Chapter 11
Honokiol, an Active Compound of *Magnolia* Plant, Inhibits Growth, and Progression of Cancers of Different Organs

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Abstract Honokiol ($C_{18}H_{18}O_2$) is a biphenolic natural product isolated from the bark and leaves of *Magnolia* plant *spp*. During the last decade or more, honokiol has been extensively studied for its beneficial effect against several diseases. Investigations have demonstrated that honokiol possesses anti-carcinogenic, anti-inflammatory, anti-oxidative, anti-angiogenic as well as inhibitory effect on malignant transformation of papillomas to carcinomas in vitro and in vivo animal models without any appreciable toxicity. Honokiol affects multiple signaling pathways, molecular and cellular targets including nuclear factor-κB (NF-κB), STAT3, epidermal growth factor receptor (EGFR), cell survival signaling, cell cycle, cyclooxygenase and other inflammatory mediators, etc. Its chemopreventive and/or therapeutic effects have been tested against chronic diseases, such as cancers of different organs. In this chapter, we describe and discuss briefly the effect of honokiol against cancers of different organs, such as melanoma, non-melanoma, lung, prostate, breast, head and neck squamous cell carcinoma, urinary bladder cancer, gastric cancer, and neuroblastoma, etc. and describe its mechanism of action including various molecular and cellular targets. Although more rigorous in vivo studies are still needed, however it is expected that therapeutic effects and activities of honokiol may help in the development and designing of clinical trials against chronic diseases in human subjects.

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S.C. Gupta et al. (eds.), *Anti-inflammatory Nutraceuticals and Chronic Diseases*,
Advances in Experimental Medicine and Biology 928,
DOI 10.1007/978-3-319-41334-1_11

Keywords Honokiol · Ultraviolet radiation · Cancer of different organs · Cell cycle regulation · Inflammatory mediators · Tumor cell migration

Abbreviations

BCC	Basal cell carcinomas
CDK	Cyclin dependent kinases
CHS	Contact hypersensitivity
COX-2	Cyclooxygenase-2
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
HNSCC	Head and neck squamous cell carcinoma
IL	Interleukin
iNOS	Inducible nitric oxide synthase
MMP	Matrix metalloproteinase
NF-κB	Nuclear factor-kappa B
NSCLC	Non-small cell lung cancer
PCNA	Proliferating cell nuclear antigen
PG	Prostaglandin
PGE_2	Prostaglandin E2
SCC	Squamous cell carcinomas
TNF-α	Tumor necrosis factor-alpha
UVR	Ultraviolet radiation

11.1 Introduction

Honokiol, a biphenolic and bioactive small molecule phytochemical (Fig. 11.1), is isolated from the bark and leaves of *Magnolia* plant species (*Magnolia officinalis*), and has been widely used in the traditional Japanese medicine Saiboku-to for the treatment of various ailments due to its anxiolytic, antithrombotic, anti-depressant, anti-emetic, and antibacterial properties [1]. Since long, the barks and leaves from the *Magnolia* plant also have been used in traditional Chinese system of medicine,

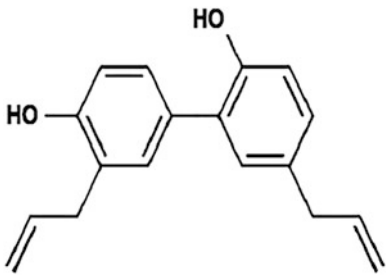

Fig. 11.1 Molecular structure of honokiol, a phytochemical from *Magnolia spp*.

therefore, in the recent past, honokiol achieved a great deal of research interest due to its diverse biologic and pharmacologic activities that include antibacterial, anti-inflammatory, anti-fungal, anti-oxidative, and anti-carcinogenic effects [1–8]. Chemically, honokiol is hydrophobic in nature but soluble in organic solvents, such as acetone. Therefore, to avoid the use of organic solvents in some topical applications and formulation, which may cause deleterious effects, the research laboratory of Dr. Katiyar has developed a topical formulation by mixing it in a hydrophilic cream, and this cream-based topical formulation is ready and easy-to-use for experimental purposes [8].

