Abstract. Accumulating evidence has indicated that corosolic acid exerts anti-diabetic, anti-obesity, anti-inflammatory, anti-hyperlipidemic and anti-viral effects. More importantly, corosolic acid has recently attracted much attention due to its anticancer properties and innocuous effects on normal cells. Furthermore, the increasing proportion of obese and/or diabetic populations has led to an epidemic of non-alcoholic fatty liver disease (NAFLD), which frequently progresses to hepatocellular carcinoma (HCC). Evidence has indicated that NAFLD is closely associated with the development of HCC and comprises a high risk factor. The present review summarizes the anticancer effects of corosolic acid in vitro and in vivo, and its related molecular mechanisms. It also describes the inhibitory effects of corosolic acid on the progression of NAFLD and its associated molecular mechanisms, providing guidance for future research on corosolic acid in NAFLD-related HCC prevention and treatment. To the best of our knowledge, a review of corosolic acid as an anticancer agent has not yet been reported. Due to its multitargeted activity in cancer cells, corosolic acid exerts anticancer effects when administered alone, and acts synergistically when administered with chemotherapeutic drugs, even in drug-resistant cells. In addition, as a novel tool to treat metabolic syndromes, corosolic acid uses the same mechanism in its action against cancer as that used in the progression of NAFLD-related HCC. Therefore, corosolic acid has been suggested as an agent for the prevention and treatment of NAFLD-related HCC.

1. Introduction

Cancer is one of the most common causes of mortality worldwide. However, it is not only a serious threat to public health, but also a global socioeconomic burden (1). An estimated 2,814,000 cases of cancer-related death and 4,292,000 new cancer cases occurred in China in 2015 (2). Based on GLOBOCAN (a global cancer statistics database), in 2018 the number of cases of cancer-related death was 9.6 million, and the number of new cancer cases was 18.1 million worldwide (3). However, data also indicate a decline in the number of new cases, which may be associated with lifestyle changes or reduced exposure to high-risk environmental factors in developed countries (4). Accumulating evidence also suggests that the proteins encoded by a variety of aberrantly-expressed regulatory genes promote tumorigenesis; these include anti-apoptotic proteins, transcription factors, growth factors and their respective receptors (5-7). Tumorigenesis is a multistep process characterized by numerous abnormalities, rather than a single mutation, during cancer initiation, promotion and progression; therefore, a single target agent is unlikely to inhibit cancer growth (8,9). Currently, the primary treatment strategies against tumors include the following: Surgery, chemoradiotherapy, immunotherapy, molecular targeted therapy and Traditional Chinese Medicine. Although chemotherapy has been proven to improve survival in patients with cancer, drug resistance and severe adverse side effects, such as damage to liver function, bone marrow suppression...
and neurotoxicity, are major obstacles that cause treatment failure (10,11). There is therefore an urgent need to develop novel and more effective drugs with fewer side effects for various types of cancer.

Due to their selective molecular targets, novel bioactive components from plant sources have emerged as new and reliable therapeutic elements for treating various types of human cancer (12,13). Indeed, over the past half century, numerous plant derivatives and secondary metabolites have been used in clinical practice for the treatment of cancer (14,15). For example, pentacyclic triterpenes constitute a group of promising anticancer drugs that comprise the lupane, oleanane and ursane groups (16,17). Since Pisha et al (18) first reported in 1995 that betulinic acid (19), a plant secondary metabolite, is a highly promising anticancer drug, experimental studies have largely focused on the cytotoxic effects of betulinic acid and other types of triterpenes, particularly their apoptosis-inducing mechanisms, initially in melanoma cell lines in vitro and in vivo (20-22). The cytotoxic effects of betulinic acid were subsequently confirmed in other cell lines, such as those derived from breast (23), colon and lung cancer (24), as well as neuroblastoma (25). In the last decade, triterpenes were also found to have additional effects on cancer through several modes of action, such as induction of apoptosis and enhancement of endoplasmic reticulum (ER) stress (23-25).

Corosolic acid, also known as 2α-hydroxyursolic acid, has a molecular formula of C_{30}H_{40}O_{4}, and a molecular weight of 472.70 g/mol (Fig. 1). As a prevalent pentacyclic triterpenoid and the principal component of Banaba leaves, corosolic acid has received a great deal of attention due to its anti-diabetic properties (26). Corosolic acid is known as a ‘phyto-insulin’ or ‘botanical insulin’ (27). It is the principal component of Lagerstroemia speciosa leaves (also called Banaba), a tropical plant found in the Philippines, Vietnam, Malaysia and Southern China (28,29). Table I lists the plant species able to biosynthesize corosolic acid (28-50). Corosolic acid has also been isolated from European and South American plants.

Experimental studies have indicated that corosolic acid plays a pivotal anticancer role in several tumorigenic processes in vitro and in vivo, including cellular proliferation, apoptosis, angiogenesis, lymphangiogenesis, metastasis and tumor immunity, and it exerts a synergistic effect when administered with other anticancer agents (2) (51-53). In addition, corosolic acid has the ability to modulate multiple cancer-related signaling pathways and processes, such as the nuclear factor kappa-B (NF-kB), phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) and Wnt/β-catenin pathways, apoptosis, nuclear factor erythroid 2-related factor 2 (Nrf2) and several other components associated with cellular proliferation or mortality (Table II) (49,51,54,55). However, more research is required to determine its potential in human clinical trials. The most recent registry data from Surveillance, Epidemiology and End Results shows that the morbidity of liver and intrahepatic bile duct cancers have risen on average 3.0% each year between 2004 and 2013 in the United States (56). In particular, hepatocellular carcinoma (HCC) is an aggressive cancer with a poor prognosis. Chronic liver diseases, such as hepatitis B and C virus infections, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) and cirrhosis are the most common underlying causes of HCC (41). NAFLD in particular, has been recognized as one of the leading etiologies for the development of HCC (57,58). NAFLD encompasses a spectrum of chronic liver diseases, ranging from simple steatosis to liver injury, which are closely associated with metabolic syndrome (MS) and are characterized by conditions such as obesity, diabetes and dyslipidemia (59-61). The understanding of the pathogenesis of NAFLD-related HCC is limited, and several possible mechanisms of NAFLD-related HCC have been described, including obesity-induced inflammation (62-64), insulin resistance (IR) (65-68), oxidative stress (69,70) and adaptive immune responses (71,72).

