There remains the indefinite yet unending possibility that there exists better preventive or therapeutic potential in the synergistic action of these terpenoid molecules. Emerging studies have shown that a combination of phytochemicals may be more effective against cancer than individual components[187-191]. Further studies are needed to explore the full potential of these multifunctional terpenoids in the combinational setting.

Possible combinations of two or more compounds could prove to be much more effective, in many cases, where use of a single agent is not effective enough. However, further studies are needed in order to provide more insight into this assumption. It is well known that the combination of several chemotherapeutic drugs offers the possibility of lowering doses of individual compounds, consequently reducing unwanted adverse effects. Considering these advantages, terpenoids may be used in combination with other chemotherapeutic drugs and

radiation therapy to enhance their therapeutic efficacy while limiting chemo- and radio-therapy-associated unwanted side effects. However, more studies are required to validate these premises.

Scanning of pertinent and extensive literature reveals a large number of *in vitro* studies demonstrating the cytotoxicity of terpenoid molecules against various liver cancer cells, yet very few compounds have been evaluated in preclinical animal models of liver cancer. The same follows suit for those natural terpenoid compounds effectively tested in animal models moving up to phase I clinical trials. One of the reasons for the limited number of preclinical liver cancer studies on triterpenoids, including lack of *in vivo* studies on agents which have already showed efficacy in cell culture systems, could be that most terpenoids are insoluble in aqueous media limiting their bioavailability in the body, an important aspect for *in vivo* efficacy. One approach to enhance the water solubility of triterpenoids could be the structural modification of naturally occurring compounds to generate more water-soluble analogs. Several other possibilities of improving the hydrophilicity of triterpenoids include the design and generation of formulations containing cyclodextrin complexes, liposomes, colloids, micelles as well as nanoparticles[192-194].

As highlighted in this review, a large number of investigations have unraveled many unique biological properties of terpenoids with the goal of evaluating their clinical potential in the prevention and therapy of liver cancer. Nevertheless, a considerable amount of work remains to be done, such as identification of novel target proteins and intercellular pathways in which they function, and development of selective end-points and surrogate biomarkers for evaluating efficacy. Long-standing epidemiological studies and well-designed clinical trials are also necessary. In conclusion, substantial experimental results from *in vitro* and *in vivo* studies presented in this review suggest that terpenoids are novel candidates in the chemopreventive and chemotherapeutic strategies to combat liver cancer.

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BRIEF ARTICLE

Binge ethanol intake in chronically exposed rat liver decreases LDL-receptor and increases angiotensinogen gene expression

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Abstract

AIM: To investigated the status of low-density lipoprotein (LDL)-receptor and angiotensionogen gene expression in rats treated chronically with ethanol followed by binge administration, a model that mimics the human scenario.

METHODS: Rats were chronically treated with ethanol in liquid diet for 4 wk followed by a single binge mode of ethanol administration (5 mg/kg body weight). Samples were processed 4 h after binge ethanol administration (chronic ethanol binge). Control rats were fed isocaloric diet. In the control for binge, ethanol was replaced by water. Expression of mRNA for angiotensinogen, c-fos and LDL-receptor, and nuclear accumulation of phospho-extracellular regulated kinases (ERK)1/2 and ERK1/2 protein were examined.

RESULTS: Binge ethanol administration in chronically treated rats caused increase in steatosis and necrosis. Chronic ethanol alone had negligible effect on mRNA levels of LDL-receptor, or on the levels of nuclear ERK1/2 and phospho-ERK1/2. But, chronic ethanol followed by binge caused a decrease in LDL-receptor mRNA, and also decreased the levels of ERK1/2 and phospho-ERK1/2 in the nuclear compartment. On the other hand, chronic ethanol-binge increased mRNA expression of angiotensinogen and c-fos.

CONCLUSION: Binge ethanol after chronic exposure, causes transcriptional dysregulation of LDL-receptor and angiotensinogen genes, both cardiovascular risk factors.

Key words: Alcoholic liver injury; Angiotensinogen; Ethanol binge; Extracellular regulated kinases1/2; Low-density lipoproteun-receptor; Plasminogen activator inhibitor-1

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INTRODUCTION

A number of epidemiological studies have indicated that moderate alcohol use protects the individual from coronary heart disease[1,2]. In contrast to this cardioprotection, overconsumption or heavy drinking of alcohol is closely correlated with cardiovascular diseases such as hypertension, hemorrhagic and thrombotic stroke, cardiac arrhythmias, cardiomyopathy and acute coronary syndrome[3-6]. A common factor in the background for progression of liver damage and vascular injury in humans is heavy ethanol binge superimposed on chronic ethanol intake[7-9]. Notably, binge drinking is on the rise worldwide[10-12]. Two contributing factors related to cardiovascular risk in humans consuming heavy amounts of alcohol are increase in plasma low-density lipoprotein (LDL)-cholesterol[13], and increase in plasma plasminogen activator inhibitor

(PAI)-1 levels[3,14]. In this regard, decreased expression of hepatic LDL-receptor[15], and increased expression of hepatic PAI-1[16], have been reported in animal models of alcoholic liver injury. We have recently reported a clinically relevant animal model of chronic ethanol binge that manifests exaggerated liver injury, activation of extracellular regulated kinases (ERK)1/2 and increased expression of PAI-1[17]. Activation of ERK1/2 is one of the signaling cascades that results in increased expression of LDL-receptor in hepatocytes[15], but the relationship of ERK1/2 activation to LDL-receptor expression after chronic ethanol binge has not been examined. Although, increased tumor necrosis factor (TNF)-α is one of the factors that can contribute to increased expression of hepatic PAI-1, TNF-α was not increased in our model, suggesting that other factors contribute to PAI-1 increase. In this regard, angiotensin Ⅱ has been shown to induce expression of PAI-1 *in vitro* and *in vivo*[18,19]. To gain molecular insight into the effects of ethanol binge in liver, we determined the effects of binge ethanol in rats treated chronically (4 wk) with ethanol, on the expression of angiotensinogen and LDL-receptor genes and the status of ERK1/2 activation.

MATERIALS AND METHODS

Materials

Male Sprague-Dawley rats, each weighing between 250-300 mg were purchased from Harlan Laboratories (Indianapolis, IN) for chronic ethanol treatment. The antibodies for phospho-ERK1/2, ERK1/2 protein were purchased from Cell Signaling (Beverly, MA). The other reagents including protease inhibitors cocktail (Sigma p8340) and anti β-actin antibody were obtained from the Sigma-Aldrich (St. Louis, MO).