The protective and therapeutic effect of phytochemicals such as honokiol may be associated with their antioxidant activity, as overproduction of reactive oxygen and nitrogen species in the human body is involved in the pathogenesis of many chronic diseases including cancer. To provide better understanding of the use of honokiol in prevention and treatment of chronic diseases such lung cancer, prostate cancer, head and neck cancer, gastric cancer, prostate cancer, urinary bladder cancer and neuroblastoma, etc., we are summarizing and explaining the investigations conducted in vitro and in vivo animal models. It is well documented that many diseases occur or initiated due to chronic and sustained inflammation in animals as well as in humans, which includes cancer of several organs and aging processes, etc. Inflammation is a localized reaction of tissue to infection, irritation, or other injury, e.g., exposure of the skin to solar ultraviolet (UV) radiation. The key features of inflammation are redness or erythema, warmth, swelling, pain, etc. Inflammation, considered as a necessary response to clear viral infection, repair tissue insults and suppress tumor initiation and progression. However, when inflammation is chronic and persists, diseases may develop, including cancer. Importantly, inflammation involves in all the three stages of tumor development: initiation, promotion/progression, and metastasis. During the initiation phase, inflammation induces the release of a variety of cytokines and chemokines (mostly pro-inflammatory) that promote the activation of inflammatory cells. These conditions change the tissue microenvironment, which resulted in the favor of increased cell survival and proliferation. Clinical and epidemiological evidences suggest a connection between inflammation and a predisposition for the development of cancer. Here, we summarize and discuss the therapeutic effects and mechanisms of action of honokiol against cancers of some specific organs with particular emphasis on ultraviolet (UV) radiation-induced skin cancer development.

11.2 Effect of UVR Exposure on the Skin: Inflammation and Immune Suppression

Exposure of the skin to solar UV radiation, specifically UVB (290–320 nm) spectrum, induces inflammatory mediators and generates oxidative stress, which all together have been implicated in various skin diseases including the initiation and progression of non-melanoma and melanoma skin cancers [9–13]. UVB-induced

inflammatory responses are characterized by the development of edema, erythema, hyperplastic responses, increases in the expression levels of inducible cyclooxygenase-2 (COX-2) and production of prostaglandin (PG) metabolites [9]. UV-induced inflammation is considered as an early and important event in tumor promotion and the growth of skin tumors, and is associated with all the three stages of tumor development, i.e., initiation, promotion and progression [9]. The prostaglandin (PG) metabolites have been implicated in UVB-induced immunosuppression as well as implicated in the development of melanoma and non-melanoma skin cancers. This involvement has been supported by the facts that nonsteroidal anti-inflammatory drugs exert their effects through COX-2 inhibition and can reverse the immunosuppressive effects of UV radiation [14, 15]. Among different PG metabolites, PGE_2 is produced abundantly by keratinocytes in UVB-exposed skin. It is a major and most effective metabolite generated by COX-2 activity and considered as a potent mediator of inflammatory responses. The role of PGE_2 in UV-induced immunosuppression is supported by the evidence that COX-2-deficient mice are resistant to UVB-induced suppression of contact hypersensitivity (CHS) response, whereas the treatment of UVB-exposed COX-2-deficient mice with PGE_2 resulted in suppression of CHS response, thus suggesting the role of PGE_2 in UVB-induced immunosuppression [16]. CHS response is considered as a prototype of T-cell mediated immune reaction/response. Hence, the regulation of UVB-induced inflammatory responses has been considered as an important strategy in reducing the risk of skin cancer. To determine the anti-inflammatory effect of honokiol, studies have been conducted using in vivo mouse models and subsequently its effect on UVB radiation-induced skin tumor development.

11.3 Treatment of Honokiol Inhibits UVB-Induced Inflammatory Mediators in the Skin

A characteristic response of skin keratinocytes to UVB irradiation is enhanced COX-2 expression and a subsequent increase in the production of PG metabolites in the skin [9, 16, 17]. The exposure of the skin to UV radiation triggers the release of PGs, such as PGD_2, $PGF_{2\alpha}$, and PGE_2, which are produced from arachidonic acid by the action of COX-2 [17, 18]. Vaid et al. [8] have shown that chronic exposure of the mouse skin to UVB radiation resulted in greater expression of COX-2 as compared with the skin of the non-UVB-exposed normal skin. Topical treatment of mice with honokiol, whether applied before or after UVB-irradiation, resulted in a suppression of COX-2 expression as compared to the expression in non-honokiol-treated UVB-irradiated mouse skin. The levels of PG metabolites in the skin with a particular emphasis on PGE_2 were also analyzed. The levels of PGE_2 in UVB-irradiated skin were significantly greater as compared with the skin of the non-UVB-irradiated mouse skin, however, the levels of