Accumulating experimental evidence has suggested that corosolic acid possesses a variety of biological properties, exerting anti-diabetic, anti-obesity, anti-hyperlipidemic, anti-viral, anti-inflammatory and anticancer effects (26,73,74). Therefore, the present review describes the anticancer effects and related molecular mechanisms of corosolic acid, highlighting its ability to inhibit NAFLD progression, and providing guidelines for future research on its use as an agent in NAFLD-related HCC prevention and treatment.

2. Corosolic acid exerts anticancer effects in vitro

Effects and mechanisms of corosolic acid in neoplastic cell lines from the digestive system. Cancer cell migration is a critical process in tumor development and metastasis (75,76), and is closely associated with vascular growth factor receptor (VEGFR) signaling (57,58); thus, the inhibition of VEGFR, and VEGFR2 in particular, is considered an important treatment approach for HCC and prevent HCC metastasis (77-79). Ku et al (48) showed that the half-maximal inhibitory concentration (IC_{50}) for corosolic acid was 2.5 µM for migratory ability, and 50 µM for cytotoxicity on the HCC Huh7 cell line. In addition, corosolic acid treatment resulted in a decrease in Huh7 cell migration in a dose-dependent manner, and corosolic acid at a dose of 2.5 µM induced low cytotoxicity for 24 h (IC_{50} cytotoxicity/IC_{50} migration=20), compared to the untreated control (48). The authors further demonstrated that the cytotoxic effects observed with corosolic acid might be associated with the markedly suppression of the VEGFR2/steroid receptor coactivator/focal adhesion kinase (FAK)/cell division cycle42 (cdc42) signaling pathway and the inhibition of the kinase activity of VEGFR2. On the other hand, Xu et al (80) reported that corosolic acid had reduced efficacy in treating liver cancer, since it accelerated the degradation of the transcription factors of Yes-associated protein (YAP) by enhancing large tumor suppressor gene 1-induced phosphorylation and β-transducin repeat containing protein (βTrCP)-dependent ubiquitination. However, Xu et al (80) also demonstrated that corosolic acid-induced apoptosis of liver cancer cells was enhanced by combined treatment with actinomycin D, which resulted in elevated YAP protein levels and decreased βTrCP protein activity. This study suggests that the effectiveness of liver cancer treatment with corosolic acid (at a final concentration of 10 µM) might be improved by its combined administration with 5 µg/ml actinomycin D for 24 h (80).

In gastric cancer cells, corosolic acid has been shown to effectively inhibit the progression of carcinogenesis through multiple mechanisms, including targeting of the adenosine
monophosphate-activated protein kinase (AMPK)-mammalian target of rapamycin (mTOR) signaling pathway, the inhibition of the NF-κB pathway, the downregulation of EGFR2/neu oncogene, the promotion of the anticancer activities of 5-fluorouracil (5-FU) via mTOR inhibition, and the reduction of 5-FU chemoresistance through the activation of the AMPK pathway (49,81,82). In human gastric cancer NCI-N87 cells, corosolic acid has been shown to inhibit the expression of human epidermal growth factor receptor 2 (HER2) and AMPK-mTOR signal phosphorylated proteins, such as Akt and extracellular signal-regulated protein kinase (ERK), which are involved in signaling pathways downstream of HER2, with the inhibitory effect of corosolic acid being both dose- and time-dependent (25 µM for 12, 24 and 48 h, and 50 µM for 24 h) (81). Furthermore, corosolic acid has been found to induce G_{1}/G_{0} arrest, which was associated with the induction of cyclin-dependent kinase inhibitor 1B and the downregulation of cyclin D1 (81). In addition, Lee et al (81) found that corosolic acid could effectively inhibit cell proliferation in both a dose- and time-dependent manner (1, 5, 10 and 50 µM for 24 h, and 25 µM for 3, 6, 12, 24 and 48 h). Furthermore, corosolic acid has been shown to induce cell cycle arrest and apoptosis through the downregulation of the HER2/neu oncogene, suggesting that it may play a role in patients with HER2-amplified gastric cancers (81). Moreover, at an IC_{50} value of 16.9±2.9 µM, corosolic acid has been shown to inhibit the proliferation of human gastric cancer SNU-601 cells via AMPK-mTOR signaling (82). Another study has reported that corosolic acid treatment at a concentration of 10, 20, 40 and 80 mg/ml for 72 h induces apoptosis in human gastric cancer BGC823 cells in a dose-dependent manner (49). This effect is achieved by inhibiting the NF-κB (p65 subunit) pathway, by decreasing the mRNA and protein expression of p65, apoptosis antigen 1 (Fas), second mitochondria derived activator of caspase, and B-cell lymphoma-2 (Bcl-2), whilst increasing that of Bcl-2 associated X (Bax), inhibitor of NF-κB (IκB) α and survivin (49). In addition, the experimental data of Sung et al (83) provides insights into the molecular mechanisms through which corosolic acid induces the apoptosis of colorectal cancer cells. Corosolic acid, at an IC_{50} value of 24 µM for 24 h, inhibits the viability of colorectal cancer HCT116 cells by inducing apoptotic cell death in a dose-dependent manner, through a molecular mechanism associated with the upregulation of the proapoptotic proteins Bax, Fas and Fas ligand (FasL), and the downregulation of the anti-apoptotic proteins Bcl-2 and survivin. Of note, corosolic acid was proven to be an ideal antagonist of the Wnt/β-catenin pathway (51). Corosolic acid decreased the level of intracellular β-catenin and suppressed the proliferation of colon cancer HCT-15 and DLD-1 cells with an APC mutation in a dose-dependent manner (20, 40 and 60 µM for 8 h), which was achieved by promoting N-terminal phosphorylation and degrading the proteasomes of β-catenin (Table II) (51).

