REVIEW

Honokiol targets mitochondria to halt cancer progression and metastasis

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Cancer continues to be the leading cause of death worldwide. Plants have a long history of use in the treatment of cancer. Honokiol (HNK) is an important bioactive compound found in the bark of Magnolia tree, and has been shown to inhibit cancer growth and metastasis in many cell types in vitro and in animal models. Resistance to chemotherapy and radiotherapy is the major obstacle for cure of cancer. Combination of HNK with many traditional chemotherapeutic drugs as well as radiation sensitizes cancer cells to apoptotic death, suggesting that HNK not only directly inhibits primary cancers and metastasis, but also has potential to overcome drug resistance. Ultimately, this may mean that HNK could be combined with traditional chemotherapies administered at lower doses to significantly reduce toxicity, meanwhile enhance efficacy. As a natural compound, HNK is composed of polyphenols and has been described in many studies targeting multiple key cell signaling molecules. Mitochondria are the main hub for cellular energy production and play an important role in cell survival, and are the key target identified for HNK to mediate cancer cell death, survival, and metastasis. In this review, we have summarized different aspects of HNK's anti-cancer effects from recent accumulated literature, as well as the underlying molecular mechanisms. This review is primarily focused on the effects of HNK on epidermal growth factor receptor (EGFR) and signal transduction and activator of transcription 3 (STAT3) signaling, as well as the broader regulation of mitochondrial function and cancer cell metabolism.

Keywords:
Honokiol / Mitochondria / Cancer Progression / Cancer Metastasis / cancer cell metabolism

1 Background

Cancer continues to be the leading cause of death worldwide. Current estimates from the American Cancer Society and from the International Union Against Cancer indicate that 12 million cases of cancer were diagnosed last year, with 7 million deaths worldwide; these numbers are expected to double by 2030 (27 million cases with 17 million deaths) [1]. Natural products have been at the core of cancer chemotherapy for the past several decades [1]. HNK is a key bioactive compound present in Magnolia bark extracts, which has been used as a folk remedy for centuries in China, South Korea, and Japan to treat gastrointestinal disorders, cough, anxiety, stroke, and

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Abbreviations: **HNK**, Honokiol; **EGFR**, Epidermal growth factor receptor; **STAT3**, signal transduction and activator of transcription 3; **BBB**, blood-brain barrier; **BCSFB**, blood-cerebrospinal fluid barrier; **mTor**, mammalian target of rapamycin; **UAB**, Ultraviolet B; **DMBA**, 7,12-dimethylbenz[a]anthracene; **OCR**, oxygen consumption rate; **NF-kB**, nuclear factor kappa B; **TNF**α, Tumor necrosis factor α; **HCoEpiCS**, human colonic epithelial cells; **PGE2**, prostaglandin E2; **SCC**, squamous cell cancer; **VEGF**, vascular endothelial growth factor; **FDA**, Food and Drug Administration; **TMZ**, Temozolomide; **MGMT**, O6-methylguanine-DNA methyltransferase; **HUVECS**, human umbilical vein endothelial cells; **RCC**, Renal cell carcinoma; **CRT**, Calreticulin; **ER**, Endoplasmic reticulum; **CSC**, Cancer stem cell; **I.P**, intraperitoneal; **MAPK**, mitogen-activated protein kinase; **AKT**, v-akt murine thymoma viral oncogene homologue 1; **TPL2**, tumor progression locus 2; **EMT**, epithelial-mesenchymal-transition; **HNSCC**, Head and Neck Squamous Cell Carcinoma; **HCC**, Hepatocellular Carcinoma; **CNS**, central nervous system; **ROS**, Reactive oxygen species; **AMPK**, AMP-activated protein kinase; **MTAs**, Mitochondria-targeted agents; **TPP**+, Triphenylphosphonium ion; **ATP**, adenosine triphosphate

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allergic diseases [2]. Magnolia bark extract was found to be nontoxic in both short-term toxicity and subchronic toxicity studies [3]. No toxicity (i.e. body weight loss, mean daily food intake decrease, altered hematological values, serum biochemical values, and tissue pathologic changes) was observed when Sprague–Dawley rats were given HNK intravenously at doses ranging from 20 to 80 mg/kg body weight, once a day for 14 days [4]. Furthermore, HNK exhibit protective effects on chemical-induced hepatocyte [5] and neuronal injury models [6].

HNK has been reported to have a variety of broad mechanisms of action, such as anti-inflammatory activity [7,8], along with antiangiogenic [9] and cardio-beneficiating [7,10,11], and neuroprotective activity [6], without appreciable toxicity. HNK has also been identified as a naturally occurring PPAR-γ agonist; however, HNK does not appear to trigger adipogenesis-one of the primary side effects of other PPAR-γ agonists. In line with this, long-term consumption of HNK ameliorates body fat accumulation and insulin resistance [12]. In recent years, HNK has also been demonstrated as a potential antitumor agent in many cancer types.

2 Pharmacokinetics and metabolites

As previously reviewed [13], HNK has a rapid absorption (T_{max} = 20min) and slow elimination ($t_{1/2z}$ = 290min) after single dose of oral gavage at 40 mg/kg in healthy rats [14]. In a slimier study, rats were administrated with oral administration of Magnolol/HNK emulsion (4:1) at 50 mg/kg, HNK attained peak plasma concentration at 1.2 h, and half-life was 3.1 h, and the absolute bioavailability for HNK is calculated as 5.3% [15]. HNK distributed into the organs rapidly after oral administration in rats, with the highest concentrations being found in the liver, followed by brain and kidney [14], which is inconsistent with the finding in tumor-bearing mice, where highest C_{max} found in liver, but followed with kidney and lung [10], this may due to the species variation and also tumor-burdened mice may also affects drug distribution. Another study using iv administration of HNK, and found the organ distribution is in the order of: lungs > plasma > liver > brain > kidney > heart > spleen, and most importantly, reported for the first time that HNK can effectively cross both blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB). Metabolites of HNK were also intensively studies in recent years, liver is the major place for HNK's in vivo biotransformation, over 50 different metabolites were found using ^{13}C labeling approach [17–19], glucuronidation and sulfation are the main metabolic pathways for HNK [20], this extensive biotranformation of HNK may partially responsible for the low bioavailability, and further studies are warranted on whether these HNK metabolites has any biological activity, and whether these conjugation extend HNK's half-life and maintaining its biological properties [20].

3 Anticancer function of honokiol

HNK has been shown to effectively inhibit cell proliferation across multiple cancer cell lines in tissue culture and in xenographs. As reviewed previously [21], the range of disease sites that HNK appears to show efficacy against is broad, including bladder cancer, head and neck squamous cell carcinoma (HNSCC), chondrosarcoma, gastric cancer, pancreatic cancer, hepatocellular carcinoma, prostate cancer, colorectal cancer, oral cancer, and leukemia. In addition to its rapid absorption in multiple organs, HNK is also capable of crossing the BBB [16], which has made this natural product effective against cancers of the central nervous system, when many standard drugs are ineffective or less effective due to inability to cross the BBB [16]. In most of these studies, induction of cancer cell apoptosis is believed to be the primary means by which HNK acts to inhibit tumor growth. There are several potential molecular means by which HNK induces apoptosis, which may partially be dependent on the cell line or disease site tested. HNK promotes human glioblastoma cancer cell apoptosis via regulation of Ca(2+) channels, and also induces apoptotic death in neuroblastoma cells through a Bax-mitochondrion-cytochrome c-caspase protease pathway [22]. In a rat intracerebral gliosarcoma model, oral administration of HNK was demonstrated to reduce brain tumor growth by 50%, resulting in significantly improved overall survival [16]. HNK is also reported to be highly effective in inhibiting melanoma cancer cells growth by attenuating AKT/mammalian target of rapamycin (mTOR) and Notch signaling [23], and topical treatment with HNK protects skin against ultraviolet B (UVB) - induced or 7,12-dimethylbenz[a]anthracene (DMBA) / 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin cancer, delays the malignant progression of papillomas to carcinomas, and reduces skin tumor incidence by 40% [24].