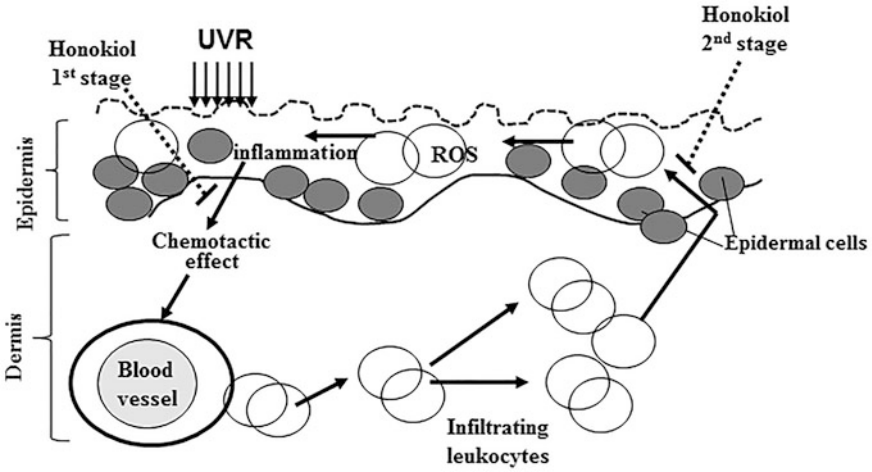

Fig. 11.2 This generalized schematic diagram depicts the mechanism of UVR-induced inflammation in the skin. UVR exposure induces inflammatory responses, including overexpression of COX-2 and PGs production and reactive oxygen species (ROS) generation at early time points (within few hours) of irradiation. Inflammatory mediators and ROS act as chemotactic factors and stimulate the infiltration of leukocytes, particularly activated macrophages and neutrophils, at UV irradiated skin site. Peak time of infiltration is in between 48 and 72 h after UV irradiation of the skin. Activated infiltrating leukocytes are the major source of inflammatory mediators as well as ROS. Topical treatment of the skin with honokiol inhibits UVR-induced effects both at 1st stage (early stage), and 2nd stage (48–72 h after UV) through inhibition of leukocyte infiltration. Inhibition of UVR-induced inflammatory mediators as well as ROS by honokiol treatment contributes to the prevention of UVR-induced skin tumor development

PGE_2 were significantly lower in the UVB-irradiated mouse skin, which was treated with honokiol. Skin exposure to UV radiation induces infiltration of inflammatory leukocytes in the skin, and most prominently are the activated macrophages and neutrophils. These infiltrating leukocytes (majority of them are $CD11b^+$ cell subset) have a role in UV-induced immune suppression, and are the major source of inflammatory mediators, such as prostaglandins and pro-inflammatory cytokines at the UV-exposed site, as demonstrated in Fig. 11.2. In addition, these infiltrating $CD11b^+$ leukocytes have been shown to have suppressive effects on immune system. UVB irradiation also induces production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, etc., in the skin. Topical treatment of mouse skin with honokiol resulted in a significant reduction in UVB-induced production of TNF-α, IL-1β, IL-6 as compared to non-honokiol-treated UVB-exposed mouse skin.

11.4 Honokiol Inhibits UVB-Enhanced Expression of Proliferating Cell Nuclear Antigen (PCNA) in the Skin

Chronic inflammation caused by UV irradiation promotes cellular proliferation in skin cells, and it is commonly measured by determining the levels of PCNA in the skin. Treatment of the skin with honokiol inhibited UVB-induced expression of PCNA in skin. Uncontrolled cellular proliferation may give rise to tumor growth and its development. Based on the above information, it can be suggested that honokiol acts as an anti-inflammatory agent, which is an initial step of skin carcinogenesis.