### Effects and mechanisms of corosolic acid on tumor cells from the urogenital system

Accumulating evidence has suggested that activated Nrf2 plays a critical role in the proliferation and survival of tumor cells, making its inhibition a promising therapeutic strategy for cancer treatment (84-87). A previous report on several Nrf2 inhibitors showed that these are promising therapeutic agents (88). Of note, corosolic acid at a concentration of 0.25-32 µM for 3 or 5 days inhibited the proliferation of TRAMP-C1 cells, a type of anchorage-independent human prostate cancer (PCa) cell line with increased levels of mRNA and
protein expression of Nrf2, heme oxygenase-1 (HO-1) and nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1; however, corosolic acid did not exert the same inhibitory effect in Nrf2-knockout TRAMP-C1 cells (54). These findings indicate that the significant cytotoxic effect of corosolic acid might be associated with its ability to restore the expression of Nrf2 via epigenetic modification (54). In addition, in the PCa, PC‑3 and DU145 cell lines, (ER) stress was activated by 0, 5, 10 and 15 µM corosolic acid after 24 and 48 h, through two proapoptotic signaling pathways: The inositol-requiring ER-to-nucleus signal kinase 1/apoptosis signal regulating kinase 1/Jun N-terminal kinase (JNK) pathway and the protein kinase RNA-like ER kinase/eukaryotic initiation factor 2 α/activating transcription factor 4/C/EBP-homologous protein signaling pathway, which induced apoptosis and suppressed cell proliferation (89). However, Woo et al (90) found that the corosolic acid-induced death of human renal carcinoma Caki cells (at 10 µM for 24 h) was inhibited by the use of α-tocopherol (a hydrophobic anti-oxidant that prevents free radical damage), but was not inhibited by benzyloxy-carbonyl-Val-Ala-Asp-fluoromethyl ketone (an apoptosis inhibitor), necrostatin-1 (a necroptosis inhibitor), ferrostatin-1 or deferoxamine (ferroptosis inhibitors) (90). Furthermore, corosolic acid induces lipid oxidation, and α-tocopherol markedly prevents corosolic acid-induced lipid peroxidation and cell death. Anti-chemotherapeutic effects of α-tocopherol are dependent on inhibition of lipid oxidation rather than inhibition of ROS production (90). It was therefore speculated that corosolic acid induced the non-apoptotic cell death associated with lipid peroxidation in cancer cells (90). Furthermore, in renal carcinoma ACHN and A498 cells, treatment with 10 µM corosolic acid for 24 h induced non-apoptotic cell death (90). Xu et al (91) reported that treating human cervix adenocarcinoma HeLa cells with 40 µM corosolic acid for 24 h could induce cell cycle arrest at the S phase, and promote apoptosis by activating caspases-8, -9 and -3 and disrupting mitochondrial membrane potential (91). In another report on CaSki human cervical cancer cells, the results indicated that 10, 50 and 100 µM corosolic acid treatment for 12, 24 and 48 h effectively inhibited proliferation in a dose- and time-dependent manner (55). In addition, the results revealed that the cytotoxic effects of corosolic acid inhibited tumor cell proliferation by inducing apoptosis and cell cycle arrest, and suppressing the PI3K/Akt signaling pathway (55). It has also been demonstrated that in epithelial ovarian cancer (92), glioma and lymphoma (93,94) cells, the activation of signal transducer and activator of transcription 3 (STAT3) was induced by co-culturing the cells with M2, but not M1 macrophages. However, Fujiwara et al (95) demonstrated that corosolic acid, at a minimum of 30 µM for 48 h, suppressed STAT3 activation in co-culture experiments with epithelial ovarian cancer ES-2 cells treated with bromodeoxyuridine (used to abrogate macrophage differentiation into the M2 phenotype), and that STAT3 inhibition was associated with the prevention of M2 macrophage polarization. In addition, the epithelial ovarian cancer cell line SKOV3 treated with 20 µM corosolic acid for 24 h, showed no effect on the viability of these cells, suggesting that corosolic acid have no anticancer properties at this concentration. By contrast, 20 µM corosolic acid enhanced the inhibitory effect of paclitaxel (PTX; 10 µM) on the proliferation of the epithelial ovarian cancer cell lines SKOV3, RMG-1 and ES-2. These results demonstrated that corosolic acid enhances the anticancer activity of anticancer drugs such as PTX in epithelial ovarian carcinoma cells (95). Notably, the combination of 20 µM corosolic acid and 10 µM paclitaxel for 24 h also inhibited STAT3 activity in the epithelial ovarian cancer cells, but corosolic acid alone or PTX alone had lesser effects on the STAT3 activity (95). These data suggested that corosolic acid enhanced cancer cell chemosensitivity and effectively inhibited cancer cell proliferation, which was also found to be associated with the prevention of M2 polarization via the suppression of STAT3 activation (95). These findings were similar to those showing that corosolic acid (30 µM for 1 h) suppressed the M2 macrophage polarization and proliferation of U373 and
Table II. Summary of the effects of corosolic acid in different types of cancers in vitro.