Animal feeding for chronic ethanol-binge model of alcoholic liver injury

Male Sprague-Dawley rats, each initially weighing 300 g, were housed under a 12-h/12-h light/dark cycle and were permitted ad libitum consumption of standard laboratory rat chow. After a 1-wk equilibration period, the animals were fed Lieber-DeCarli liquid diet (Dyets, Inc., Bethlehem, PA)[20]. Ethanol was progressively introduced into the liquid diet starting at 1.25% (w/v) for day 1, increased to 1.67% (w/v) for day 2 and to 2.5% (w/v) for days 3 and 4, and, finally, maintained at 5% (w/v) for 4 wk. Weight-matched littermates were pair-fed on the same liquid diet, except the ethanol was replaced by dextrin/maltose (control) to maintain the isocaloric intake in the two groups. Each day, the previous day's intake was measured, and the pair-fed rat was fed same calorie of dextrin/maltose. After 4 wk, rats were divided into four groups: control, chronic ethanol, control water, chronic ethanol binge. The chronic ethanol binge group had single binge intragastric administration of ethanol (5 mg/kg, body weight) for 4 h. In the control group for chronic ethanol binge, ethanol was replaced by water (control water). The animal care and protocol for their use were approved by the University of Missouri Animal Care and Use Committee.

Preparation of whole liver extracts, nuclear extracts and immunoblotting

Frozen liver was homogenized with hypotonic buffer containing 10 mmol/L HEPES, pH 7.4, 10 mmol/L β-glycerophosphate, 0.5 mmol/L EDTA, 0.5 mmol/L EGTA, 10 mmol/L sodium fluoride, 2.5 mmol/L sodium pyrophosphate 1 mmol/L Na-orthovanadate, 1.5 mmol/L $MgCl_2$, 1 mmol/L dithiothreitol (DTT), Sigma p8340 protease inhibitor cocktail and 10% glycerol. The proteins in the homogenate were extracted and denatured by adding SDS to 1%. After boiling for 5 min, the homogenate was sonicated for 5 s and centrifuged at 12000 g for 10 min. The supernatant was used for protein assay and western blotting. Protein concentration was measured using the Bio-Rad DC protein assay kit. The nuclear extracts were obtained following our previously published protocols[21,22]. The total liver extracts and nuclear fractions (60 to 80 μg protein) were combined with equal volume of 2 × Laemelli buffer and fractionated on 10% polyacrylamide gels and immunoblotting was performed as described earlier[22]. Equal loading of protein was confirmed by determining β-actin levels for whole cell extracts and histone H3 protein levels for nuclear extracts. Levels of β-actin or histone H3 did not change after chronic ethanol or binge treatments.

Histopathology and determination of alanine amino transfease

For light microscopy, formalin fixed specimens were sectioned and stained with hematoxylin and eosin. Serum alanine amino transfease (ALT) was determined in an autoanalyzer by kinetic assay.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from the livers using the TRIzol reagent (Invitrogen) followed by DNase I treatment and clean up in Qiagen RNeasy midi kit (Qiagen). First strand cDNA was synthesized from one microgram of total RNA using the cDNA synthesis kit (Applied Biosystems). Reverse transcriptase-polymerase chain reaction (RT-PCR) reaction was performed using SYBR green supemix from Biorad using primers as shown in Table 1. Thermal cycling conditions were 95℃ for 3 min as initial denaturation and enzyme-activating step followed by 40 cycles of 95℃ for 15 s denaturation, and 57℃ for 1 min annealing and extension. After amplification, a melting curve analysis was performed by increasing the temperature by 0.5℃ increments from 55℃ to 95℃ and measuring fluorescence at each temperature for a period of 10 s. All cDNA samples were analyzed in triplicate and each run contained a relative standard curve. The expression of each gene was normalized to GAPDH and calculated to relative pair fed control using comparative cycle threshold method[23].

Statistical analysis

All results are expressed as mean ± SD and were obtained by combining data from individual experiments.

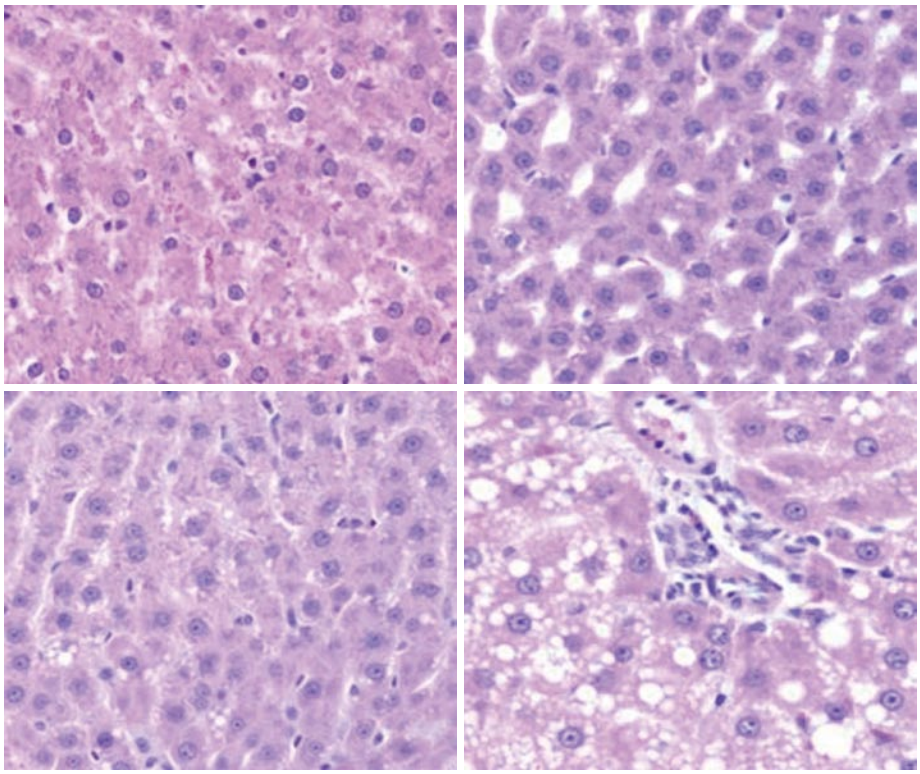

Figure 1 Histology of chronic and chronic ethanol-single binge liver samples. Rats were fed ethanol in liquid diet chronically for 4 wk and then given a single ethanol binge dose (5 mg/kg, 4 h). Sections of liver samples were stained with hematoxylin and eosin. Control -water represents pair-fed animals given water for binge control.

Table 1 Primers used for real time polymerase chain reaction

GAPDH
Forward: 5'-AGACAGCCGCATCTTCTTGT-3'
Reverse: 5'-CTTGCCGTGGGTTAGAGTCAT-3'
LDL-receptor
Forward: 5'-TTCTTCAGGTTGGGGATCAG-3'
Reverse: 5'-CAGCTCTGTGTGAACCTGGA-3'
Angiotensinogen
Forward: 5'-GAGGCAAGAGGTGTAGCCAG-3'
Reverse: 5'-GCAGTCTCCCTCCTTCACAG-3'
c-fos
Forward: 5'-GCGGACTACGAGGCGTCAT-3'
Reverse: 5'-GGAGGAGACCAGAGT-3'

Statistical analyses were made using the Student *t* test (two-tailed, paired, and unpaired). Differences with a *P* value of < 0.05 were considered significant.