11.5 Honokiol Treatment Inhibits UV Radiation-Induced Skin Tumor Development and Malignant Progression of Papillomas to Carcinomas in Mice

Non-melanoma skin cancer (NMSC) is the most common cancer in the United States. The majority of NMSCs is environmentally induced and caused by excessive exposure to solar UVB radiation which induces inflammation, oxidative stress, suppression of immune system, and DNA damage. NMSC is composed of two types of skin cancer; basal cell carcinomas (BCCs), and squamous cell carcinomas (SCCs). The incidence of SCC has increased approximately 200 % over the past three decades, and represents about 20 % of NMSC [19, 20]. As topical treatment of honokiol prevents UVB-induced inflammation and their mediators in the mouse skin, the effect of honokiol was assessed against UVB-induced skin tumor development in SKH-1 hairless mouse model [8]. To induce tumors, mice were exposed to UVB radiation (180 mJ/cm^2) 3 times a week for 24 weeks. It was observed that topical treatment of honokiol significantly inhibits UVB-induced initiation and progression of skin tumors [8]. Honokiol treatment also increased the latency period of skin tumor development. The tumor multiplicity and tumor size were significantly reduced in the group of UVB-irradiated mice that were treated with honokiol than in the control group of mice that were UVB-irradiated but not treated with honokiol. When data were compared in terms of average tumor volume/tumor bearing mouse between honokiol-treated and non-honokiol-treated groups, a significant reduction was observed after the treatment of honokiol. Similar observations were also noted by Chilampalli et al. [21] wherein chemopreventive effect of honokiol was determined on UVB-induced skin carcinogenesis. However, these investigators have determined the chemopreventive effect of topical treatment of honokiol at lower dose (30 μg/200 ml acetone/mouse) as well as lower dose of UVB exposure (30 mJ/cm^2). This study also concluded that honokiol treatment affords photoprotection in terms of tumor multiplicity. In addition to the inhibition of skin tumor development in UVB-exposed mouse skin, the malignant progression of papillomas to carcinomas were also significantly prevented by the

topical treatment of honokiol in mice. Histochemical analysis revealed that most of the carcinomas were identified as kerato-acanthomas and squamous cell carcinomas [8].

11.5.1 Honokiol Controls Cell Cycle Regulators in UVB-Induced Skin Tumors

Enhanced expression of cell cycle regulatory proteins such as cyclin-dependent kinases (CDKs) and cyclins or decreased expression of CDK inhibitors have been implicated in UV-induced skin carcinogenesis [22, 23]. The cell cycle deregulation affects skin carcinogenesis under the influence of UV-induced inflammatory mediators. Regulation of cyclin-CDK complexes plays a key role in cell cycle progression at different phases in which CDKs are negatively regulated by a group of functionally related proteins known as CDK inhibitors, such as Kip/Cip family members [24, 25]. Cip1/p21 is a universal CDK inhibitor, and binds with PCNA to inhibit PCNA function in DNA replication process [26], while Kip1/p27 is upregulated in response to anti-proliferative signals. The expression levels of cyclins (cyclin D1, D2, and E) and CDKs (CDK2, CDK4, and CDK6) were considerably higher in UVB-induced skin tumors compared with non-UVB-irradiated normal skin from age-matched control mice. However, treatment of the skin with honokiol resulted in inhibition of UVB-induced expression levels of cyclins (cyclins D1, D2, and E) and CDKs in skin tumors compared to skin tumors obtained from non-honokiol-treated mice. Further, tumor suppressor genes or proteins are also involved in tumor development. Cip1/p21 also act as tumor suppressor, and regulates cell cycle progression. Treatment of honokiol enhances the levels of Cip1/p21 in skin tumors compared with non-honokiol-treated skin tumors. Cell cycle arrest in tumor cells could lead to the reduction in proliferation potential of cells as observed by a decrease in the levels of PCNA in UVB-exposed skin and skin tumors. Thus, the modulation in cell cycle progression and inhibition of cell proliferation could be one of the possible mechanisms through which honokiol inhibits UVB-induced skin tumor development [8]. Kip1/p27 is another important CDK inhibitor that regulates CDK-cyclin activity at G1-S transition of cell cycle [22]. The level of Kip1/p27 was also upregulated in the skin tumors obtained from honokiol-treated mice. These observations reflect the molecular mechanism through which honokiol acts as an anti-inflammatory and anti-skin carcinogenic agent.