Lung cancer is one of the most difficult to treat cancers; 5-year survival rates have remained near 15% for the past several decades. HNK has been reported to inhibit class I histone deacetylases [25], results in the cell cycle arrest, which ultimately leads to cell death in multiple non-small cell lung cancer (NSCLC) cell lines in tissue culture. In addition, HNK by oral administration significantly inhibited the growth of xenografted tumors produced by S.C. injection of A549 human lung adenocarcinoma cells into athymic nude mice [26]. HNK also triggers apoptotic death in human lung squamous cell cancer (SCC) cells by modulation of Bcl-XL and Bad proteins, release of mitochondrial cytochrome c and activation of caspase-3 [27]. We were the first to demonstrate that HNK is a potent chemopreventive agent against lung SCC development in a carcinogen-induced lung SCC murine model [28]. Our study also demonstrated for the first time that HNK may directly target the mitochondria. HNK treatment results in a decreased oxygen consumption rate (OCR) in whole intact cells, rapidly, and persistently inhibiting mitochondrial respiration, which leads to the induction of apoptosis in lung cancer cells and ultimately attenuates lung squamous

carcinoma growth in a chemically induced lung SCC murine model [28].

4 Honokiol as a chemo- and radiosensitizer

Chemotherapy and radiotherapy remain the major treatment options for many types of cancer, however, the emergence of drug-resistant cancer cells and strong side effects of these cytotoxic chemotherapies have greatly limited their clinical efficacy. It is therefore imperative to develop novel strategies to overcome chemoresistance in cancer, while reducing toxicity of chemotherapeutic drugs to enhance their antitumor efficacy.

Recent studies have revealed several critical survival signaling elements that are important not only for cancer progression, but which also confer chemoresistance. Some of these signaling pathways include mediators such as Ras, Akt, and nuclear factor kappa B (NF-κB) [29–31]. It has been shown that targeting some of these signaling nodes can be useful in inhibiting tumor growth and progression as well as in restoring the sensitivity of tumor cells to the cytotoxic drugs [32, 33]. The striking aspect of HNK as an anticancer drug is its potential to inhibit multiple important survival pathways, such as NF-κB and Akt [34, 35]. Therefore, various studies were conducted to investigate the possibility of HNK as sensitizer for traditional chemotherapy or radiotherapy (Summarized in Table 1).

Combined treatment with HNK and paclitaxel synergistically augmented cytotoxicity in the multi-drug resistant human squamous cancer cell line KB compared with treatment with either agent alone in vitro [36]. Importantly, this combined treatment significantly inhibited in vivo growth of KB-8-5 cervical tumors in a xenograft model [36]. HNK also effectively sensitizes tumor necrosis factor α (TNFα)-induced apoptosis by inhibiting TNFα-induced nerve growth factor IB expression in breast cancer cells [37]. The combination of HNK with cisplatin exhibits enhanced antitumor activity against human lung cancer in a human lung tumor cell line (A549) xenograft model, human ovarian carcinoma mouse model, and colon cancer models [26]. Oxaliplatin is another platinum-based chemotherapeutic drug, widely used in the treatment of colorectal carcinomas, and clinically is not well tolerated due to its toxicity, especially dose-related neurosensory toxicity [38]. HNK could significantly enhance the antiproliferative effects of oxaliplatin in human colon cancer cells, while exhibiting no toxicity to normal human colonic epithelial cells (HCoEpiCs) [39]. Oxaliplatin combined with HNK also dramatically improved the induction of apoptosis in a human colorectal adenocarcinoma cell line (HT-29) and reduced prostaglandin E2 (PGE2) and vascular endothelial growth factor (VEGF) secretion levels [39]. Therefore, HNK can enhance the chemotherapeutic effect of oxaliplatin, while reduce its adverse effects by allowing lower doses of oxaliplatin to be used with greater efficacy.

Gemcitabine, a standard U.S. Food and Drug Administration (FDA) approved drug for pancreatic cancer therapy, is reported to be minimally effective, and improves patient survival on the order of weeks only [40]. HNK significantly potentiates the cytotoxic effects of Gemcitabine, in part, by restricting the Gemcitabine-induced nuclear accumulation of NF-κB in the treated pancreatic cancer cell lines [41]. HNK combined with gemcitabine also was found to synergistically inhibit the proliferation of human Burkett lymphoma cells and induces their apoptosis [42].

Temozolomide (TMZ) is an alkylating chemotherapeutic agent that is currently used for the treatment of glioblastoma [43, 44]. The therapeutic benefit of TMZ depends on its ability to alkylate/methylate nucleic acids. Glioma cancer stem cells upregulate O^6-methylguanine-DNA methyltransferase (MGMT), a DNA repairing enzyme to remove TMZ-DNA adduct, which may diminish the therapeutic efficacy of TMZ and confer the resistance to TMZ therapy [45, 46]. Combination of TMZ with HNK significantly enhances the induction of apoptosis, downregulation of Notch3 and its downstream expression of Hes1 in glioma cancer stem cells, and sensitizes TMZ-resistant cancer cells, suggesting that HNK might have clinical benefits for the glioblastoma patients who are refractory to TMZ treatment [47].

The combination of HNK plus cetuximab, another FDA-approved EGFR inhibitor for HNSCC, significantly enhanced growth inhibition in HNSCC cells [48]. Erlotinib-resistant HNSCC cells retain sensitivity to the growth inhibitory effects of HNK, suggesting that HNK may be an effective therapeutic agent in HNSCC, in which it can augment the effects of EGFR inhibitors and overcome EGFR inhibitor resistance [48]. Combination of HNK with adriamycin, or EGFR inhibitor lapatinib, or mTOR inhibitor rapamycin presented synergistic effects on induction of apoptosis breast cancer cells [49]. HNK alone also is effective against both adriamycin-resistant and tamoxifen-resistant breast cancer cell lines [50].

HNK, was also found to effectively radiosensitize colorectal cancer cells both in vitro and in vivo [51] Using mismatch repair defective (HCT116) and proficient (HCT116-CH3) colorectal cancer cell lines, combination of HNK and γ-irradiation treatment shows that HNK is highly effective in radiosensitizing colorectal cancer cells, especially those with a mismatch repair defect [52]. The radiosensitizing effect of HNK on lung carcinoma was also investigated by using liposomal HNK in Lewis lung carcinoma cells (LL/2) both in vitro and in vivo, treatment with Lipo-HNK for 24 h showed a radiation enhancement ratio of 1.9 compared to radiation treatment alone, and also caused an 8.7 day delay in tumor growth [53].

5 Anti-metastatic function of honokiol

HNK exhibits a biphasic pharmacokinetic profile, characterized by rapid distribution to major organs in the following order: lungs > plasma > liver > brain > kidney > heart >

Table 1. Honokiol as a chemo and radiation sensitizer

Chemo/radio therapeutics	Cancer types	Effects	Reference
Paclitaxel	Multi-drug resistant human cervical squamous Cancer	Synergistically augmented cytotoxicity	[37]
TNF-α	Breast cancer	Synergistically induced apoptosis	[38]
Cisplatin	Human lung cancer colon cancer, ovarian carcinoma	Synergistically inhibited angiogenesis and induced apoptosis	[27, 68, 115]
Oxaliplatin	Human colorectal adenocarcinoma	Significantly enhanced the anti-proliferative effects of oxaliplatin and improved the induction of apoptosis, while exhibiting no toxicity to normal human colonic epithelial cells	[40]
Gemcitabine	Human pancreatic cancer, human Burkett lymphoma	Significantly potentiated the cytotoxic effects of Gemcitabine	[42, 43]
Temozolomide (TMZ)	Human glioblastoma	Significantly enhanced the induction of apoptosis sensitizes TMZ-resistant cancer cells.	[48]
Cetuximab	Head and neck squamous cell carcinoma	Enhanced the growth inhibitory activity of cetuximab	[49]
Adriamycin	Human breast cancer	Synergistic suppression of tumor progression in vitro and in vivo	[49]
Lapatinib	Human breast cancer cell line SKBR-3	Synergistic cell growth inhibition and induction of apoptosis	[51]
Rapamycin	Human breast cancer	Synergistic induction of apoptosis	[51]
Radiation	Human colon cancer	Significantly reduced tumor burden	[52, 115]
	Human colon cancer	Highly effective in radiosensitizing colorectal cancer cells, especially those with a mismatched repair defect	[53]
	Lewis lung carcinoma	Enhanced radiation treatment and delayed tumor growth	[54]

spleen, followed by a slower elimination phase [54, 55]. Therefore, its' potential anti-metastatic activity was also explored by multiple groups (Summarized in Table 2), with the majority of these studies being conducted at the in vitro tissue culture level. Treatment with HNK significantly reduces the adhesion of glioblastoma cells (T98G) to human umbilical vein endothelial cells (HUVECs), and inhibits the invasion of T98G cells [56]. HNK inhibits cancer cell migration by targeting nitric oxide and cyclooxygenase-2 or Ras GTPase-activating-like protein (IQGAP1) [57]. HNK can induce autophagy of neuroblastoma cells and consequent apoptosis through activating the PI3K/Akt/mTOR and ERS/ROS/ERK1/2 signaling pathways and suppressing cell migration [58]. Renal cell carcinoma (RCC) is another form of cancer that is known to have a high risk of metastasis [59]. HNK suppresses the multistep process of RCC metastasis, including invasion and colony formation, by activation of KISS1/KISS1R signaling, suggesting HNK may be a natural agent against RCC metastasis [60].