RESULTS

Augmentation of ethanol binge induced injury after chronic ethanol intake

Administration of ethanol for 4 wk is known to result in mild steatosis and moderate increase in ALT. However, administration of a single ethanol binge caused a dramatic increase in steatosis (Figure 1). Ethanol binge also caused significant increase in ALT (2.2 fold increase, $P < 0.05$) compared to a moderate increase in chronic ethanol treated rats (1.4 fold, $P > 0.05$). Thus, chronic ethanol exposure increased the susceptibility of liver to binge-induced injury.

Decreased expression of LDL-receptor and decreased accumulation of ERK1/2 in the nucleus after chronic ethanol binge

The effect of chronic ethanol and ethanol binge on changes in LDL-receptor gene expression is shown in Figure 2. Although mRNA levels of LDL- receptor were not much affected by chronic ethanol, its levels significantly decreased after binge ethanol treatment. Activation of ERK1/2 is one of the important mechanisms for the expression of LDL receptor in hepatocytes[15,24]. A recent study showed expression of LDL receptor and activation of ERK1/2, were both decreased in chronic ethanol treated rats[15]. This finding is different from the chronic ethanol and chronic ethanol binge group in our earlier study[17]. In the current study, we found increased activation of ERK1/2 after chronic ethanol binge. We next determined the nuclear levels of phosphorylated ERK1/2, and ERK1/2 protein in liver from control and ethanol treated rats. In chronic ethanol treated rats, the nuclear levels of phospho-ERK1/2 were not significantly different from control rats whereas they were significantly lower in chronic ethanol binge group (Figure 3). The decrease in the levels of phospho-ERK1/2 was accompanied by decreased levels of ERK1/2 protein in nuclear extracts after chronic ethanol binge. These results suggest impaired translocation of activated ERK1/2 to the nuclear compartment after chronic ethanol-binge.

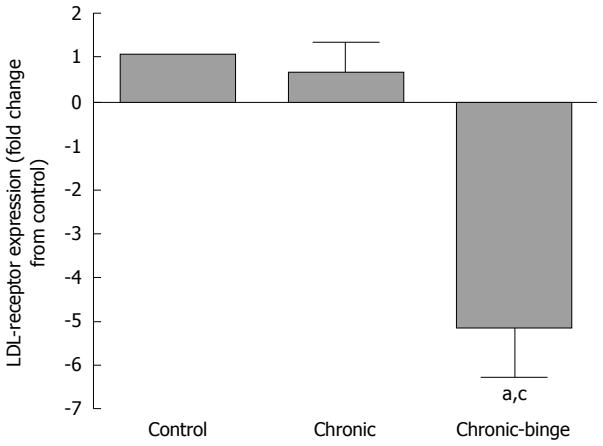

Figure 2 Low-density lipoprotein-receptor mRNA expression in chronic and chronic-binge treated rats. After 4 wk of chronic ethanol feeding, binge was administered as in Figure 1. Total RNA was isolated from liver and reverse transcribed to cDNA. Aliquots of the cDNA preparations were amplified by real time QT-PCR. The fold increase in gene expression was determined after normalizing the differences in level of GAPDH mRNA. Values are mean ± SD (n = 4). $^{a}P < 0.05$ *vs* control; $^{c}P < 0.05$ *vs* chronic ethanol group.

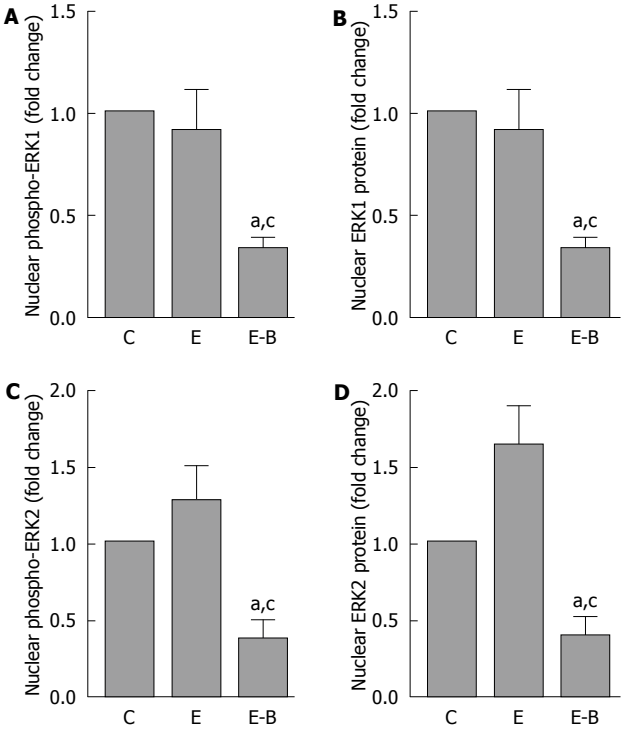

Figure 3 Levels of phosphorylated ERK1/2 (A/C) and ERK1/2 (B/D) protein in nuclear extracts in chronic and chronic-binge treated rats. The chronic ethanol feeding (4 wk) and binge (single) treatment was as in Figure 1. The nuclear extracts from liver were subjected to western blotting with respective antibodies, followed by densitometry of bands (see methods). Values are mean ± SD (n = 4). $^{a}P < 0.05$ *vs* control; $^{c}P < 0.05$ *vs* chronic ethanol group. C: Control (pair fed); E: Chronic ethanol; E-B: Chronic-ethanol binge.