11.5.2 Honokiol Inhibits Cell Survival Signals/Pathways in UVB-Induced Skin Tumors

The risk of UVB radiation-induced skin tumor development increases through various signaling pathways including the activation of the cell survival kinases,

such as PI3K/Akt [27, 28]. Studies revealed that the levels of both the catalytic (p110) and regulatory (p85) subunits of PI3K were enhanced in the UVB-induced skin tumors as compared with the non-UVB-exposed normal mouse skin; however, the levels of the p85 and p110 subunits were greatly reduced in the UV-induced skin tumors from honokiol-treated mice compared to the skin tumors of non-honokiol-treated mice. Most of the biological effects of PI3K are mediated through the activation of the downstream target Akt. Akt is a serine/threonine kinase, which has been identified as an important component of pro-survival signaling pathways [29]. It was observed that the treatment of honokiol resulted in reduction of UVB-induced phosphorylation of Akt (Ser^{473}) as compared to the skin tumors of non-honokiol-treated group. Further, cell survival signals have been associated with cellular proliferation and carcinogenesis [30–32]. The skin tumors augment UVB radiation-induced activation of PI3K and phospho-Akt as compared to the non-UVB-exposed mouse skin. The PI3K/Akt signaling pathway regulates the activity of the transcriptional factor, NF-κB, which in turn known to regulate several well-known markers of tumor promotion and tumor cell proliferation, e.g., COX-2, inducible nitric oxide synthase (iNOS) and PCNA [33, 34]. Thus, the inhibition of PI3K/p-Akt pathway in skin tumors by honokiol may have a role in inhibition of UVB-induced skin tumor growth in mouse model.

11.6 Honokiol Inhibits Metastatic Potential of Melanoma Cells

Melanoma is a leading cause of skin cancer-related deaths due to its propensity to metastasize at distant organs of the body, and the average survival of patients with advanced stage melanoma is less than 1 year. The American Cancer Society indicated that the incidence of melanoma is increasing, and increasing particularly in children [35]. The oxidative stress plays a significant role in cancer cell progression and migration. Nox1 is a multi-protein complex that consists of cytosolic ($p47^{phox}$, $p40^{phox}$, and $p67^{phox}$) and membrane-bound proteins ($p22^{phox}$, $gp91^{phox}$) that when assembled becomes activated, and initiates respiratory bursts or generation of oxidative stress [36, 37]. A recent study by Prasad et al. [38] reported that honokiol treatment inhibits the migration capacity of melanoma cells and this effect was associated with the inhibition of Nox1 expression and NADPH oxidase activity in melanoma cells. Honokiol not only reduces the NADPH oxidase activity, but also reduces oxidative bursts in melanoma cells. Treatment of melanoma cells with honokiol blocks the interaction between membrane-bound and cytosolic-bound proteins. The $p22^{phox}$ protein is the binding partner of $p47^{phox}$ [39, 40], the subunits required for oxidase assembly. Failure of this interaction between membrane-bound and cytosolic-bound proteins would lead to the inactivation of Nox in melanoma cells, and that would result in the reduction of oxidative stress, which is responsible for invasive or metastatic phenotype of melanoma cells. The inhibitory effect of

honokiol on migratory potential of melanoma cells was supported by an action of diphenyleneiodonium chloride, a Nox 1 inhibitor, in melanoma cells. The treatment of melanoma cells with diphenyleneiodonium chloride also blocked or reduced melanoma cell migration. Activation of matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9, plays crucial roles in tissue matrix degradation, and thus, paves the way for cell migration. Prasad et al. [38] also found that honokiol treatment reduces the expression of MMP-2 and MMP-9 in Hs294t and SK-Mel28 melanoma cells, thus suggesting a mechanism of action by honokiol.