Many studies demonstrate that HNK can reverse the epithelial-mesenchymal-transition (EMT) process, which is a key step during embryogenesis, cancer invasion, and metastasis, and is an attractive target for therapeutic interventions directed against tumor metastasis [61, 62]. HNK treatment significantly downregulates Calreticulin (CRT) in highly metastatic gastric cancer cell lines grown in vitro and metastatic animal by activating endoplasmic reticulum (ER) stress, and blocking EMT. HNK also inhibits EMT in breast cancer cells, results in significant downregulation of mesenchymal marker proteins and concurrent upregulation of epithelial markers [63]. Experimental EMT induced by exposure to TGF-β and TNF-α in spontaneously immortalized nontumorigenic human mammary epithelial cells can be completely reversed by HNK treatment as evidenced by morphological as well as molecular changes [64]. HNK effectively blocked human glioblastoma cell U87MG invasion by reducing membrane permeability and the EMT [65]. HNK reduced the expression levels of Snail, N-cadherin and β-catenin, which are mesenchymal markers, but increased E-cadherin, an epithelial marker, resulting in the inhibition of metastasis by targeting the interaction between U87MG and bone marrow microvascular endothelial cells (BMECs), regulating the adhesion of U87MG to BMECs by inhibiting VCAM-1, and regulating the invasion of U87MG through BMECs by reducing membrane permeability and EMT processes of U87MG cells [65]. HNK inhibits NSCLC cell migration by targeting PGE2-mediated activation of β-catenin signaling [66]. Leptin, a major adipocytokine produced by adipocytes, is emerging as a key molecule linking obesity with breast cancer, and it is important to find effective strategies to antagonize oncogenic effects of leptin to disrupt the obesity-cancer axis [67, 68]. Recent studies on breast cancer cells provide evidence that HNK is a potential leptin-antagonist, which

Table 2. Anti-metastatic function of Honokiol

Cancer types	Effects	Reference
Brain	Significantly reduced the adhesion of glioblastoma cells (T98G) to human umbilical vein endothelial cells (HUVECs), and inhibited the invasion of T98G cells	[55]
	Inhibited migration of neuroblastoma cells	[57]
	Inhibited invasion process of U87MG human glioblastoma cells through brain microvascular endothelial cells (BMECs) by reducing membrane permeability and EMT processes of U87MG cells	[64]
Renal	Suppressed invasion and colony formation by activation of KISS1/KISS1R signaling	[59]
	Suppressed renal cancer cells ' metastasis via blocking EMT and suppressing cancer stem cell (CSC) characteristics	[70]
Lung	Significantly inhibited tumor-associated lymphangiogenesis and metastasis with a remarkable delay in tumor growth and prolonged life span	[73]
	Inhibited in vitro cell migration by targeting PGE2-mediated activation of β-catenin signaling	[65]
Gastric	Significantly downregulated Calreticulin (CRT) in highly metastatic gastric cancer cell lines grown in vitro and metastatic animal by activating endoplasmic reticulum (ER) stress, and blocking EMT. Also inhibited EMT in breast cancer cells, results in significant downregulation of mesenchymal marker proteins and concurrent upregulation of epithelial markers	[62]
	Significantly decreased tumor growth, peritoneal dissemination, and peritoneum or organ metastasis by induction of ER stress	[63]
Breast	Inhibited migration by targeting nitric oxide and Cox-2 or Ras GTPase-activating-like protein (IQGAP1)	[56]
	Inhibited leptin-, TGF-β- or TNF-α-induced EMT, cell migration, and invasion in vitro and in vivo, along with a reduction in the expression of stemness factors	[63, 68, 69]
Bone	No inhibition on the primary tumor, but reduced lung and liver micrometastases and macrometastases by 69 and 80%, respectively	[72]
	Inhibited the growth of metastatic human prostate cancer cells grafted into bone	[74]

inhibits leptin-induced EMT, and mammosphere-formation along with a reduction in the expression of stemness factors, Oct4, and Nanog, and more strikingly, inhibits leptin-induced breast cancer cell migration invasion and leptin-induced breast-tumor-xenograft growth by upregulating miR-34a in an LKB1-dependent manner [69]. Mechanistic studies revealed that HNK inhibits phosphorylation and activation of key molecules of leptin-signaling pathways, such as Wnt1 and β-catenin [70]. HNK was also reported to suppress renal cancer cells ' metastasis via blocking EMT and suppressing cancer stem cell (CSC) characteristics [71].

HNK's anti-metastatic function has been further tested in several mouse models. HNK significantly decreased tumor growth, peritoneal dissemination, and peritoneum or organ metastasis of orthotopically implanted human gastric cancer MKN-45 cells by induction of ER stress [72]. HNK-treated tumors showed increased signatures such as E-cadherin, cytokeratin-18 and ER stress marker, and decreased expression of vimentin, Snail and tumor progression locus 2 (Tpl2) [72]. Antimetastatic activity of HNK in osteosarcoma was tested in syngraft C3H mice model subcutaneously injected with LM8 osteosarcoma cells, daily intraperitoneal treatment of mice with HNK showed no inhibition on the primary tumor, however, treatment reduced the number of micrometastases and macrometastases in both lung and liver by 69 and 80%, respectively [73]. The authors concluded that HNK has considerable potential for the treatment of metastasizing osteosarcoma. Lymph node metastasis of tumor could be a crucial early step in the metastatic process [74]. Liposomal HNK significantly inhibited tumor-associated lymphangiogenesis and metastasis in a Lewis lung carcinoma orthotopic model, which was also associated with a remarkable delay in tumor growth and prolonged life span [74]. In an experimental prostate cancer bone metastasis murine model, androgen-independent prostate cancer cell PCa, C4-2 cells were implanted directly into the bones of mice. Daily intraperitoneal (i.p.) injection of HNK inhibited the growth of metastatic human prostate cancer cells grafted into bone, while also suppressing serum prostate-specific antigen (PSA), a broad circulating marker that can be associated with prostate cancers [75]. These findings have increased interest in bringing HNK to the clinic as a novel chemotherapeutic agent. HNK, either alone or in combination with other therapeutics, has the potential to become a new, promising approach for cancer treatment based on its ability to inhibit both primary and metastatic tumors, overcome drug resistance, and diminish toxicity associated with high dose usage of chemotherapeutic drugs.

6 Molecular mechanism of actions of honokiol

The molecular mechanisms of action of HNK have been described in many research as well as review papers. HNK is a natural compound composed of polyphenols that tend to have many potential targets, so all the suggested mechanisms of action of HNK could be physiologically relevant (as

Table 3. Molecular mechanisms of HNK in cancers

Tissue types	Molecular targets	Effects
HNSCC, breast cancers	EGFR phosphorylation	HNK binds to EGFR, inhibits HNSCC progression through inhibition of EGFR-mTOR signaling pathway (25) HNK inhibited HNSCC and breast cancer progression through inhibition of EGFR phosphorylation [26,27]
HCC, breast cancers, lung cancers	STAT3 phosphorylation	HNK inhibited HCC, breast cancers, and lung cancers progression through inhibition of STAT3 phosphorylation [28,29]
Brain metastatic lung cancers	STAT3 phosphorylation	HNK inhibited STAT3 phosphorylation and STAT3 knock-down abrogates the anti-metastatic effects of HNK in brain metastatic lung cancer cells both in-vitro and in-vivo (to be published)
Lung cancers	Mitochondria	HNK inhibited lung cancers through mitochondria-mediated apoptosis via AMPK activation (34)
Brain metastatic lung cancers	Mitochondrial complex I subunits	HNK inhibited lung cancer brain metastasis in-vivo through inhibition of mitochondrial complex I subunits expression (to be published)

shown in Table 1). However, many research papers used non-physiologically achievable doses to show the effects of HNK in-vitro and in-vivo mouse models, so it is also possible that some of the suggested mechanisms of actions of HNK may not work in humans. Nevertheless, HNK has been described as nontoxic and safe natural agents with superior effects in many types of cancers, including late stage cancers. In this review, we will focus on the effects of HNK in EGFR, STAT3, and especially mitochondrial function that recently have been shown to be a target of HNK in both primary and metastatic cancers (Summarized in Table 3).