<table>
<thead>
<tr>
<th>First author/s, year</th>
<th>Cancer type</th>
<th>Cell types</th>
<th>Molecular mechanism</th>
<th>Effects</th>
<th>Drug synergism</th>
<th>(Refs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu et al, 2017 and</td>
<td>Liver cancer</td>
<td>Huh7</td>
<td>βTrCP-dependent Ubiquitination of YAP (↑ YAP, ↓ βTrCP) VEGFR2/Src/FAK pathway (VEGFR2, Src, FAK)</td>
<td>↑ Apoptosis ↓ Migration activity, cell motility</td>
<td>Actino-mycin DNA</td>
<td>(48,80)</td>
</tr>
<tr>
<td>Ku et al, 2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woo et al, 2018</td>
<td>SK-Hep1, Huh7</td>
<td></td>
<td>↑ Lipid peroxidation</td>
<td>↑ Non-apoptotic cell death</td>
<td>NA</td>
<td>(90)</td>
</tr>
<tr>
<td>Lee et al, 2010</td>
<td>Gastric cancer</td>
<td>NCI-N87</td>
<td>HER2/neu oncogene</td>
<td>↑ Cell cycle arrest and apoptosis pathway (↓ HER2, Akt, Erk; ↑ P27Kip1, cyclin D1)</td>
<td>Adria-mycin, 5-FU</td>
<td>(81)</td>
</tr>
<tr>
<td>Cheng et al, 2017</td>
<td>BGC823</td>
<td></td>
<td>NF-κB pathway (↓ P65, Fas, FasL, Bcl-2, Smac; ↑ IκBα, Bax, survivin)</td>
<td>↑ Cell cycle arrest and apoptosis</td>
<td>NA</td>
<td>(49)</td>
</tr>
<tr>
<td>Lee et al, 2010</td>
<td>SNU-601</td>
<td></td>
<td>AMPK-mTOR pathway (↑ AMPK; ↓ mTOR)</td>
<td>↑ Cell cycle arrest and apoptosis</td>
<td>NA</td>
<td>(82)</td>
</tr>
<tr>
<td>Lee et al, 2015</td>
<td>SNU-620</td>
<td></td>
<td>mTOR signaling pathway (↓ mTOR); caspase-dependent pathway (↑ caspase-8,-9 and -3)</td>
<td>↑ Cell cycle arrest and apoptosis</td>
<td>5-FU</td>
<td>(106)</td>
</tr>
<tr>
<td>Yamaguchi et al, 2006</td>
<td>SNU-620/5-FU®</td>
<td></td>
<td>AMPK-mTOR pathway (↓ Bcl-2, TS, mTOR/4EBP1, PARP; ↑ AMPK, Bim, caspase-3, poly-ADP-ribose)</td>
<td>5-FU</td>
<td></td>
<td>(109)</td>
</tr>
<tr>
<td>Sung et al, 2014</td>
<td>Colon cancer</td>
<td>HCT116</td>
<td>caspase-dependent pathway (↑ caspase-8,-9 and -3, Bax, Fas, FasL; ↓ Bcl-2, survivin)</td>
<td>↑ Apoptotic cell death</td>
<td>NA</td>
<td>(83)</td>
</tr>
<tr>
<td>Yoo et al, 2015</td>
<td>CT-26</td>
<td></td>
<td>FAK pathway (↓ angiopoetin-1, FAK, ERK1/2); caspase-dependent pathway (↑ caspase-8,-9 and -3)</td>
<td>↓ Tumor proliferation; ↑ cell cycle arrest and apoptosis</td>
<td>NA</td>
<td>(53)</td>
</tr>
<tr>
<td>Kim et al, 2014</td>
<td>APC-mutated HCT15</td>
<td></td>
<td>Wnt/β-catenin pathway (↓ β-catenin)</td>
<td>↓ Tumor proliferation</td>
<td>NA</td>
<td>(51)</td>
</tr>
<tr>
<td>First author/s, year</td>
<td>Cancer type</td>
<td>Cell types</td>
<td>Molecular mechanism</td>
<td>Effects</td>
<td>Drug synergism</td>
<td>(Refs.)</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------</td>
<td>-------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>-----------------------------------</td>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Nho et al, 2013</td>
<td>Lung cancer</td>
<td>A549</td>
<td>Mitochondrial/caspase-dependent pathway ($\uparrow$ caspase -7,-8,-9 and -3, ROS; $\downarrow$ Bcl-2, survivin, Bid)</td>
<td>$\uparrow$ Apoptotic cell death</td>
<td>NA</td>
<td>(99)</td>
</tr>
<tr>
<td>Woo et al, 2018</td>
<td>Kidney cancer</td>
<td>Caki, ACHN, A498</td>
<td>$\uparrow$ Lipid peroxidation</td>
<td>$\uparrow$ Non-apoptotic cell death</td>
<td>NA</td>
<td>(90)</td>
</tr>
<tr>
<td>Woo et al, 2018</td>
<td>Breast cancer</td>
<td>MDA-M, B231</td>
<td>$\uparrow$ Lipid peroxidation</td>
<td>$\uparrow$ Non-apoptotic cell death</td>
<td>NA</td>
<td>(90)</td>
</tr>
<tr>
<td>Ma et al, 2018</td>
<td>Prostate cancer</td>
<td>PC-3, DU145</td>
<td>ER stress; IRE-1/ASK1/JNK signaling pathway and PERK/eIF2alpha/ATF4/CHOP signaling pathway ($\uparrow$ IRE-1, PERK, CHOP, TRIB3; $\downarrow$ AKT)</td>
<td>$\uparrow$ ER stress-dependent apoptosis</td>
<td>NA</td>
<td>(89)</td>
</tr>
<tr>
<td>Yang et al, 2017</td>
<td>TRAMP-C1</td>
<td></td>
<td>Nrf2/HO-1 pathway ($\uparrow$ H3KK27ac; $\downarrow$ DNMTs, HDACs, H3k27me3)</td>
<td>$\uparrow$ Epigenetic alterations</td>
<td>NA</td>
<td>(54)</td>
</tr>
<tr>
<td>Xu et al, 2009</td>
<td>Cervical cancer</td>
<td>HeLa</td>
<td>mitochondrial pathway and caspases activation ($\uparrow$ Bax/Bcl-2 ratio, caspase-8,-9 and -3)</td>
<td>$\uparrow$ Cell cycle arrest and apoptosis</td>
<td>NA</td>
<td>(91)</td>
</tr>
<tr>
<td>Yong et al, 2016</td>
<td>Ovarian Cancer</td>
<td>CaSki</td>
<td>PI3K/Akt signaling pathway ($\downarrow$ PI3K/Akt)</td>
<td>$\uparrow$ Cell cycle arrest and apoptosis</td>
<td>NA</td>
<td>(55)</td>
</tr>
<tr>
<td>Fujiwara et al, 2013</td>
<td>Ovarian cancer</td>
<td>SKOV3, RMG-1 and ES-2 SKOV3</td>
<td>STAT3 pathway ($\downarrow$ STAT3) and $\downarrow$ M2 macrophage polarization</td>
<td>$\downarrow$ Chemoresistance; $\downarrow$ Tumorigenic macrophages</td>
<td>PTX, CDDP and DOX</td>
<td>(95)</td>
</tr>
<tr>
<td>Fujiwara et al, 2011</td>
<td>Glioblastoma</td>
<td>U373, T98G</td>
<td>JAK-STAT3, NF-κB pathway ($\uparrow$ T lymphocytes infiltration; $\downarrow$ MDSC, COX2 mRNA, CCL-2 mRNA, M2 polarization)</td>
<td>$\uparrow$ Antitumor immunity</td>
<td>NA</td>
<td>(96)</td>
</tr>
<tr>
<td>Cai et al, 2011</td>
<td>Osteosarcoma</td>
<td>MG-63</td>
<td>Mitochondria-mediated apoptosis pathway ($\uparrow$ caspase-3/9)</td>
<td>$\uparrow$ Mitochondria-mediated apoptosis</td>
<td>Adriamycin, cisplatin</td>
<td>(97)</td>
</tr>
<tr>
<td>Wang et al, 2018</td>
<td>Retinoblastoma</td>
<td>Y-79</td>
<td>MELK-FoxM1 signaling ($\uparrow$ MELK, FoxM1)</td>
<td>$\uparrow$ Cell cycle arrest and apoptosis; $\uparrow$ Cytotoxicity</td>
<td>NA</td>
<td>(101)</td>
</tr>
</tbody>
</table>