11.7 Honokiol Promotes Cell Death of Neuroblastoma Cells

In children, several childhood cancers such as leukemia, neuroblastoma, Wilms tumor, lymphoma, rhabdomyosarcoma, retinoblastoma, bone cancer including osteosarcoma and Ewing sarcoma are the leading cause of deaths [35]. Among various tumor types diagnosed in children, neuroblastomas are the most common solid cancer and develop from neural crest elements of the sympathetic nervous system [41]. Based on severity of disease, neuroblastomas are classified into three risk categories as low, intermediate, and high. Unfortunately, neuroblastomas diagnosed in children, belongs to high-risk category [42], and it is a severe problem in pediatric oncology. Children with neuroblastoma achieve a long-term treatment, and which usually causes other serious complications such hearing loss, cardiac dysfunction, infertility, and secondary malignancies after therapy [43]. Studies indicated that honokiol has beneficial effect against neuroblastoma as it can pass through blood brain barrier and kill neuroblastoma cells without affecting too much viability of normal brain cells [44]. Autophagy is a self-degradative physiological process in the body that deals with cell's destruction and maintains homeostasis or normal functioning. Studies have shown that honokiol induces autophagy in various types of tumor cells including neuroblastoma via diverse mechanisms [45–48]. DNA fragmentation and cell cycle arrest at the sub-G1 phase are two typical characteristics that indicate that cells are undergoing apoptosis. In human neuroglioma H4 cells, honokiol have been reported to cause cell cycle arrest and induce apoptosis through an activation of p53 [49]. The mammalian target of rapamycin (mTOR) signaling is a negative regulator of cellular autophagy [50]. Following phosphorylation, mTOR inhibits activation of downstream protein kinases, including ULK1 and ATG13, and subsequently suppress cellular autophagy. The study by Yeh et al. [45] revealed that treatment of neuroblastoma cells with honokiol caused significant downregulation of mTOR phosphorylation which leads to induction of autophagy of neuroblastoma cells.

11.8 Honokiol Inhibits the Growth of Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma (HNSCC) is a commonly occurring malignancy worldwide and account approximately 20,000 deaths in the United States annually [51, 52]. In addition to several other factors, the over expression of epidermal growth factor receptor (EGFR) has been commonly reported and observed in more than 90 % cases of HNSCC. Overexpression of EGFR is associated with poor clinical outcomes of HNSCC [53–55]. Therefore, EGFR is considering as a promising target for the treatment of patients suffering from HNSCC. Honokiol treatment significantly decreased the cell viability and induced apoptosis in various HNSCC cell lines, such as derived from tongue, larynx, oral cavity, and pharynx, and suggests that honokiol possesses broad therapeutic effect on this malignancy. A recent study published by Singh et al. [56] reported that honokiol targets EGFR signaling to inhibit HNSCC growth. Treatment of HNSCC cell lines with honokiol decreases the expression levels of total EGFR as well as p-EGFR and its downstream target mTOR signaling. An activation of mTOR signaling has been shown to contribute in tumor growth and progression. To confirm the findings, authors further verified the inhibition of mTOR and its downstream targets using rapamycin, an inhibitor of mTOR, and its effect on cell viability. It was found that treatment of cells with rapamycin results in significant inhibition of cell viability of HNSCC cells as well as decrease in the levels of mTOR and its downstream targets. Based on these observations, it appears that honokiol acts as an antagonist and/or causes increased turnover of EGFR, thus accounting for decreased expression of EGFR in HNSCC cells. The iNOS is involved in tumorigenesis [57]. Studies reported that honokiol treatment not only inhibits HNSCC cell proliferation, but also induced apoptosis in HNSCC cell lines through decreasing the expression level of iNOS at the protein as well as mRNA levels. NO is an important regulator of various MMPs, which are over expressed in metastatic cancers and promote cancer cell migration [58]. Studies also indicated that honokiol treatment reduces metastatic potential of HNSCC cells by targeting MMPs and inhibiting nuclear translocation of NF-κB [59]. A proteomic study based on LC-MS/MS analysis revealed that out of 181 identified proteins, 96 proteins were differentially expressed in honokiol-treated HN22 cells. Cho et al. [59] list the biological functions of several identified proteins. The endoplasmic reticulum protein 44 (ERp44) acts as an anchoring protein for ER-resident proteins that lack an ER retention signal and in many instances, a family of ER oxidoreductases including ERp44 catalyzes oxidative protein folding. The treatment of honokiol significantly reduces ERp44 expression in HNSCC cell lines. After ERp44 degradation, ER calcium ion flows into the cytoplasm. ERp44 is a key regulator of protein secretion, calcium signaling and redox regulation via interaction with IP3R1 in a calcium ion, redox and pH dependent manner [60]. In pathologic conditions, excessive influx of cytosolic calcium ion into the mitochondria triggers dysfunction of the mitochondrial membrane permeabilization with mitochondrial ROS induction [61]. Studies

have identified that honokiol treatment induced a significant release of cytochrome c from the mitochondria, followed by an increase in mitochondrial Bax and a significant decrease in the expression levels of the pro-apoptotic proteins Bid, Bcl-xL that results in death of HNSCC cells.