6.1 Effects of HNK in the inhibition of EGFR signaling pathway in cancers

EGFR is 170 kDa transmembrane receptor tyrosine kinases (RTKs) that are frequently deregulated in many types of cancers via either mutation or over-expression [76]. Over-expression or activating mutations in EGFR can lead to abnormal metabolism, increased cell proliferation, and cell survival through activation of the downstream mitogen-activated protein kinase (MAPK) and v-akt murine thymoma viral oncogene homolog 1 (AKT) signaling pathways [77–81]. It is also estimated that 40–50% of lung cancer patients and significant number of other types of cancer patients develop brain metastasis during a typical course of the disease [82,83].

HNK has been shown to inhibit EGFR signaling pathway through either inhibition of EGFR expression [84] or inhibition of EGFR phosphorylation [48,85]. Singh, et al. [84] demonstrated that HNK inhibits HNSCC in both in-vitro and in-vivo xenograft mouse models via inhibition of EGFR expression. They also demonstrated HNK has better binding affinity to EGFR than EGFR tyrosine kinase inhibitor, gefitinib, via molecular docking analysis thereby inhibiting EGFR phosphorylation and its downstream signaling pathways such as the mTOR signaling pathway [84]. In addition, two other groups have shown that HNK inhibits HNSCC and breast cancer progression via inhibition of EGFR phosphorylation without affecting EGFR expression [48,85]. The only available therapies to address central nervous system (CNS) metastases include whole brain/CNS irradiation or surgical resection in eligible patients, and treatment with anti-EGFR agents in patients with EGFR mutations [82]. These therapies are purely palliative and have significant toxicity. Therefore, HNK treatment could be a potential way to treat EGFR-driven cancers as well as metastatic cancers with greater efficacy with minimal side effects.

6.2 Effects of HNK in STAT3 signaling pathway in cancers

STAT3 (signal transducer and activator of transcription 3) is a well-known oncogene that is regulated by receptor tyrosine kinases (Fig. 2), G-protein-coupled receptors, and interleukin families via phosphorylation. Phosphorylated STAT3 undergoes dimerization and translocation to either the nucleus or mitochondria to mediate its activity resulting in enhanced cell proliferation, survival, and invasion for many cancer types [86,87]. STAT3 can also localize into the mitochondria and mediate mitochondrial biogenesis [88,89].

STAT3 is a major downstream mediator of many signaling pathways involved in cancers. In fact, STAT3 is one of the key downstream mediators of EGFR signaling pathways [90–93]. Several mechanisms of action have been suggested for HNK as discussed, STAT3 has recently been demonstrated as another potential target of HNK [64, 94]. There is no evidence that HNK directly regulates STAT3 activity, but it is likely that HNK regulates STAT3 activity via either

EGFR or other receptor tyrosine kinases such as Tropomysoin receptor tyrosine kinases (Trks), hepatocyte-derived growth factor receptor (HGFR), and interleukins (ILs) [95–98]. We also observed that the anti-proliferative and anti-invasive effects of HNK are mediated by inhibiting STAT3 activity in both EGFR-dependent and EGFR-independent manner (unpublished observations). HNK inhibited STAT3 phosphorylation, nuclear translocation, and DNA binding of STAT3 in either an EGF or JAK1/2-dependent manner, which resulted in the inhibition of hepatocellular carcinoma (HCC) cell proliferation and survival [94]. Also, HNK-treated HCC cells exhibited downregulation of STAT3 target genes, such as Cyclin D1, Bcl-2, Bcl-xL, Mcl-1, surviving, and VEGF compared to that of vehicle control treated HCC cells [94]. Another study showed that HNK inhibited proliferation, migration, EMT, and invasion of breast cancer cells (MCF7 and MDA-MB-213) through the inhibition of STAT3 phosphorylation [64]. Epithelial markers, such as fibronectin and vimentin were downregulated by HNK treatment while mesenchymal markers such as cytokeratin 18 (CK-18) and occludin were upregulated by HNK treatment in breast cancer cells [64]. In a recent study, we demonstrated that HNK treatment suppresses mitochondrial respiration and increases generation of reactive oxygen species (ROS) in the mitochondria, leading to the induction of apoptosis in lung cancer cells [28]. Specifically, HNK was found to inhibit mitochondrial complex I activity, stimulate ROS generation, AMPK (AMP-activated protein kinase) activation, and to inhibit both nuclear (p-STAT3^{T705}) and mitochondrial STAT3 phosphorylation (p-STAT3^{S727}) in lung cancer cells. Moreover, STAT3 knockdown abrogated the anti-proliferative and anti-invasive effects of HNK in brain metastatic lung cancer cells (unpublished observations). Bioenergetic analysis via Seahorse experiment indicated that STAT3 knocked-down lung cancer cells have low oxygen consumption rate (OCR) compared to that of control vector lung cancer cells (unpublished observations). Therefore, we believe that HNK inhibits lung cancer brain metastasis via inhibition of mitochondria complex I-STAT3 signaling pathway (Fig. 2). These new discoveries, for the first time, link regulating STAT3-mitochondrial complex I activity as a key mechanism of action of HNK in cancer chemoprevention and cancer therapeutics (unpublished observations).

6.3 Effects of HNK in mitochondria function

Mitochondria are the main hub for cellular energy production and play an important role in cell survival. Many studies described the important functional roles of mitochondria in cancers and new work is increasingly suggesting that the role of mitochondria and metabolic changes in cancer extends beyond the observed Warburg effect. Mutations, dysregulated mitochondrial mass, and functional defects in mitochondria are associated with and can cause cancerous phenotypes [99–101]. In addition, chemical agents that target mitochondrial function have been shown effective in the treatment of cancers. Mitochondrial dysfunction also has been demonstrated as a resistance mechanism to chemotherapeutic agents in cancers.

HNK has been shown to inhibit mitochondrial function [28, 102–104]. The importance of mitochondrial function in tumorigenesis and metastasis has been emphasized [105, 106]. Recently, Li et al. [103] demonstrated that HNK induces mitochondrial permeability transition of pore-mediated cell death in various types of cancer cells. Also, the ability of HNK to cross the BBB and induction of apoptosis in neuroblastoma cells through mitochondrial-mediated cytochrome C and cleaved caspase-3,6 and 9 were demonstrated [103]. Activation of mitochondrial deacetylase sirtuin 3 (Sirt3) has also been reported to be regulated by HNK [104]. HNK can bind Sirt3 and block cardiac hypertrophy in a mouse model that naturally develops cardiac hypertrophy over time [89]. Sirt3 has been suggested as a tumor suppressor that plays an important function in mitochondria-mediated metabolism [107, 108]. Our group recently demonstrated that HNK inhibits lung tumorigenesis via inhibition of mitochondrial function both in-vitro and in-vivo mouse lung SCC model [28]. We demonstrated that HNK treatment suppresses mitochondrial respiration and increases generation of ROS in the mitochondria, leading to the induction of apoptosis in lung cancer cells [28]. Also, HNK treated mice showed significantly lower mitochondrial complex I related gene expression levels compared to that of mice treated with control vehicle (unpublished observations). The fact that HNK's effect is primarily mediated by inducing apoptosis through a mitochondrial-dependent mechanism provides a supportive rationale for the idea that conjugating to a targeting agent that will drive HNK to the mitochondria can dramatically increase its preventive and therapeutic efficacy. Our unpublished data demonstrate that a mitochondria-targeted HNK is a significantly more potent inhibitor of lung cancer cell proliferation and metastasis than HNK. Mitochondria-targeted agents including mitochondria-targeted HNK that target cancer mitochondrial bioenergetics are promising for inhibiting tumor growth and appear to be less toxic to normal cells [109, 110]. Increased negative plasma membrane and mitochondrial transmembrane potentials in cancer cells facilitate the selective accumulation and retention of delocalized lipophilic cations (DLC) such as Rhodamine 123 [109, 110]. Several mitochondria-targeted agents (MTAs) containing a triphenylphosphonium ion (TPP^+) attached to various bioactive molecules (e.g., Mito-CP, Mito-Vit-E, and Mito-Q) decreased ATP levels more selectively in cancer cells compared to normal cells, and inhibited cancer cell proliferation at nontoxic sub-micromolar levels [109–111]. Although tumor cells rely on aerobic glycolysis as the major source of adenosine triphosphate (ATP) to fuel cell proliferation (the Warburg effect), there is increasing evidence demonstrating that mitochondria are indeed functional in most tumor cells [99, 112–114]. Detailed profiling of cellular bioenergetics has

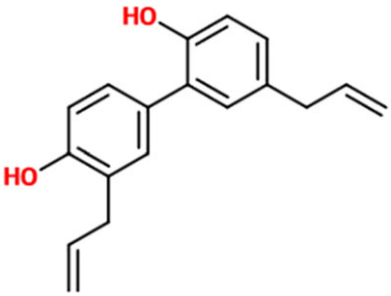

Figure 1. Chemical structure of honokiol.

recently provided new insight on the critical role of mitochondrial metabolism in tumor cells [112, 115].