NA, not applicable; HER2, human epidermal growth factor receptor 2; AMPK, adenosine monophosphate; mTOR, activated protein kinase-mammalian target of rapamycin; CCL-2, chemokine (C-C motif) ligand 2; Fas, apoptosis antigen 1; VEGFR, vascular growth factor receptor; Src, steroid receptor coactivator; FAK, focal adhesion kinase; cdc42, cell division cycle42; Smac, second mitochondria derived activator of caspase; Bax, B-cell lymphoma-2 associated X; NF-κB, nuclear factor kappa-B; IκBα, inhibitor of NF-κB; ER, endoplasmic reticulum; IRE-1, inositol-requiring ER-to-nucleus signal kinase 1; ASK1, apoptosis signal regulating kinase 1; JNK, Jun N-terminal kinase; PERK, protein kinase RNA-like ER kinase; eIF2alpha, eukaryotic initiation factor 2alpha; ATF4, activating transcription factor 4; CHOP, C/EBP-homologous protein; p27kip1, cyclin-dependent kinase inhibitor 1B; MELK, maternal embryonic leucine-zipper kinase; FoxM1, forkhead box M1; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; STAT3, signal transducer and activator of transcription 3; MDSCs, myeloid-derived suppressor cells; COX-2, cyclooxygenase-2; Akt, protein kinase B; ERK, extracellular signal-regulated protein kinase; YAP, Yes-associated protein; Fasl, TNF ligand superfamily member 6; P65, NF-κB subunit; S-Fu, 5-Fluorouracil; TS, thymidine synthase; Bim, Bcl-2 interacting mediator of cell death; PARP, poly ADP-ribose polymerase; Bid, BH3 interacting domain death agonist; ROS, reactive oxygen species; H3KK27ac, lysine H3K27 acetylation; DNMTs, DNA methyltransferases; HDACs, histone deacetylases; H3K27me3, trimethylation of lysine 27 on histone 3; PTX, paclitaxel; CDDP, Cisplatin; DOX, doxorubicin; $\uparrow$, indicates upregulation; $\downarrow$, indicates downregulation.
T98G glioblastoma cells in parallel with inhibiting both STAT3 and NF-κB activation (Table II) (96).

Effects and mechanisms of corosolic acid in neoplastic cell lines from osteosarcoma and lung metastasis. The response of osteosarcoma MG-63 cells to corosolic acid treatment has been previously reported (97,98). The results shared by both studies indicate that the viability of osteosarcoma MG-63 cells was significantly inhibited by corosolic acid (35 µM for 12 h, and 20, 30 and 40 µM for 24 h), and that corosolic acid induced apoptosis through the activation of caspases-3 and -9 to cause mitochondrial dysfunction (97,98). Moreover, using human osteosarcoma Saos2 and HSOS-1 cell lines and the murine osteosarcoma LM8 cell line, Horlad et al (52) reported that treatment with 30 µM corosolic acid for 24 h inhibited lung metastasis by inhibiting STAT3 activation, increasing the number of infiltrating lymphocytes in the tumor tissues and abrogating the immunosuppressive effect of myeloid-derived suppressor cells (MDSCs) through the decreased expression of cyclooxygenase-2 (COX-2) and chemokine (C-C motif) ligand 2 (CCL2) mRNA in these MDSCs (52) (Table II).