Increased expression of angiotensionogen and c-fos expression

We have previously shown that PAI-1 gene expression is increased after chronic ethanol binge[17]. TNF-α is one of the cytokine that has been implicated in the up regulation of PAI-1 expression[16]. However, we did not find

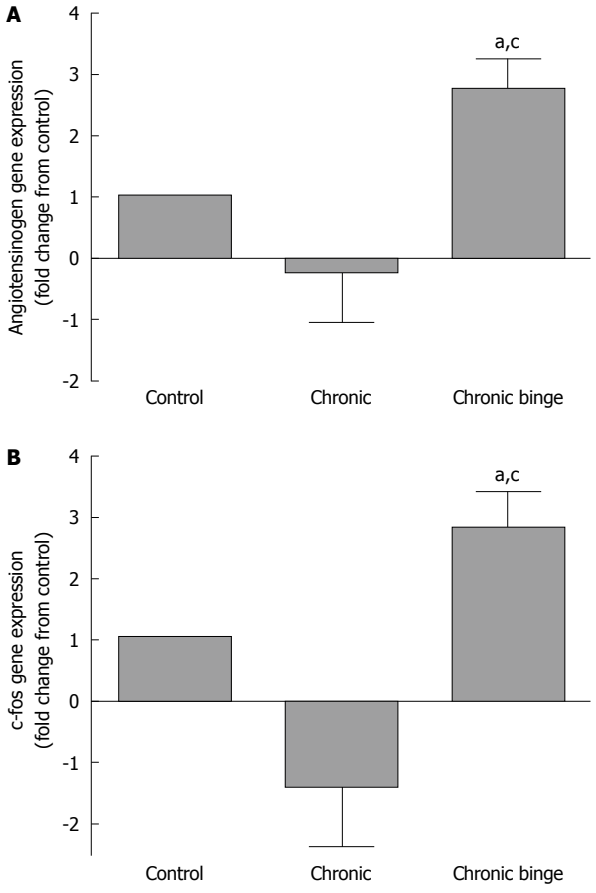

Figure 4 Angiotensionogen (A) and c-fos mRNA (B) expression in chronic and chronic-binge treated rats. After 4 wk of chronic ethanol feeding, single binge was administered as in Figure 1. Total RNA was isolated from liver and reverse transcribed to cDNA. Aliquots of the cDNA preparations were amplified by real time QT-PCR. The fold increase in gene expression was determined after normalizing the differences in level of GAPDH mRNA. Values are mean ± SD (n = 4). $^{a}P < 0.05$ *vs* control; $^{c}P < 0.05$ *vs* chronic ethanol group.

increased expression of TNF-α after ethanol binge[17]. PAI-1 gene expression has been shown to be induced by angiotensin Ⅱ[18,19], and angiotensin Ⅱ levels in plasma are increased by ethanol binge after chronic ethanol consumption in humans[25], but no such studies have been done in animal models of alcoholic liver injury. Therefore, we determined the level of angiotensinogen gene expression in liver samples. Angiotensinogen gene expression was not altered after chronic ethanol treatment, whereas its expression was significantly increased after chronic ethanol-binge treatment (Figure 4). In this regard, c-fos, is one of the transcription factors regulating PAI-1 expression, and angiotensin Ⅱ has been shown to stimulate c-fos expression in hepatocytes *in vitro*[26]. Although the expression of c-fos was not altered by chronic ethanol treatment, its expression was significantly increased by binge (Figure 4) and the pattern of changes in c-fos expression was similar to that of angiotensionogen.

DISCUSSION

This is the first report demonstrating changes in two important factors, known to contribute to cardiovascular risk

associated with heavy ethanol consumption in humans, in a clinically relevant chronic ethanol-binge rat model. Chronic ethanol-binge was characterized by decreased expression of LDL-receptor and increased expression of angiotensionogen gene. Decreased expression of hepatic LDL-receptor in humans is associated with increased plasma LDL-cholesterol levels and heavy alcohol consumption is associated with increased plasma LDL-cholesterol levels[13]. ERK1/2 activation is reported to be involved in the induction of LDL-receptor expression in hepatocytes *in vitro*[15,24]. We have recently reported activation of ERK1/2 by chronic ethanol binge[17], but observed a decrease in LDL-receptor expression (Figure 2). In order to address these apparently contradictory findings, we have examined the nuclear levels of phosphorylated ERK1/2 in the chronic ethanol binge group and have found an impaired accumulation of nuclear phospho-ERK1/2 and ERK1/2 protein. Decreased expression of LDL receptor by inhibition of mitogen-activated protein kinase (MAPK) signaling in HepG2 cells under basal conditions[15], or after agonist stimulation[24], coupled with decreased nuclear ERK1/2 as reported here, implies dysregulation of compartmentalization of MAPK signaling by chronic ethanol binge. Although, the mechanism underlying impaired accumulation is not known at present, exaggerated oxidative stress may be one of the determining factors for impaired translocation of ERK1/2 to the nucleus. In this regard, hydrogen peroxide has been shown to cause impaired accumulation of ERK1/2 in cultured rat hepatocytes[27], and chronic ethanol administration followed by intraperiotoneal administration of ethanol has been shown to exaggerate oxidative stress in the liver[28].

Increased plasma PAI-levels were more correlated to stetaosis in humans than adipose tissue fat accumulation, suggesting liver is one of the major sources for circulating PAI-1 levels[29]. Increased levels of circulating PAI-1 have been reported after heavy ethanol binge in people with prolonged ethanol consumption[3,5]. Angiotensin Ⅱ is one of the agonists known to stimulate the expression of angiotensinogen gene in the liver, and plasma levels of angiotensin Ⅱ are correlated to hepatic angiotensinogen expression[19]. Plasma angiotensin Ⅱ levels were reportedly increased to significantly higher levels compared to acute binge type of ethanol administration in humans[27]. In the present study, chronic ethanol binge was accompanied by a significant increase in angiotensiogen gene expression thereby suggesting a possible relationship between PAI-1 gene expression and angiotensiongen gene expression. These data pave the way for future studies to correlate this binge effect to measures of vascular injury *in vivo*. It should be noted that the major source of angiotensinogen, PAI-1, and LDL-receptor is hepatocytes. Therefore, studies on whole liver homogenates are fairly representative of hepatocyte injury; since hepatocytes account for more than 80% of liver cells. Although, involvement of non-parenchymal cells cannot be excluded at present, the results suggest a role of liver as a whole in the dysregulation of cardiovascular risk factors, by chronic ethanol binge. Although mechanisms of angiotensinogen expression are not clear, inhibition of ERK1/2 activation was associated with increased expression of the angiotensinogen gene in renal tubular cells in an oxidative stress-dependent manner[30]. This raises the possibility that decreased nuclear translocation of ERK1/2, observed in the present study, may modulate angiotensinogen gene expression. Expression of PAI-1 has been linked to ERK1/2 activation *in vitro* and *in vivo*[16,31], but in this study, nuclear accumulation of ERK1/2 was decreased after chronic ethanol binge (Figure 3). However, regulation of PAI-1 expression also occurs by ERK1/2-independent mechanisms[32]. In this regard, expression of c-fos, one of the transcription factors regulating PAI-1 expression, can also occur in an ERK-independent but redox-sensitive manner[33]. Angiotensin Ⅱ causes activation of NAPDH oxidase and oxidative stress[34]. Increased expression of c-fos after ethanol binge and the similar pattern of changes in c-fos expression and angiotensionogen supports a relationship between the expression of these two genes.