7 Summary

HNK is a natural compound that exhibits anticancer effects with the ability to penetrate the BBB. HNK have been demonstrated to have wide range of molecular targets that may depend on the treated cell and tissue type. Unlike other chemopreventive or chemotherapeutic agents, HNK has no known side effects and no substantial reported toxicities- making this agent a potentially good clinical candidate. In this review, we have summarized the anticancer, chemo/radiosensitizing, and anti-metastatic effects of HNK in different types of cancers. First, HNK treatment inhibited glioblastoma, melanoma, and lung cancer progressions via primarily inducing apoptosis through regulation of Ca+2 channels, AKT/mTOR pathway, and mitochondrial-dependent pathways. Second, HNK treatments increased the efficacy of both chemotherapy and radiotherapy in many types of cancers. Third, HNK showed superior anti-metastatic effects in in-vitro and in-vivo glioblastoma, breast and lung cancers through inhibition of PI3K/Akt/ mTOR, EMT, and Wnt signaling pathways. Recent unpublished works from our lab also suggest that HNK is effective against established lung cancer cell metastasis present in the brain, and can also prevent lung cancer cell metastasis to the brain in mouse models. In addition, we summarized many of the molecular mechanisms of HNK in cancers elucidated to date. HNK have been demonstrated to inhibit the EGFR signaling pathway via inhibition of either EGFR expression or EGFR phosphorylation in HNSCC and breast cancers. Also, STAT3 has been suggested as a target of HNK in HCC, breast, and lung cancers. Unpublished data from our laboratory also demonstrate that HNK inhibits STAT3 phosphorylation and STAT3 knock-down abrogated the anticancer and anti-metastatic effects of HNK in lung cancer cells. Last, we summarized the effects of HNK in mitochondrial function. Our group demonstrated that HNK

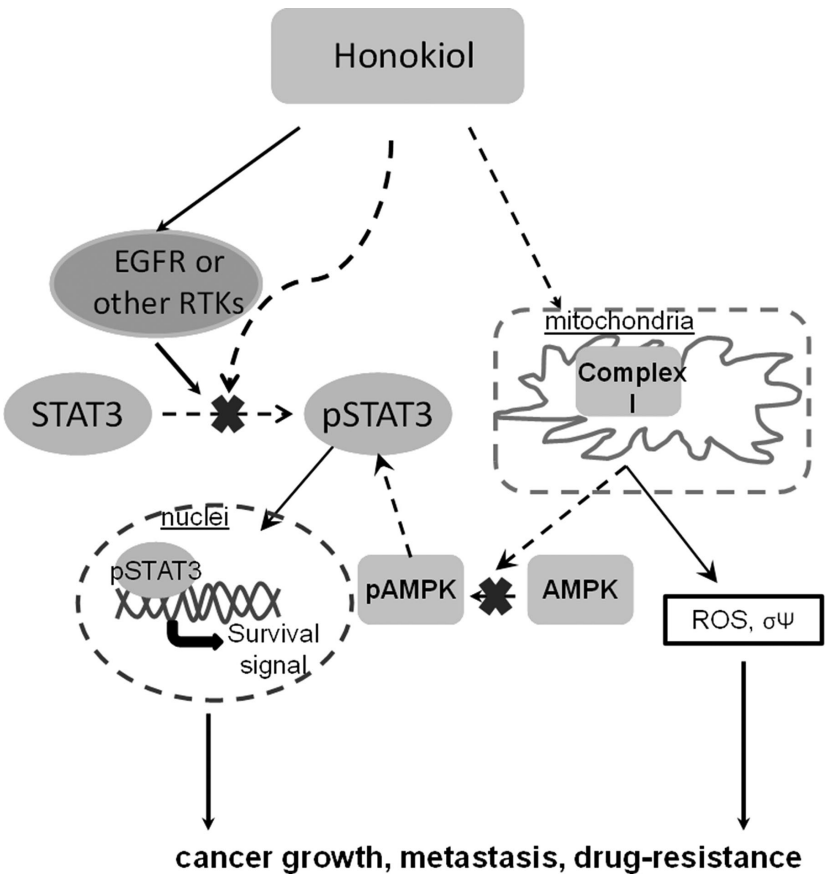

Figure 2. Proposed molecular mechanisms for honokiol. Molecular mechanism for honokiol proposed from literature was illustrated as solid lines and arrows; new mechanism for honokiol through targeting mitochondria from our own unpublished data was illustrated as dotted lines and arrows.

inhibited mitochondrial respiration and induced apoptosis of cancer cells in a mitochondria-STAT3 dependent manner. Unpublished data from our laboratory also demonstrate that HNK inhibits mitochondrial function via regulation of mitochondrial complex I subunits (as shown in Fig. 1). Thus, targeting of mitochondrial function and cellular bioenergetics with HNK could provide an effective way to prevent and to treat cancers including advanced metastatic cancers.

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8 References

[1] Abhishek Bhanot, R. S., Noolvi, M. N., Natural sources as potential anti-cancer agents: a review. *Internat. J. Phytomed.*, 2011, *3*, 17.

[2] Pan, J., Zhang, Q., Li, K., Liu, Q., et al., Chemoprevention of lung squamous cell carcinoma by ginseng. *Cancer Prev. Res. (Phila)*, 2013, *6*, 530–539.

[3] Liu, Z., Zhang, X., Cui, W., Zhang, X., et al., Evaluation of short-term and subchronic toxicity of magnolia bark extract in rats. *Regul. Toxicol. Pharmacol.*, 2007, *49*, 160–171.

[4] Zhang, Q., Li, J., Zhang, W., An, Q., et al., Acute and subchronic toxicity studies of honokiol microemulsion. *Regul. Toxicol. Pharmacol.*, 2015, *71*, 428–436.

[5] Park, E. J., Zhao, Y. Z., Na, M., Bae, K., et al., Protective effects of honokiol and magnolol on tertiary butyl hydroperoxide- or D-galactosamine-induced toxicity in rat primary hepatocytes. *Planta Med.*, 2003, *69*, 33–37.

[6] Hoi, C. P., Ho, Y. P., Baum, L., Chow, A. H., Neuroprotective effect of honokiol and magnolol, compounds from Magnolia officinalis, on beta-amyloid-induced toxicity in PC12 cells. *Phytother. Res.*, 2010, *24*, 1538–1542.

[7] Hsu, C. C., Chen, L. F., Lin, M. T., Tian, Y. F., Honokiol protected against heatstroke-induced oxidative stress and inflammation in diabetic rats. *Int. J. Endocrinol.*, 2014, *2014*, 134575.

[8] Liu, J., Zhang, C., Liu, Z., Zhang, J., et al., Honokiol downregulates Kruppel-like factor 4 expression, attenuates inflammation, and reduces histopathology after spinal cord injury in rats. *Spine (Phila Pa 1976)*, 2015, *40*, 363–368.

[9] Fried, L. E. and Arbiser, J. L., Honokiol, a multifunctional antiangiogenic and antitumor agent. *Antioxid. Redox Signal*, 2009, *11*, 1139–1148.

[10] Han, M., Yu, X., Guo, Y., Wang, Y., et al., Honokiol nanosuspensions: preparation, increased oral bioavailability and dramatically enhanced biodistribution in the cardio-cerebro-vascular system. *Colloids Surf. B Biointerfaces*, 2014, *116*, 114–120.

[11] Lo, Y. C., Teng, C. M., Chen, C. F., Chen, C. C., Hong, C. Y., Magnolol and honokiol isolated from *Magnolia officinalis* protect rat heart mitochondria against lipid peroxidation. *Biochem. Pharmacol.*, 1994, *47*, 549–553.

[12] Kim, Y. J., Choi, M. S., Cha, B. Y., Woo, J. T., et al., Long-term supplementation of honokiol and magnolol ameliorates body fat accumulation, insulin resistance, and adipose inflammation in high-fat fed mice. *Mol. Nutr. Food Res.*, 2013, *57*, 1988–1998.

[13] Arora, S., Singh, S., Piazza, G. A., Contreras, C. M., et al., Honokiol: a novel natural agent for cancer prevention and therapy. *Curr. Mol. Med.*, 2012, *12*, 1244–1252.