Effects and mechanisms of corosolic acid in the lung cancer A549 cell line. Corosolic acid (10-40 µM for 6-48 h) had a significant inhibitory effect on A549 cells, a human lung adenocarcinoma cell line, in a concentration- and time-dependent manner (99). Exposure to corosolic acid induced cell cycle arrest at the sub-G1 stage and caused apoptotic death in A549 cells (99). In addition, corosolic acid also activated caspases-3/-7, -8 and -9 and poly (ADP-ribose) polymerase, and increased the levels of reactive oxygen species (ROS). Corosolic acid-induced apoptosis was inhibited by exposure to the ROS scavenger N-acetylcysteine (99). These results indicate that corosolic acid induced apoptosis through mitochondria-mediated and caspase-dependent processes in a ROS-dependent manner (99). In addition, under CoCl2-stimulated hypoxic conditions, corosolic acid (IC50 of 12.5 µg/ml for 48 h) induced marked cytotoxicity in cancerous cells, and its action was associated with the suppressed expression of hypoxia-inducible factor-1 α and β and its downstream target genes (Table II) (100).

Effects and mechanisms of corosolic acid in the retinoblastoma Y79 cell line. The response of human retinoblastoma Y-79 cells to corosolic acid was investigated (101). The results showed that corosolic acid (10 µM for 24 h) could induce cell cycle arrest and apoptosis through its cytotoxic activity (IC50 of 4.15 µM for 24 h or 3.37 µM for 48 h) in a dose-dependent manner (101). The results also showed that the transcriptional activity of forkhead box M1 (FoxM1) was self-induced or driven by maternal embryonic leucine-zipper kinase (MELK), and that corosolic acid inhibited the expression levels of MELK and FoxM1 (101). The report established a promising therapeutic target of human retinoblastoma via MELK-FoxM1 signaling (Table II) (101).

3. Corosolic acid exerts anticancer effects in vivo

Banno et al (37) published the first study on the cancer-preventing and anti-inflammatory activities of corosolic acid in vivo. Corosolic acid exhibited a marked anti-inflammatory effect, with an IC50 of 0.09-0.3 mg per ear on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation (1 µg/ear) in mice; however, corosolic acid with an IC50 of 0.09-0.3 mg per ear did not exhibit an anticancer activity in a mouse tumor model. In vivo experiments in a murine sarcoma model showed that subcutaneous tumor development and lung metastasis was significantly suppressed by orally administered corosolic acid (17.5 mg/kg, 2 times/week for 21 days) (102). Corosolic acid was indicated as a potential new anticancer agent, as it targets macrophage polarization (102). In a murine osteosarcoma model, it was shown that orally administered corosolic acid (17.5 mg/kg/day for 7 days) significantly suppressed subcutaneous tumor development and pulmonary metastasis (52). It was also indicated that corosolic acid has a potential anticancer effect through targeting macrophage polarization and the immunosuppressive activity of MDSCs (52) Corosolic acid (20 µM) also displayed synergistic effects with anticancer agents, such as adriamycin (10 µM) and cisplatin (10 µM) after 24 h (52). In a mouse model of colon carcinoma, 5 and 25 mg/kg/day corosolic acid, administered via a peritumoral injection for 12 days inhibited allograft colon tumor growth. The results found that corosolic acid reduced the final tumor volume and the blood and lymphatic vessel densities of tumors, indicating that it suppresses in vivo angiogenesis and lymphangiogenesis (53). This was the first report of the anti-angiogenic and anti-lymphangiogenic effects of corosolic acid (53). Ma et al (89) established a xenograft tumor model of castration-resistant prostate cancer, and 10 and 20 mg/kg corosolic acid every 2 days for 14 days, administered via an intraperitoneal injection, was found to reduce tumor growth. Ku et al (48) reported that 5 mg/kg/day corosolic acid for 21 days effectively inhibited HCC Huh7 tumor growth in a male NOD/SCID mice model, and combined treatment of corosolic acid with sorafenib showed a synergistic inhibitory effect on tumor growth (corosolic acid 2.5 mg/kg/day with sorafenib 10 or 20 mg/kg/day) compared with corosolic acid alone, for 21 days in a mouse model (Table III).

4. Corosolic acid exerts synergistic anticancer activity with chemotherapeutic drugs

Accumulating experimental evidence has highlighted the pivotal role of STAT3 activation in the resistance to chemotherapy and radiotherapy in the thyroid cancer-derived CD133+ cells (103) and human epithelial ovarian cancer cells (104). It is thought that inhibiting STAT3 might be effective for treating patients with malignant tumors (103-105). A report by Fujiwara et al (95) suggested that 20 µM corosolic acid, as a selective STAT3 inhibitor, is able to increase sensitivity to chemotherapeutic agents, including paclitaxel (10 µM), cisplatin (10 µM) and doxorubicin (10 µM), in epithelial ovarian cancer SKOV3, RMG-1 and ES-2 cell lines for 24 h. In addition, the results of a study by Lee et al (81) showed that 25 µM corosolic acid enhances the inhibitory effect on human gastric cancer NCI-N87 cell proliferation when combined with adriamycin (0.01 to 2 mg/ml) and 5-FU (0.1 to 50 mg/ml), but not with docetaxel (0.01 to 2 mg/ml) or paclitaxel (0.01 to 6 mg/ml). Lee et al (106) indicated that corosolic acid...
ZHAO et al: ANTICANCER ROLE OF COROSOLIC ACID

(50 µM) enhances the anticancer activity of 5-FU (20 µg) after 24 h in human gastric carcinoma SNU-620 cells in an mTOR inhibition-dependent manner. In addition, a report by Fujiwara et al (102) showed that corosolic acid (20 µM) also displayed synergistic effects with anticancer agents, such as adriamycin (10 µM) and cisplatin (10 µM) 24 h. Furthermore, in a study by Park et al (107), a 5-FU-resistant gastric cancer cell line (SNU-620/5-FUR) was established, which had a marked reduced AMPK phosphorylation when compared with the parental cell line, SNU-620. Cell treatment with 25 µM corosolic acid for 24 h was found to enhance the chemosensitivity of 5-FU-resistant gastric cancer cells, and the reduction of AMPK phosphorylation by compound c (AMPK inhibitor) was revealed to be associated with increased 5-FU-resistant cell viability (107). Corosolic acid treatment significantly reduced cell viability while compound c reversed corosolic acid-induced cell growth inhibition (107). The corosolic acid-induced AMPK activation was markedly increased by additional 5-FU treatment, while compound c reversed AMPK phosphorylation (107). These results imply that corosolic acid can activate AMPK and sensitize gastric cancer to 5-FU (150 µM; Table II).