In summary, the present study offers the first evidence that ethanol binge, after chronic ethanol intake, is associated with decreased LDL-receptor and increased angiotensinogen gene expression. Decreased expression of LDL-receptor is accompanied by decreased accumulation of phosphorylated ERK1/2 in the nuclear compartment, whereas increase in angiotensionogen gene expression is accompanied by increased expression of transcription factor c-fos. These ethanol binge-induced changes have significant implications for cardiovascular risk.

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COMMENTS

Background

Binge drinking of ethanol is on the rise worldwide. Epidemiological studies indicate that this has caused alarming increase in liver and cardiovascular damage. The molecular mechanisms of this damage are not well defined. This article focuses onto this aspect.

Research frontiers

The angiotensin and low-density lipoprotein (LDL) receptor are important players in the cardiovascular complications. Both are generated in the liver and therefore highlight the importance of the contribution of liver to vascular events. Binge ethanol is shown here to influence both components.

Innovations and breakthroughs

This is the first report in a clinically relevant animal model demonstrating that binge ethanol in chronically consuming rats caused dramatic alterations in genes for angiotensinogen and LDL receptor. This offers a new mechanistic understanding of the binge ethanol effect relevant to cardiovascular complications observed in alcoholics.

Applications

The observations have clinical ramifications for future development of tools to control cardiovascular problems in chronic binge drinkers.

Terminology

Binge ethanol is repeat episodic drinking of alcohol.

Peer review

This is a very elegant study on the mechanism of liver damage from binge alcohol consumption.

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CASE REPORT

Simultaneous occurrence of malignant fibrous histiocytoma and hepatocellular carcinoma in cirrhotic liver: A case report

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Abstract

Primary hepatic malignant fibrous histiocytoma (MFH) is rarely encountered. There have been no reports to date of hepatic MFH associated with liver cirrhosis. The presence of liver cirrhosis is considered an adjunctive feature favoring sarcomatoid hepatocellular carcinoma (HCC) in the diagnosis of spindle cell tumors in liver. We describe here a 59-year-old man with liver cirrhosis due to hepatitis B virus infection 20 years ago. On abdominal computed tomography scanning, two distinct hepatic masses were identified in the background of cirrhosis, which had different radiological features from conventional HCC. He underwent segmentectomy for removal of the tumors. The pathological examination of surgically resected specimen revealed the large malignant spindle cell tumor and small conventional HCC. Additional tissue sampling and immunohistochemical stainings demonstrated that the spindle cell tumor was consistent with MFH. On the post-operative follow-up for 21 mo, a round mass showing similar radiological findings for the previous MFH was appeared on the surface of resection margin, suggesting the recurrence. Despite its rarity, hepatic MFH should be considered during differential diagnosis, even in cirrhotic patients, and extensive tissue sampling and immunohistochemical analyses are necessary in the diagnosis of hepatic spindle cell tumors.

Key words: Liver neoplasm; Malignant fibrous histiocytoma; Hepatocellular carcinoma; Liver cirrhosis

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INTRODUCTION

Primary sarcoma of the liver is a rare entity, representing < 1% of primary hepatic malignancies[1]. Malignancies of vascular differentiation, such as angiosarcomas and epithelioid hemangioendotheliomas, are the most common

of these tumors. However, other types of sarcomas, such as leiomyosarcomas, embryonal sarcomas, and fibrosarcomas, may arise in the liver, despite being exceedingly rare[2].

Malignant fibrous histiocytoma (MFH), also known as undifferentiated pleomorphic sarcoma, is the most common type of sarcoma seen in adults aged at least 50 years[3]. MFH occurs primarily as a mass on the extremities; the involvement of the retroperitoneum is relatively unusual and the presence in a visceral organ is extremely rare. Like other types of primary hepatic sarcoma, primary hepatic MFH, initially described in 1985[4,5], is very rare, with only 38 patients described to date in the English language literature. However, hepatic MFH has not been reported to be associated with advanced liver cirrhosis and hepatocellular carcinoma (HCC). We describe here a liver cirrhosis patient who developed synchronous MFH and HCC in liver and we review the literature.

CASE REPORT

A 59-year-old male was admitted to our hospital for treatment of a newly developed mass in his liver. He had a history of liver cirrhosis (Child-Pugh class A) due to hepatitis B virus identified 20 years earlier and had taken lamivudine 100 mg/d for 2 years. Two years earlier, he had undergone transarterial chemoembolization for HCC in segment Ⅳ detected by dynamic magnetic resonance images. Follow-up imaging, including a computed tomography (CT) scan 4 mo earlier, showed no evidence of tumor recurrence. He had no other significant medical history on follow-up.

At presentation, he had no symptoms or abnormal physical signs. He was serologically positive for HBsAg but had no other significant laboratory abnormality, including serum transaminase, serum bilirubin and serum α-fetoprotein (AFP) (0.7 ng/mL, normal range 0.00-8.10 ng/mL) concentrations. A CT scan showed an ill-defined, heterogeneous, low-attenuated lesion measuring 4 cm in diameter in the right anterior segment of his liver (Figure 1A-C). This lesion had irregular geographical margins with no bulge onto the hepatic capsule. It was not well enhanced in the late arterial phase or portal venous phase. A second small ill-defined, low-attenuated lesion measuring 1.5 cm was also observed in segment Ⅴ; this lesion had a similar enhancing pattern as the main mass (Figure 1D). There were no other significant lesions in his abdominal cavity. Based on a clinicoradiological diagnosis of infiltrating HCC, we performed a right anterior segmentectomy.

Macroscopically, we observed a relatively well demarcated ovoid mass, measuring 3.8 cm × 3.0 cm × 3.0 cm, on a background of cirrhosis. The mass had yellowish-white color, rubbery consistency and lobulated contour with central tumor necrosis. The lesion was confined to the resected hepatic parenchyma. The second nodule measured 1.4 cm × 1.1 cm × 0.6 cm. Both lesions correlated with the low-attenuated lesions seen in the preoperative CT scan (Figure 2A).

Microscopic examination revealed that the large ovoid mass was totally composed of spindle cells showing fascicular and storiform growth patterns embedded in collagenous background (Figure 2B). The nuclei of the tumor cells were fairly pleomorphic with brisk mitotic figures (more than 20 mitoses in 10 high-power fields). Abnormal mitotic figures and bizarre giant cells were frequently found (Figure 2C). On immunohistochemical stainings, the spindle cells expressed vimentin and CD68 in their cytoplasm (Figure 3A and B) but they did not react with antibodies to AFP, hepatocyte paraffin-1 antigen (Hep-Par-1), cytokeratins (AE1/AE3, 7 and 18) and endothelial markers (CD31 and CD34). Some of the tumor cells showed weak positive staining for α-smooth muscle actin (Figure 3C) but were negative for desmin and myogenic differentiation-1 (MyoD1). A small number of tumor cells were positive for S-100 protein (Figure 3D). The final pathological diagnosis for the large mass was pleomorphic MFH.