[14] Jun-Jun, W., Xiao-Lei, M., Jing-Ya, C., Yong, C., The pharmacokinetics and tissue distribution of honokiol and its metabolites in rats. *Eur. J. Drug Metab. Pharmacokinet.*, 2015, doi: 10.1007/s13318-015-0281-6.

[15] Sheng, Y. L., Xu, J. H., Shi, C. H., Li, W., et al., UPLC-MS/MS-ESI assay for simultaneous determination of magnolol and honokiol in rat plasma: application to pharmacokinetic study after administration emulsion of the isomer. *J. Ethnopharmacol.*, 2014, *155*, 1568–1574.

[16] Wang, X., Duan, X., Yang, G., Zhang, X., et al., Honokiol crosses BBB and BCSFB, and inhibits brain tumor growth in rat 9L intracerebral gliosarcoma model and human U251 xenograft glioma model. *PLoS One*, 2011, *6*, e18490.

[17] Dong, Y., Tang, M., Song, H., Li, R., et al., Characterization of metabolic profile of honokiol in rat feces using liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry and (13)C stable isotope labeling. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2014, 953–954, 20–29.

[18] Lai, H., Tang, M., Liu, J., Dong, Y., et al., Identification of honokiol metabolites in rats by the method of stable isotope cluster technique and ultra-high performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2013, *931*, 157–163.

[19] Liu, J., Tang, M., Lai, H., Dong, Y., et al., Identification of metabolites of honokiol in rat urine using 13C stable isotope labeling and liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry. *J. Chromatogr. A*, 2013, *1295*, 48–56.

[20] Bohmdorfer, M., Maier-Salamon, A., Taferner, B., Reznicek, G., et al., In vitro metabolism and disposition of honokiol in rat and human livers. *J. Pharm. Sci.*, 2011, *100*, 3506–3516.

[21] Lin, L., Liu, A., Peng, Z., Lin, H. J., et al., Curcumin: a novel Stat3 pathway inhibitor for chemoprevention of lung cancer. *Eur. J. Cancer Prev.*, 2012, *21*, 407–412.

[22] Zhai, H., Nakade, K., Mitsumoto, Y., Fukuyama, Y., Honokiol and magnolol induce Ca2+ mobilization in rat cortical neurons and human neuroblastoma SH-SY5Y cells. *Eur. J. Pharmacol.*, 2003, *474*(2-3), 199–204.

[23] Kaushik, G., Venugopal, A., Ramamoorthy, P., Standing, D., et al., Honokiol inhibits melanoma stem cells by targeting notch signaling. *Mol. Carcinog.*, 2015, *54*, 1710–1721.

[24] Chilampalli, S., Zhang, X., Fahmy, H., Kaushik, R. S., et al., Chemopreventive effects of honokiol on UVB-induced skin cancer development. *Anticancer Res.*, 2010, *30*, 777–783.

[25] Singh, T., Prasad, R., and Katiyar, S. K., Inhibition of class I histone deacetylases in non-small cell lung cancer by honokiol leads to suppression of cancer cell growth and

induction of cell death in vitro and in vivo. *Epigenetics*, 2013, *8*, 54–65.

[26] Jiang, Q. Q., Fan, L. Y., Yang, G. L., Guo, W. H., et al., Improved therapeutic effectiveness by combining liposomal honokiol with cisplatin in lung cancer model. *BMC Cancer*, 2008, *8*, 242.

[27] Yang, S. E., Hsieh, M. T., Tsai, T. H., Hsu, S. L., Down-modulation of Bcl-XL, release of cytochrome c and sequential activation of caspases during honokiol-induced apoptosis in human squamous lung cancer CH27 cells. *Biochem. Pharmacol.*, 2002, *63*, 1641–1651.

[28] Pan, J., Zhang, Q., Liu, Q., Komas, S. M., et al., Honokiol inhibits lung tumorigenesis through inhibition of mitochondrial function. *Cancer Prev. Res. (Phila)*, 2014, *7*, 1149–1159.

[29] Antoon, J. W., White, M. D., Slaughter, E. M., Driver, J. L., et al., Targeting NFkB mediated breast cancer chemoresistance through selective inhibition of sphingosine kinase-2. *Cancer Biol. Ther.*, 2011, *11*, 678–689.

[30] Jansen, B., Schlagbauer-Wadl, H., Eichler, H. G., Wolff, K., et al., Activated N-ras contributes to the chemoresistance of human melanoma in severe combined immunodeficiency (SCID) mice by blocking apoptosis. *Cancer Res.*, 1997, *57*, 362–365.

[31] Yu, L. L., Dai, N., Yu, H. G., Sun, L. M., Si, J. M., Akt associates with nuclear factor kappaB and plays an important role in chemoresistance of gastric cancer cells. *Oncol. Rep.*, 2010, *24*, 113–119.

[32] Falasca, M., PI3K/Akt signalling pathway specific inhibitors: a novel strategy to sensitize cancer cells to anti-cancer drugs. *Curr. Pharm. Des.*, 2010, *16*, 1410–1416.

[33] Wu, Y., Yu, D. D., Hu, Y., Cao, H. X., et al., LXR ligands sensitize EGFR-TKI-resistant human lung cancer cells in vitro by inhibiting Akt activation. *Biochem. Biophys. Res. Commun.*, 2015, *467*, 900–905.

[34] Choi, S. S., Cha, B. Y., Lee, Y. S., Yonezawa, T., et al., Honokiol and magnolol stimulate glucose uptake by activating PI3K-dependent Akt in L6 myotubes. *Biofactors*, 2012, *38*, 372–377.

[35] Kim, B. H. and Cho, J. Y., Anti-inflammatory effect of honokiol is mediated by PI3K/Akt pathway suppression. *Acta Pharmacol. Sin.*, 2008, *29* , 113–122.

[36] Pan, J., Zhang, Q., Xiong, D., Vedell, P., et al., Honokiol enhances paclitaxel efficacy in multi-drug resistant human cancer model through the induction of apoptosis. *PLoS One*, 2014, *9*, e86369.

[37] Xie, L., Jiang, F., Zhang, X., Alitongbieke, G., et al., Honokiol sensitizes breast cancer cells to TNFalpha induction of apoptosis by inhibiting Nur77 expression. *Br. J. Pharmacol.*, 2015.

[38] Cournede, A., Ries, P., Richard, K., Guillain, A., et al., Oxaliplatin neurotoxicity: a report of three cases with post-operative exacerbation. *Gastroenterol. Clin. Biol.*, 2005, *29*, 461–464.

[39] Hua, H., Chen, W., Shen, L., Sheng, Q., Teng, L., Honokiol augments the anti-cancer effects of oxaliplatin in colon cancer cells. *Acta Biochim. Biophys. Sin. (Shanghai)*, 2013, *45*, 773–779.

[40] Zhang, Y. D., Yang, Q., Jiang, Z. M., Ma, W., et al., Overall survival of patients with advanced pancreatic cancer improved with an increase in second-line chemotherapy after gemcitabine-based therapy. *JOP*, 2011, *12*, 131–137.

[41] Arora, S., Bhardwaj, A., Srivastava, S. K., Singh, S., et al., Honokiol arrests cell cycle, induces apoptosis, and potentiates the cytotoxic effect of gemcitabine in human pancreatic cancer cells. *PLoS One*, 2011, *6*, e21573.

[42] Zhang, M. W., Xu, X. J., Fan, J. X., Hung, Y. X., et al., Honokiol combined with Gemcitabine synergistically inhibits the proliferation of human Burkitt lymphoma cells and induces their apoptosis. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*, 2014, *22*, 93–98.

[43] Bae, S. H., Park, M. J., Lee, M. M., Kim, T. M., et al., Toxicity profile of temozolomide in the treatment of 300 malignant glioma patients in Korea. *J. Korean Med. Sci.*, 2014, *29*, 980–984.

[44] Friedman, H. S., Kerby, T., and Calvert, H., Temozolomide and treatment of malignant glioma. *Clin. Cancer Res.*, 2000, *6*, 2585–2597.

[45] Watanabe, R., Nakasu, Y., Tashiro, H., Mitsuya, K., et al., O6-methylguanine DNA methyltransferase expression in tumor cells predicts outcome of radiotherapy plus concomitant and adjuvant temozolomide therapy in patients with primary glioblastoma. *Brain Tumor Pathol.*, 2011, *28*, 127–135.

[46] Jiang, X., Reardon, D. A., Desjardins, A., Vredenburgh, J. J., et al., O6-methylguanine-DNA methyltransferase (MGMT) immunohistochemistry as a predictor of resistance to temozolomide in primary CNS lymphoma. *J. Neurooncol.*, 2013, *114*, 135–140.