5. Corosolic acid exerts anti-inflammatory and anti-MS effects

Nelson et al (108) first reported that corosolic acid (2 µmol twice-weekly over a 2-week period) may be an effective anti-inflammatory agent. Yamaguchi et al (109) further explored corosolic acid isolated from Banaba leaves and found that it prevented oxidative stress and reduced the inflammation caused by MS. In SHR-cp rats with characteristics that included obesity, hyperglycemia, hyperlipidemia, hypertension, hyperinsulinemia, oxidative stress and inflammation, a

---

Table III. Summary of the effects and mechanisms of corosolic acid in different types of cancer in vivo.

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Cancer model type</th>
<th>Corosolic acid mechanism of action</th>
<th>Corosolic acid dose; administration</th>
<th>Effects</th>
<th>(Refs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horlad et al, 2013</td>
<td>Murine sarcoma xenograft model</td>
<td>↓ STAT3 activation, ↑ CD4⁺ and CD8⁺ lymphocytes, ↓ the suppressive effect of MDSC</td>
<td>17.5 mg/kg/day; oral</td>
<td>Impaired subcutaneous tumor development and lung metastasis</td>
<td>(52)</td>
</tr>
<tr>
<td>Ku et al, 2015</td>
<td>Mouse HCC Huh7 xenograft model</td>
<td>VEGFR2/Src/FAK pathway (↓ VEGFR2, Src, FAK, ↓ phosphorylation of VEGFR2 and FAK)</td>
<td>5 mg/kg/day; intraperitoneal injection</td>
<td>85% reduction in tumor mass compared to the control group</td>
<td>(48)</td>
</tr>
<tr>
<td>Yoo et al, 2015</td>
<td>Mouse xenograft colon CT-26 model</td>
<td>Anti-angiogenic and anti-lymphangiogenic effects</td>
<td>5 or 25 mg/kg/day; peritumor rejection</td>
<td>Reduced the final tumor volume and blood and lymphatic vessel density of tumors</td>
<td>(53)</td>
</tr>
<tr>
<td>Ma et al, 2018</td>
<td>Mouse xenograft PC-3 model</td>
<td>ER stress, IRE-1/ASK1/JNK signaling pathway, and PERK/EIF2α/ATF4/CHOP signaling pathway (↑ IRE-1, PERK, CHOP, TRIB3; ↓ AKT)</td>
<td>10 or 20 mg/kg/2 days; intraperitoneal injection</td>
<td>Reduced the final tumor volume in a dose-dependent manner</td>
<td>(89)</td>
</tr>
<tr>
<td>Fujiwara et al, 2014</td>
<td>Mouse LM8 sarcoma model</td>
<td>Inhibits macrophage polarization to M2 phenotype by suppressing STAT3 activation</td>
<td>17.5 mg/kg, 2 times/week; oral</td>
<td>Reduced the final tumor volume</td>
<td>(102)</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; MDSCs, myeloid-derived suppressor cells; Src, steroid receptor coactivator; FAK, focal adhesion kinase; cdc42, cell division cycle42; ER, endoplasmic reticulum; STAT3, signal transducer and activator of transcription 3; VEGFR2, VEGFR, vascular growth factor receptor 2; IRE-1, inositol-requiring ER-to-nucleus signal kinase 1; ASK1, apoptosis signal regulating kinase 1; JNK, Jun N-terminal kinase; PERK, protein kinase RNA-like ER kinase; eIF2α, eukaryotic initiation factor 2α; ATF4, activating transcription factor 4; CHOP, C/EBP-homologous protein; TRIB3, tribbles pseudo-kinase 3; ↑, indicates upregulation; ↓, indicates downregulation.
diet rich in 0.072% corosolic acid for 14 weeks ameliorated hypertension, regulated hyperlipidemia, prevented oxidative stress and ameliorated inflammation (109). A report by Chen et al (110) suggested that 6 µM corosolic acid treatment for 30 min was able to inhibit monocyte chemotactrant protein-1 expression, and that 2 µg/kg/day corosolic acid for 10 days ameliorated atherosclerosis by regulating the nuclear factor-kB signaling pathway in apolipoprotein E-deficient mice. Furthermore, Kim et al (111) reported that exposure of lipopolysaccharide (LPS)-pretreated bone marrow-derived monocytes to corosolic acid downregulated the NF-kB target genes pyrin domain-containing protein 3 (NLRP3) and interleukin-1 (IL-1), which was similar to the effects observed for LPS-pretreated bone marrow-derived monocytes with an inhibitor of IL-1 receptor-associated kinase (IkBα). Ser phosphorylation were abolished, indicating that corosolic acid ameliorated IR and inhibited inflammation through the activation of AMPK in a liver kinase B1-dependent manner (27) (Fig. 3).