Contrary to the large mass, the small nodule was composed of cords or nests of polygonal epithelial cells showing mixed eosinophilic granular and clear cytoplasm (Figure 2D). Some of these cells had enlarged and pleomorphic nuclei but most had uniform round nuclei. Most of these tumor cells were positively stained for cytokeratin 18 and HepPar-1, indicating hepatocytic differentiation (Figure 3E and F). The final pathological diagnosis for the small nodule was HCC.

The patient recovered well and was discharged 16 d after surgery. The simple X-ray for both upper and lower extremities and additional chest CT scan showed no abnormal findings, confirming that the sarcoma had originated from the liver. We could not find any evidence of recurrence during the post-operative follow-up of 21 mo. However, a low-attenuated round mass had appeared on resection margin of right posterior segment on abdominal CT scan at that time. Although histological confirmation was not tried, similar contrast enhancement pattern with that of resected MFH on the dynamic image study was suggestive of the recurrence of MFH (Figure 4A and B).

DISCUSSION

Pleomorphic MFH, regarded as the most common soft tissue sarcoma in adults, was formerly defined as pleomorphic malignant spindle cell neoplasms showing fibroblastic and facultative histiocytic differentiation. However, recent advances in ultrastructural and immunohistochemical techniques have shown that several pleomorphic sarcomas with definite lines of differentiation may share these morphological characteristics[6,7]. According to the World Health Organization classification of soft tissue sarcomas, pleomorphic MFH is defined as pleomorphic spindle cell tumors with unclear lines of differentiation[8].

The clinicopathological criteria for primary hepatic MFH include: (1) solitary or multifocal hepatic tumors without evidence of primary lesions in other parts of the body; (2) lesions histologically compatible with the

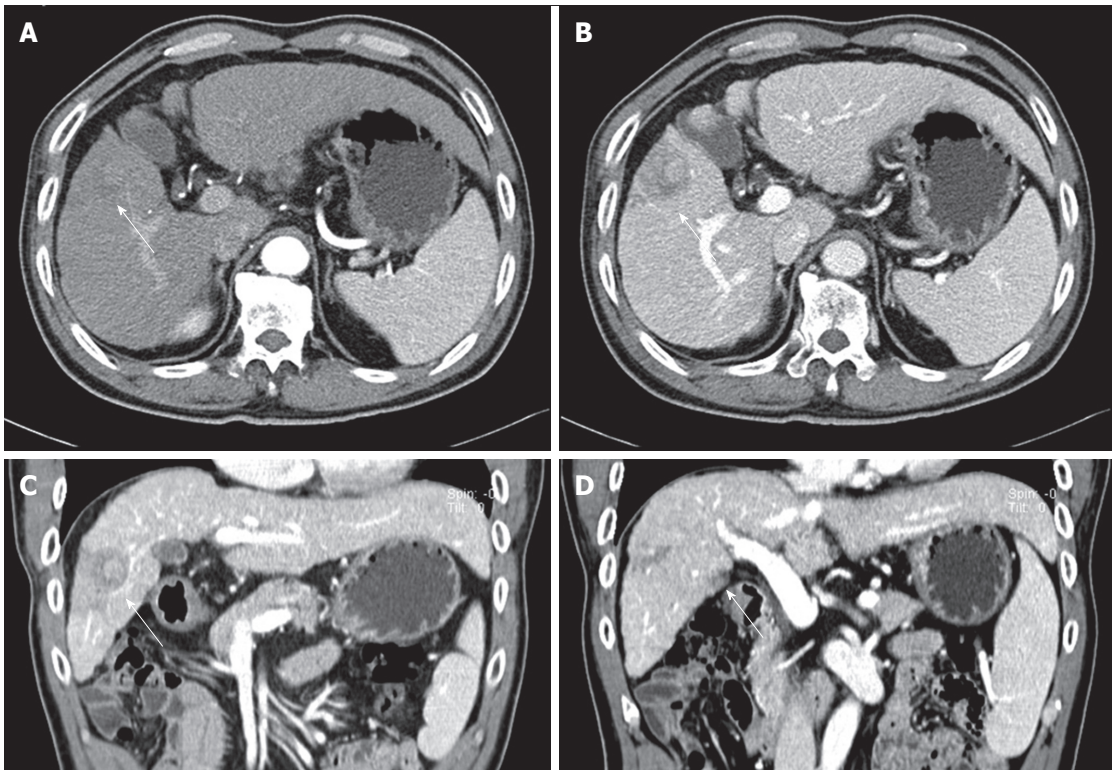

Figure 1 Computed tomography scan results. A: An ill-defined, low-attenuated lesion was observed in the right anterior segment on late arterial phase image (arrow); B: The lesion was more clearly seen as a low-attenuated, poorly enhanced lesion in the portal phase. Central high-attenuated portion was observed; C: Coronal reformat image in the portal phase, showing the low-attenuated lesion pushing against the segmental portal vein, resulting in its narrowing (arrow); D: Another coronal reformat image in the portal phase, showing a low-attenuated, poorly enhanced lesion in segment Ⅴ (arrowhead).