[47] Lai, I. C., Shih, P. H., Yao, C. J., Yeh, C. T., et al., Elimination of cancer stem-like cells and potentiation of temozolomide sensitivity by Honokiol in glioblastoma multiforme cells. *PLoS One*, 2015, *10*, e0114830.

[48] Leeman-Neill, R. J., Cai, Q., Joyce, S. C., Thomas, S. M., et al., Honokiol inhibits epidermal growth factor receptor signaling and enhances the antitumor effects of epidermal growth factor receptor inhibitors. *Clin. Cancer Res*, 2010, *16*, 2571–2579.

[49] Hou, W., Chen, L., Yang, G., Zhou, H., et al., Synergistic antitumor effects of liposomal honokiol combined with adriamycin in breast cancer models. *Phytother. Res*, 2008, *22*, 1125–1132.

[50] Liu, H., Zang, C., Emde, A., Planas-Silva, M. D., et al., Antitumor effect of honokiol alone and in combination with other anti-cancer agents in breast cancer. *Eur. J. Pharmacol.*, 2008, *591*, 43–51.

[51] He, Z., Subramaniam, D., Zhang, Z., Zhang, Y., Anant, S., Honokiol as a radiosensitizing agent for colorectal cancers. *Curr. Colorectal Cancer Rep.*, 2013, *9*.

[52] He, Z., Subramaniam, D., Ramalingam, S., Dhar, A., et al., Honokiol radiosensitizes colorectal cancer cells: enhanced activity in cells with mismatch repair defects. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 2011, *301*, G929–G937.

[53] Hu, J., Chen, L. J., Liu, L., Chen, X., et al., Liposomal honokiol, a potent anti-angiogenesis agent, in combination

with radiotherapy produces a synergistic antitumor efficacy without increasing toxicity. *Exp. Mol. Med.*, 2008, *40*, 617–628.

[54] Chen, F., Wang, T., Wu, Y. F., Gu, Y., et al., Honokiol: a potent chemotherapy candidate for human colorectal carcinoma. *World J. Gastroenterol.*, 2004, *10*, 3459–463.

[55] Tsai, T. H., Chou, C. J., Cheng, F. C., Chen, C. F., Pharmacokinetics of honokiol after intravenous administration in rats assessed using high-performance liquid chromatography. *J. Chromatogr. B Biomed. Appl.*, 1994, *655*, 41–45.

[56] Jeong, J. J., Lee, J. H., Chang, K. C., Kim, H. J., Honokiol exerts an anticancer effect in T98G human glioblastoma cells through the induction of apoptosis and the regulation of adhesion molecules. *Int. J. Oncol.*, 2012, *41*, 1358–1364.

[57] Singh, T. and Katiyar, S. K., Honokiol, a phytochemical from Magnolia spp., inhibits breast cancer cell migration by targeting nitric oxide and cyclooxygenase-2. *Int. J. Oncol.*, 2011, *38*, 769–776.

[58] Yeh, P. S., Wang, W., Chang, Y. A., Lin, C. J., et al., Honokiol induces autophagy of neuroblastoma cells through activating the PI3K/Akt/mTOR and endoplasmic reticular stress/ERK1/2 signaling pathways and suppressing cell migration. *Cancer Lett.*, 2015.

[59] Milowsky, M. I. and Nanus, D. M., Advanced renal cell carcinoma. *Curr. Treat. Options Oncol.*, 2001, *2*, 437–445.

[60] Cheng, S., Castillo, V., Eliaz, I., Sliva, D., Honokiol suppresses metastasis of renal cell carcinoma by targeting KISS1/KISS1R signaling. *Int. J. Oncol.*, 2015, *46*, 2293–2298.

[61] Li, L. and Li, W., Epithelial-mesenchymal transition in human cancer: comprehensive reprogramming of metabolism, epigenetics, and differentiation. *Pharmacol. Ther.*, 2015, *150*, 33–46.

[62] Voulgari, A. and Pintzas, A., Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic. *Biochim. Biophys. Acta*, 2009, *1796*, 75–90.

[63] Liu, S. H., Lee, W. J., Lai, D. W., Wu, S. M., et al., Honokiol confers immunogenicity by dictating calreticulin exposure, activating ER stress and inhibiting epithelial-to-mesenchymal transition. *Mol. Oncol.*, 2015, *9*, 834–849.

[64] Avtanski, D. B., Nagalingam, A., Bonner, M. Y., Arbiser, J. L., et al., Honokiol inhibits epithelial-mesenchymal transition in breast cancer cells by targeting signal transducer and activator of transcription 3/Zeb1/E-cadherin axis. *Mol. Oncol.*, 2014, *8*, 565–580.

[65] Joo, Y. N., Eun, S. Y., Park, S. W., Lee, J. H., et al., Honokiol inhibits U87MG human glioblastoma cell invasion through endothelial cells by regulating membrane permeability and the epithelial-mesenchymal transition. *Int. J. Oncol.*, 2014, *44*, 187–194.

[66] Singh, T. and Katiyar, S. K., Honokiol inhibits non-small cell lung cancer cell migration by targeting PGE(2)-mediated activation of beta-catenin signaling. *PLoS One*, 2013, *8*, e60749.

[67] Ray, A., Adipokine leptin in obesity-related pathology of breast cancer. *J. Biosci.*, 2012, *37*, 289–294.

[68] Surmacz, E., Obesity hormone leptin: a new target in breast cancer? *Breast Cancer Res.*, 2007, *9*, 301.

[69] Avtanski, D. B., Nagalingam, A., Bonner, M. Y., Arbiser, J. L., et al., Honokiol activates LKB1-miR-34a axis and antagonizes the oncogenic actions of leptin in breast cancer. *Oncotarget*, 2015, *6*, 29947–29962.

[70] Avtanski, D. B., Nagalingam, A., Kuppusamy, P., Bonner, M. Y., et al., Honokiol abrogates leptin-induced tumor progression by inhibiting Wnt1-MTA1-beta-catenin signaling axis in a microRNA-34a dependent manner. *Oncotarget*, 2015, *6*, 16396–16410.

[71] Li, W., Wang, Q., Su, Q., Ma, D., et al., Honokiol suppresses renal cancer cells ' metastasis via dual-blocking epithelial-mesenchymal transition and cancer stem cell properties through modulating miR-141/ZEB2 signaling. *Mol. Cells*, 2014, *37*, 383–388.

[72] Pan, H. C., Lai, D. W., Lan, K. H., Shen, C. C., et al., Honokiol thwarts gastric tumor growth and peritoneal dissemination by inhibiting Tpl2 in an orthotopic model. *Carcinogenesis*, 2013, *34*, 2568–2579.

[73] Steinmann, P., Walters, D. K., Arlt, M. J., Banke, I. J., et al., Antimetastatic activity of honokiol in osteosarcoma. *Cancer*, 2012, *118*, 2117–2127.

[74] Wen, J., Fu, A. F., Chen, L. J., Xie, X. J., et al., Liposomal honokiol inhibits VEGF-D-induced lymphangiogenesis and metastasis in xenograft tumor model. *Int. J. Cancer*, 2009, *124*, 2709–2718.

[75] Shigemura, K., Arbiser, J. L., Sun, S. Y., Zayzafoon, M., et al., Honokiol, a natural plant product, inhibits the bone metastatic growth of human prostate cancer cells. *Cancer*, 2007, *109*, 1279–1289.

[76] Yarden, Y. and Shilo, B. Z., SnapShot: EGFR signaling pathway. *Cell*, 2007, *131*, 1018.

[77] Okamoto, K., Okamoto, I., Hatashita, E., Kuwata, K., et al., Overcoming erlotinib resistance in EGFR mutation-positive non-small cell lung cancer cells by targeting survivin. *Mol. Cancer Therapeut.*, 2012, *11*, 204–213.

[78] Bhatia, V., Garg, P. K., Agarwal, V., Sharma, M., Ray, S., Inflammatory papillary stenosis due to Giardia lamblia in a patient with hyper-immunoglobulin M immunodeficiency syndrome. *Gastrointest. Endosc.*, 2007, *66*, 181–182; discussion 182.

[79] Sordella, R., Bell, D. W., Haber, D. A., Settleman, J., Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science*, 2004, *305*, 1163–1167.

[80] Lynch, T. J., Bell, D. W., Sordella, R., Gurubhagavatula, S., et al., Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.*, 2004, *350*, 2129–2139.