6. Proposed mechanisms underlying the inhibition of NAFLD-related HCC progression by corosolic acid

The characteristics of NAFLD include obesity, IR, hypertension and dyslipidemia, which are also the most common characteristics observed in livers affected by MS (112). Furthermore, the development of NAFLD-related HCC is increasingly recognized, since patients with NAFLD are at high risk of developing HCC (112). NAFLD-associated HCC has been estimated to account for 10-12% of HCC cases in Western populations and 1-6% of HCC cases in Asian populations from 42 sites in 14 countries from 2005 to 2012 (58). Based on multiple studies, accumulated evidence has suggested that type 2 diabetes mellitus (T2DM) and obesity are independent risk factors for the development of HCC in patients with NAFLD.
activity (26,73,74). On the other hand, as aforementioned, corosolic acid has shown an ability to modulate multiple cancer-related signaling pathways, including the adenosine monophosphate-activated protein kinase (AMPK), NF-κB, PI3K/Akt/mTOR, Wnt/β-catenin, FAK, ERK1/2, STAT3 in MDSCs, Nrf2/HO-1 and numerous other signaling pathways associated with cell proliferation and cell death, among other cellular processes in multiple types of malignant tumors (as observed in preclinical in vitro and in vivo experiments) (48,49,51,52,54,55,81-83,91-96,107). Due to its anticancer and anti-immunity activities, corosolic acid has attracted growing attention. A schematic plot of the proposed mechanisms of the corosolic acid-induced inhibition of NAFLD-related HCC progression is presented in Fig. 5. The release of an increased number of proinflammatory cytokines, such as TNF-α and IL-6, NF-κB, JNK1, STAT3 and mTOR. (B) Corosolic acid ameliorates IR through the activation of AMPK, and downregulates IGF-1, c-fos, c-Jun and JNK1. (C) Corosolic acid downregulates the activation of P38 and JNK1, and increases the number of infiltrating CD4+ T lymphocytes via the inhibition of ROS. (D) Corosolic acid increases the number of infiltrating CD4+ T lymphocytes, CD8+ T lymphocytes and NK cells. NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; IR, insulin resistance; AMPK, adenosine monophosphate-activated protein kinase; IL-6, interleukin-6; NF-κB, nuclear factor kappa-B; TNF-α, tumor necrosis factor-α; JNK1, Jun N-terminal kinase 1; mTOR, adenosine monophosphate-activated protein kinase; STAT3, signal transducer and activator of transcription 3; IGF-1, insulin-like growth factor-1; AMPK, adenosine monophosphate-activated protein kinase; ROS, reactive oxygen species; NK, natural killer; ↑, indicates upregulation; ↓, indicates downregulation.
factor-α (TNF-α) and interleukin-6 (IL-6), is promoted by obesity and adipose tissue expansion (62). NF-κB, JNK, mTOR and extracellular signal-related kinases, such as those associated with pro-oncogenic pathways, are stimulated by TNF-α (63). It is highly likely that hepatocytes with previously acquired oncogenic mutations will continue the malignant transformation that is induced by the chronic activation of the IL-6/STAT3 axis (64). As aforementioned, as an agent with anticancer and anti-inflammatory activity, corosolic acid plays vital roles in the inhibition of proinflammatory cytokine and mTOR expression, and the downregulation of ERK, while as a STAT3 and NF-κB inhibitor, it can enhance anticancer activity (53,95,111). NAFLD promotes systemic and hepatic IR with the resultant hyperinsulinemia-activated proinflammatory cytokines and lipotoxic activity in obesity and T2DM (112). A previous report showed that the production of IRS-1 and insulin-like growth factor-1 (IGF-1) was increased by IR and hyperinsulinemia (65). IGF-1 promotes cell proliferation, inhibits apoptosis and stimulates cell growth (65). Furthermore, IGF-1 contributes to the upregulated expression of the proto-oncogenes c-fos and c-Jun in vitro, and the downregulation of AMPK, which is associated with the development of HCC (66). JNK, another important intracellular marker, is closely linked to obesity, IR, NAFLD and HCC (67). It has also been indicated that JNK-induced phosphorylation and activation of IRS-1 are responsible for obesity-induced IR (67). A report by Chang et al (68) showed that JNK signaling might play a pivotal role in hepatocarcinogenesis, where an increased JNK1 activation was detected by immunostaining in 17/31 HCC samples relative to their paired adjacent normal tissues. In addition, recent studies have revealed the potential role of the adaptive immune system in the development of NAFLD-related HCC (71,72). A report by Ma et al (71) revealed that hepatocytes exhibit increased linoelic acid secretion and mitochondria-derived ROS, both of which led to enhanced carcinogenesis. The same report also found that CD4+ T lymphocytes have greater mitochondrial mass than CD8+ T lymphocytes and generate higher levels of mitochondrially derived ROS. The disruption of mitochondrial function by free fatty acids such as palmitic acid accumulated in NAFLD, caused more oxidative damage and in turn promoted selective depletion of CD4+ T lymphocytes. In addition, blockade of ROS reversed NAFLD-induced hepatic CD4+ T lymphocyte decrease and delayed NAFLD-promoted HCC in mouse models of NAFLD-associated HCC. Wolf et al (72) developed a mouse model recapitulating key features of human metabolic syndrome, non-alcoholic steatohepatitis, and HCC by feeding mice a diet mimicking metabolic syndrome when administered along with chemotherapeutic drugs, even in drug-resistant cells. In addition, parts of the same corosolic acid mechanism that ameliorates MS also induces anticancer activity and suppresses the progression of NAFLD-related HCC.

Therefore, corosolic acid, a potential tool in MS treatment, is being considered as a possible agent in NAFLD-related HCC prevention and treatment (Figs. 3 and 5).

**Acknowledgements**

Not applicable.

**Funding**

This work was supported by the Fund for Science & Technology Development of Jilin Province (grant nos. 20200201544JC, 20160101060JC and 20150101108JC), the National Key R&D Program of China (grant nos. 2017YFD0502200 and 2016YFD0501302), and the Project of the Education Department of Jilin Province (grant no. 2016444).

**Availability of data and materials**

Not applicable.

**Authors' contributions**

JZ, HZ, YA and KS participated in the design and interpretation of the studies, the revision of the manuscript. JZ, HZ, YA and KS wrote the review. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**


Mitogenic properties of dual inhibition of Raf and Nrf2 redirects stress.


Yong QX, Jian HZ, and Xing SY: Corosolic acid impairs tumor development and lung metastasis by inhibiting the immunosuppressive activity of myeloid-derived suppressor cells. Mol Nutr Food Res 57: 1046-1054, 2013.