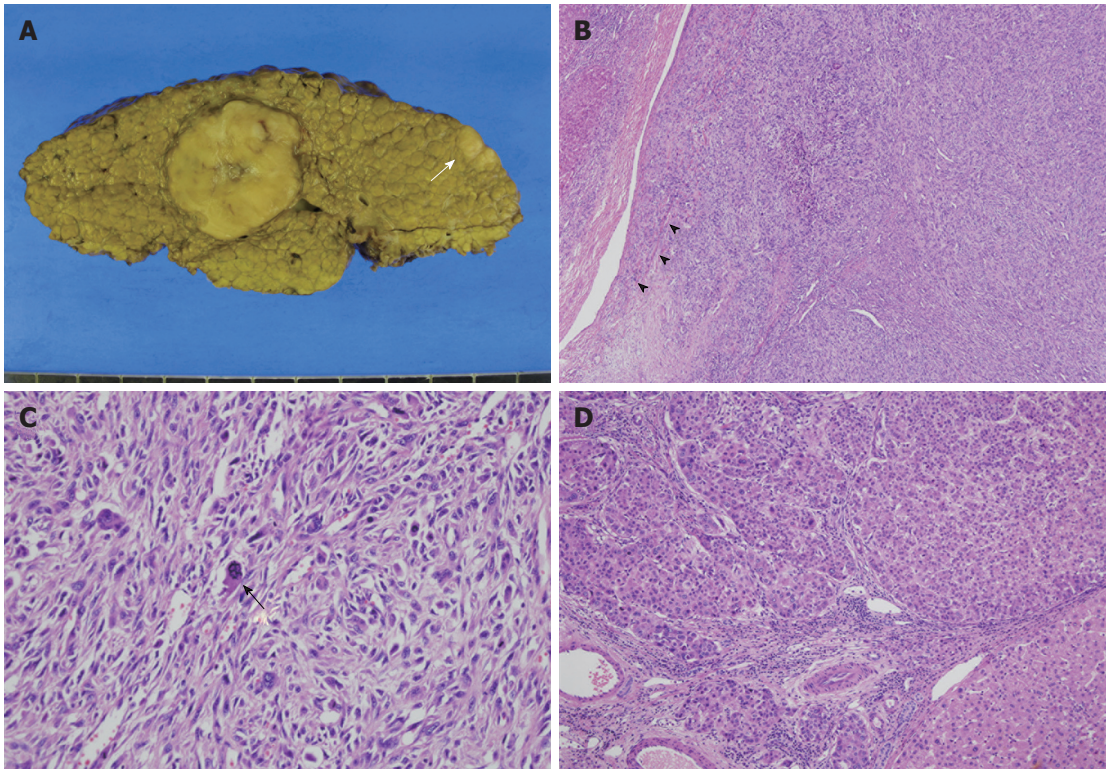

Figure 2 Histopathological findings of the tumors. A: Gross photography of the resected specimen, showing a large round lobulated mass and a smaller oval-shaped mass (white arrow). Typical multinodular cirrhotic change in background liver was evident; B: Photomicrography of the large round mass, consisting of closely packed spindle cells forming a storiform pattern. Infiltration into the segmental portal vein was also observed (arrowheads) (HE, × 40); C: High power examination, showing pleomorphic spindle cells with bizarre-shaped giant cells, along with numerous mitoses with atypical figures (black arrow) (HE, × 200); D: Photomicrography of the small oval-shaped mass showing polygonal epithelial cells forming trabeculae and round cell nests, consistent with a conventional HCC (HE, × 100).

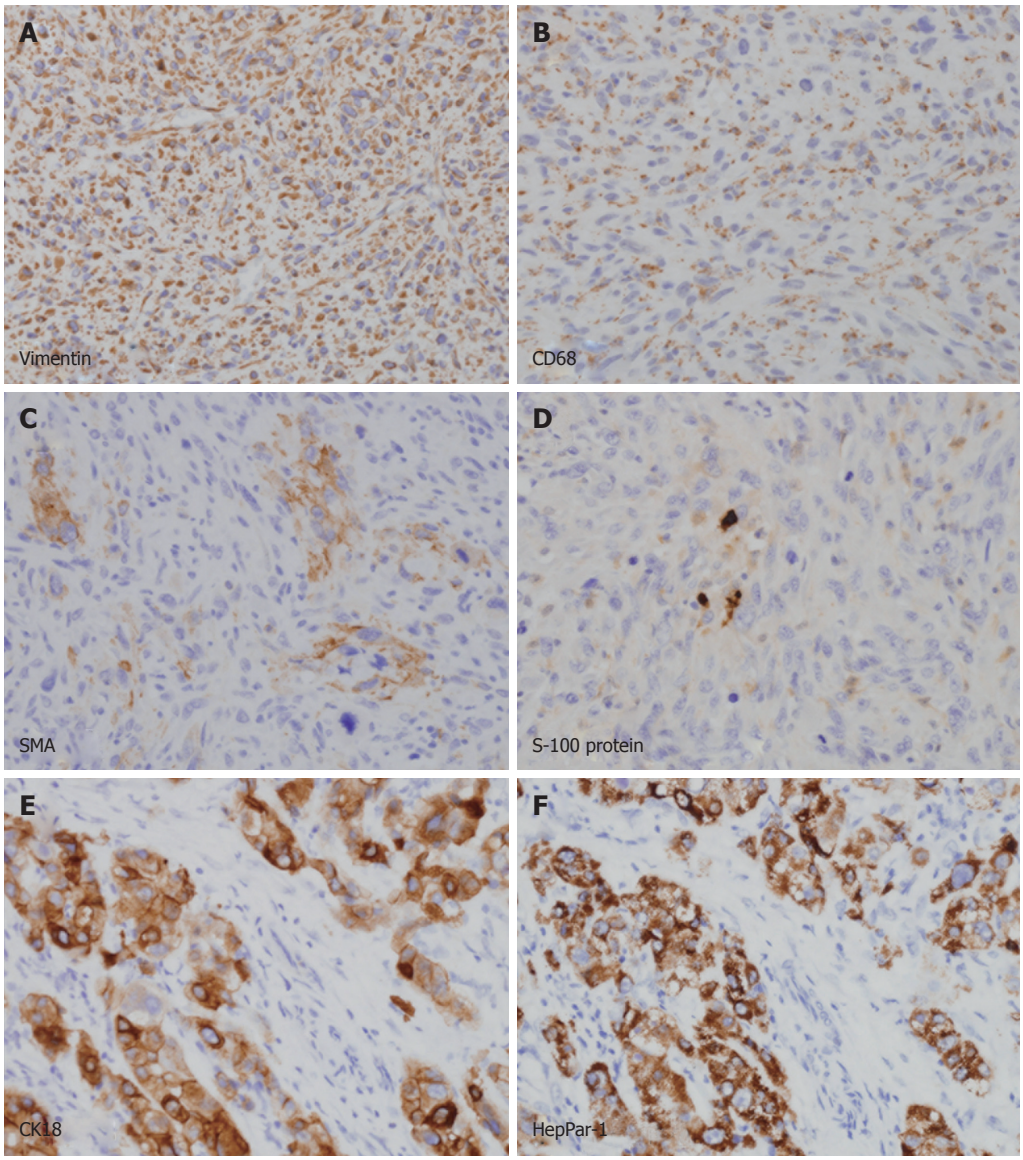

Figure 3 Immunohistochemical analyses of the tumors. A-D: Spindle cells in the large mass were diffusely immunopositive for vimentin (A) and CD68 (B) in their cytoplasm. A few cells (less than 5%) were also positive for α-smooth muscle actin (C) and S-100 protein (D). All cells were negative for cytokeratins, hepatocyte paraffin-1 antigen (HepPar-1), desmin and myogenic differentiation-1, excluding cells of epithelial and myogenic differentiation (not shown); E, F: Polygonal cells in small mass were strongly immunopositive for cytokeratin 18 (E) and HepPar-1 (F), consistent with a typical hepatocellular carcinoma.