[81] Faber, A. C., Wong, K. K., and Engelman, J. A., Differences underlying EGFR and HER2 oncogene addiction. *Cell Cycle*, 2010, *9*, 851–852.

[82] Goldberg, S. B., Contessa, J. N., Omay, S. B., Chiang, V., Lung cancer brain metastases. *Cancer J.*, 2015, *21*, 398–403.

[83] Schuster, D. P., Kovacs, A., Garbow, J., Piwnica-Worms, D, Recent advances in imaging the lungs of intact small animals. *Am. J. Respir. Cell Mol. Biol.*, 2004, *30*, 129–138.

[84] Singh, T., Gupta, N. A., Xu, S., Prasad, R., et al., Honokiol inhibits the growth of head and neck squamous cell carcinoma by targeting epidermal growth factor receptor. *Oncotarget*, 2015. *6*, 21268–21282.

[85] Park, E. J., Min, H. Y., Chung, H. J., Hong, J. Y., et al., Down-regulation of c-Src/EGFR-mediated signaling activation is involved in the honokiol-induced cell cycle arrest and apoptosis in MDA-MB-231 human breast cancer cells. *Cancer Lett.*, 2009, *277*, 133–140.

[86] Lin, L. et al., STAT3 is necessary for proliferation and survival in colon cancer-initiating cells. *Cancer Res.*, 2011, *71*, 7226–7237.

[87] Yang, H., Yamazaki, T., Pietrocola, F., Zhou, H., et al., STAT3 inhibition enhances the therapeutic efficacy of immunogenic chemotherapy by stimulating type 1 interferon production by cancer cells. *Cancer Res.*, 2015, *75*, 3812–3822.

[88] Zhou, L. and Too, H. P., Mitochondrial localized STAT3 is involved in NGF induced neurite outgrowth. *PloS One*, 2011, *6*, e21680.

[89] Tammineni, P., Anugula, C., Mohammed, F., Anjaneyulu, M., et al., The import of the transcription factor STAT3 into mitochondria depends on GRIM-19, a component of the electron transport chain. *J. Biolog. Chem.*, 2013, *288*, 4723–4732.

[90] Vigneron, A., Gamelin, E., and Coqueret, O., The EGFR-STAT3 oncogenic pathway up-regulates the Eme1 endonuclease to reduce DNA damage after topoisomerase I inhibition. *Cancer Res.*, 2008, *68*, 815–825.

[91] Sen, M., Joyce, S., Panahandeh, M., Li, C., et al., Targeting Stat3 abrogates EGFR inhibitor resistance in cancer. *Clin. Cancer Res.*, 2012, *18*, 4986–4996.

[92] Gao, S. P., Mark, K. G., Leslie, K., Pao, W., et al., Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. *J. Clin. Invest.*, 2007, *117*, 3846–3856.

[93] Wu, J., Patmore, D. M., Jousma, E., Eaves, D. W., et al., EGFR-STAT3 signaling promotes formation of malignant peripheral nerve sheath tumors. *Oncogene*, 2014, *33*, 173–180.

[94] Rajendran, P., Li, F., Shanmugam, M. K., Vali, S., et al., Honokiol inhibits signal transducer and activator of transcription-3 signaling, proliferation, and survival of hepatocellular carcinoma cells via the protein tyrosine phosphatase SHP-1. *J. Cell. Physiol.*, 2012, *227*, 2184–2195.

[95] Miranda, C., Fumagalli, T., Anania, M. C., Vizioli, M. G., et al., Role of STAT3 in in vitro transformation triggered by TRK oncogenes. *PLoS One*, 2010. *5*, e9446.

[96] Ng, Y. P., Cheung, Z. H., and Ip, N. Y., STAT3 as a downstream mediator of Trk signaling and functions. *J. Biol. Chem.*, 2006, *281*, 15636–15644.

[97] Zhang, Y. W., Wang, L. M., Jove, R., Vande Woude, G. F., Requirement of Stat3 signaling for HGF/SF-Met mediated tumorigenesis. *Oncogene*, 2002, *21*, 217–226.

[98] Dvorak, K., Chavarria, M., Payne, C. M., Ramsey, L., et al., Activation of the interleukin-6/STAT3 antiapoptotic pathway in esophageal cells by bile acids and low pH: relevance to Barrett's esophagus. *Clin. Cancer Res.*, 2007, *13*, 5305–5313.

[99] Cheng, G., Zielonka, J., McAllister, D., Hardy, M., et al., Antiproliferative effects of mitochondria-targeted cationic antioxidants and analogs: role of mitochondrial bioenergetics and energy-sensing mechanism. *Cancer Lett.*, 2015, *365*, 96–106.

[100] Garama, D. J., Harris, T. J., White, C. L., Rossello, F. J., et al., A synthetic lethal interaction between glutathione synthesis and mitochondrial reactive oxygen species provides a tumor-specific vulnerability dependent on STAT3. *Mol. Cell Biol.*, 2015, *35*, 3646–3656.

[101] Stewart, J. B., Alaei-Mahabadi, B., Sabarinathan, R., Samuelsson, T., et al., Simultaneous DNA and RNA mapping of somatic mitochondrial mutations across diverse human cancers. *PLoS Genet.*, 2015, *11*, e1005333.

[102] Li, L., Han, W., Gu, Y., Qiu, S., et al., Honokiol induces a necrotic cell death through the mitochondrial permeability transition pore. *Cancer Res.*, 2007, *67*, 4894–4903.

[103] Lin, J. W., Chen, J. T., Hong, C. Y., Lin, Y. L., Honokiol traverses the blood-brain barrier and induces apoptosis of neuroblastoma cells via an intrinsic bax-mitochondrion-cytochrome c-caspase protease pathway. *Neurooncology*, 2012, *14*, 302–314.

[104] Pillai, V. B., Samant, S., Sundaresan, N. R., Raghuraman, H., et al., Honokiol blocks and reverses cardiac hypertrophy in mice by activating mitochondrial Sirt3. *Nat. Commun.*, 2015, *6*, 6656.

[105] Weinberg, S. E. and Chandel, N. S., Targeting mitochondria metabolism for cancer therapy. *Nat. Chem. Biol.*, 2015, *11*, 9–15.

[106] Lamb, R., Ozsvari, B., Lisanti, C. L., Tanowitz, H. B., et al., Antibiotics that target mitochondria effectively eradicate cancer stem cells, across multiple tumor types: treating cancer like an infectious disease. *Oncotarget*, 2015, *6*, 4569–4584.

[107] Quan, Y., Wang, N., Chen, Q., Xu, J., et al., SIRT3 inhibits prostate cancer by destabilizing oncoprotein c-MYC through regulation of the PI3K/Akt pathway. *Oncotarget*, 2015, *6*, 26494–26507.

[108] Jeong, S. M., Lee, J., Finley, L. W., Schmidt, P. J., et al., SIRT3 regulates cellular iron metabolism and cancer growth by repressing iron regulatory protein 1. *Oncogene*, 2015, *34*, 2115–2124.

[109] Cheng, G., Zielonka, J., Dranka, B. P., McAllister, D., et al., Mitochondria-targeted drugs synergize with 2-deoxyglucose to trigger breast cancer cell death. *Cancer Res.*, 2012, *72*, 2634–2644.

[110] Cheng, G., Zielonka, J., McAllister, D., Tsai, S., et al., Profiling and targeting of cellular bioenergetics: inhibition of pancreatic cancer cell proliferation. *Br. J. Cancer*, 2014(May), 1–9.

[111] Cheng, G., Zielonka, J., McAllister, D. M., Mackinnon, A. C., et al., Mitochondria-targeted vitamin E analogs inhibit breast cancer cell energy metabolism and promote cell death. *BMC Cancer*, 2013, *13*, 285–285.

[112] Cheng, G., Zielonka, J., Dranka, B. P., McAllister, D., et al., Mitochondria-targeted drugs synergize with 2-deoxyglucose to trigger breast cancer cell death. *Cancer Res.*, 2012, *72*, 2634–2644.

[113] Cheng, G., Zielonka, J., McAllister, D., Tsai, S., et al., Profiling and targeting of cellular bioenergetics: inhibition of pancreatic cancer cell proliferation. *Br. J. Cancer*, 2014, *111*, 85–93.

[114] Weinberg, F., Hamanaka, R., Wheaton, W. W., Weinberg, S., et al., Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. *Proc. Natl Acad. Sci. U S A*, 2010, *107*, 8788–8793.

[115] Barbi de Moura, M., Vincent, G., Fayewicz, S. L., Bateman, N. W., et al., Mitochondrial respiration–an important therapeutic target in melanoma. *PLoS One*, 2012, *7*, e40690.