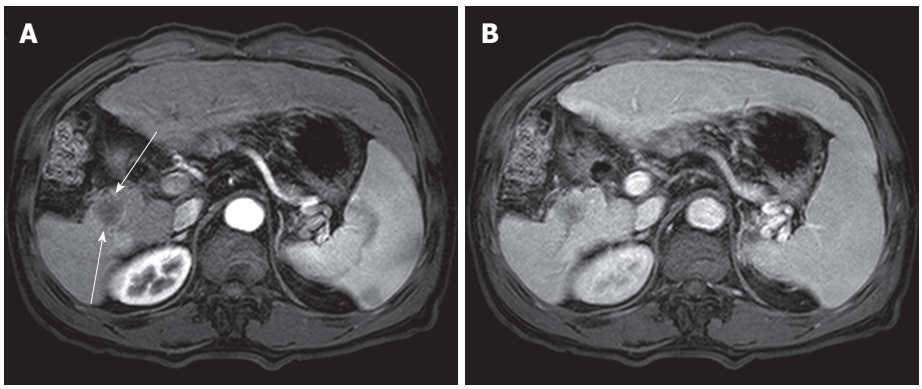

Figure 4 Computed tomography scan at follow-up of twenty-one months. A: A well demarcated low-attenuated round mass was newly found at the resection margin of right posterior segment (slender arrows). This mass was not significantly enhanced in late arterial phase; B: This mass was also not well enhanced at portal phase, which is similar pattern with the previous malignant fibrous histiocytoma.

spectrum of soft tissue MFH; (3) immunohistochemical evidence of mesenchymal differentiation, with < 5% of tumor cells showing any well-defined line of derivation; and (4) the presence of fibroblast-like, histiocyte-like, fibrohistiocytic or undifferentiated cells without evidence of any other well-differentiated cell type by electron microscopy[9]. Our patient presented with no evidence of a primary tumor except in the liver. The pleomorphic spindle cells forming a storiform pattern showed diffuse positive staining for vimentin but were negative for cytokeratins, AFP and HepPar-1, suggesting a true mesenchymal tumor. Moreover, additional immunohistochemical staining showed no significant expression of antigens specifying a lineage of differentiation; although α-smooth muscle actin was focally expressed, desmin and MyoD1 were not significantly expressed and only a small population of tumor cells (< 5%) showed faint expression of S-100 protein. Extensive tissue sampling showed no evidence of a relatively differentiated sarcomatous or carcinomatous focus. Although electron microscopy was not performed, these clinicopathological features were sufficient for a diagnosis of primary hepatic MFH.

We found that the cytoplasm of tumor cells was positive for CD68 in a granular pattern. MFH has been reported to express histiocytic markers, such as CD68, α-1-antitrypsin, α-1-antichymotrypsin and lysozymes[10]. Similar findings have been reported in cases of primary hepatic MFH[9]. Although MFH does not originate from histiocytes[11] and although malignant neoplasms other than MFH may express histiocytic markers[12], positive immunohistochemical staining for histiocytic markers in the absence of other markers for specific lineages raise the possibility of MFH in primary hepatic spindle cell tumors. To our knowledge, our case is the first described case with primary hepatic MFH arising in a cirrhotic liver. Primary hepatic MFH has been reported in three patients with viral hepatitis[13-15] but cirrhotic changes in the remaining hepatic parenchyma were not described in these patients. Thus, in contrast to common belief, hepatic MFH should also be included in the differential diagnosis of liver mass in patients with liver cirrhosis, especially when it is radiologically unusual for HCC.

Primary hepatic MFH may be indistinguishable from other hepatic malignancies, especially in a small biopsy specimen. Nevertheless, although it is extremely rare, the preoperative and/or postoperative diagnosis of hepatic MFH may be necessary in clinical practice. Sarcomatoid HCC is reported to be a slightly worse prognosis than hepatic MFH (23% *vs* 33% of 2-year survival rate) in distinct small collective studies[9,16]. Furthermore, in contrast to HCC, there is a report that chemoembolization therapy is ineffective in hepatic MFH[17], raising the need for the preoperative diagnosis. However, case reports and collective studies have failed to delineate the common radiological findings of hepatic MFH[18]. For example, our case showed liver cirrhosis and portal vein invasion, the findings favoring HCC over true sarcomas[16,19]. Furthermore, the diagnosis of hepatic MFH cannot be easily rendered by biopsy because the well-differentiated HCC component, necessary for the diagnosis of sarcomatoid HCC, might be present in another portion of tumor that is not examined. Currently, thorough microscopic examination of resected specimen and ancillary immunohistochemical stainings are requisite for the diagnosis of hepatic MFH.

In addition to hepatic MFH, we simultaneously observed a second tumor in this patient, a HCC. To date, there are only three reports of the concurrent development of hepatic sarcoma and HCC in three autopsy cases in Japan but their specific differentiation was not immunohistochemically confirmed[20-22]. To our knowledge, our case is also the first showing synchronous development of hepatic MFH and HCC. Owing to their similarity on CT scans, these two lesions were considered intrahepatic metastases from a large hepatic tumor but their histological features differed. Patients presenting with several liver masses without any significant evidence of extrahepatic primary focus should be suspected of having multiple primary hepatic neoplasms, even though this situation is rare.

In conclusion, primary hepatic MFH may appear in patients with liver cirrhosis and HCC may develop simultaneously. Even in patients with viral hepatitis and cirrhosis, thorough tissue sampling and intensive immunohistochemical analyses should be mandatory to differentiate primary hepatic MFH from other primary hepatic sarcomas and, especially, sarcomatoid HCC.

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Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011
Miami, FL 33101, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011
San Francisco, CA 94143, United States

January 27-28, 2011
Falk Workshop, Liver and Immunology, Medical University, Franz-Josef-Strauss-Allee 11
Regensburg 93053, Germany

January 28-29, 2011
9. Gastro Forum München
Munich, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference
Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

February 22, 2011-March 04, 2011
Canadian Digestive Diseases Week 2011
Vancouver, BC, Canada

February 24-26, 2011
Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation
Dublin, Ireland

March 3-5, 2011
42nd Annual Topics in Internal Medicine
Gainesville, FL 32614, United States

March 7-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings
Sarasota, FL 34234, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011
Birmingham, England, United Kingdom

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011
Jacksonville, FL 34234, United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform
Sacramento, CA 94143, United States

March 25-27, 2011
MedicReS IC 2011
Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease
San Diego, CA 94143, United States

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition
Riyadh, Saudi Arabia

May 7-10, 2011
Digestive Disease Week
Chicago, IL 60446, United States

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671
Barcelona 08028, Spain

May 21-24, 2011
22nd European Society of Gastrointestinal and Abdominal Radiology Annual Meeting and Postgraduate Course
Venise, Italy

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn, Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011
Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV
SPIGC, II ESYS
Napoli, Italy

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer
Barcelona, Spain

October 19-29, 2011
Cardiology & Gastroenterology Tahiti 10 night CME Cruise
Papeete, French Polynesia

October 22-26, 2011
19th United European Gastroenterology Week
Stockholm, Sweden

October 28-November 2, 2011
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INSTRUCTIONS TO AUTHORS

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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