11.9 Protective Effect of Honokiol in Breast Cancer

There has been growing emphasis on the importance of epithelial to mesenchymal transition (EMT), an essential normal physiological process for embryonic development, tissue remodeling, wound healing, and in cancer progression. An oncogenic EMT not only derives tumors to gain a mesenchymal phenotype but also facilitate in migration and invasion potential of cancer cells. The adoption of mesenchymal characteristics not only promotes separation of cancer cells from primary tumor sites but also provides favorable microenvironment such as increase in tumor-initiating cell characteristics including self-renewal, multi potency and resistance to conventional therapeutics [62–64]. Recent study by Avtanski et al. [65] indicates that honokiol effectively inhibits EMT in breast cancer cells as evident from morphological changes and molecular alterations of mesenchymal and epithelial genes. Breast tumors treated with honokiol also showed reduced expression of mesenchymal markers, and provide convincing evidence to support the efficacy of honokiol as a potent inhibitor of EMT. An inactivation of serine–threonine kinase liver kinase B1 (LKB1; also known as STK11), a known tumor suppressor, is correlated with poor prognosis of breast carcinoma [66], and its knockdown increases motility and invasiveness of cancer cells, through induction in the expression of many mesenchymal marker proteins indicating its possible role in EMT [67, 68]. SIRT1 and SIRT3 have been shown to deacetylate LKB1 leading to an increase in its cytoplasmic localization, binding with STRAD and MO25 and activation of kinase function. Avtanski et al. [65] have found that honokiol increases the expressions of SIRT1 and SIRT3, which leads to increase in the cytoplasmic localization of LKB1 in breast cancer cells. The miRNAs play essential roles in various biological processes, including cell proliferation, survival, and differentiation. The loss of miR-34a expression has been reported in many cancer types including breast cancer [69]. Treatment of honokiol enhances miR-34a expression and inhibits mesenchymal markers while enhancing the expression of epithelial markers in breast cancer cells. These changes may result in suppression of cell viability of breast cancer cells.

11.10 Growth Inhibitory Effect of Honokiol in Urinary Bladder Cancer

Urinary bladder cancer is one of the most common urogenital malignant cancer with an estimated 74,690 new cases and 15,580 deaths occurring in USA during 2014 [70]. Recent studies reported that cancer stem cells might account for

chemotherapy failure, which are enriched after therapy and have the ability to generate all types of differentiated cells to repopulate tumors and eventually lead to metastasis [71–73]. Histone modifications through polycomb repressive complexes also play an essential role for normal and malignant cell stemness maintenance. Deregulation of Enhancer of Zeste Homologue 2 (EZH2), an important component of polycomb repressive complex 2, is frequently detected in a variety of cancer along with urinary bladder cancer [74–76]. Activation EZH2 specifically represses the transcription of differentiation-related genes throughout the cell cycle to maintain the stemness of cells, and this make it a promising therapeutic target for cancer. Zhang et al. [77] have found that depletion of EZH2 by honokiol treatment inhibited cell proliferation and clonogenicity of urinary bladder cancer cells.

11.11 Anti-Tumorigenic Activity of Honokiol in Prostate Cancer

Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer-related death in males after skin cancer in economically developed countries [70]. The major cause of mortality in prostate cancer is associated with metastasis. Approximately, 90 % of deaths from solid tumors are caused by metastasis [78]. Studies have shown that honokiol induces autophagy in cancer cells [79, 80]. An induction of autophagy with different functional consequences has been described for a number of structurally divergent naturally occurring anticancer agents. Studies have implicated that mTOR is a negative regulator of autophagy. A study published by Hahm et al. [48] reported the suppression of mTOR and Akt phosphorylation by honokiol treatment in prostate cancer cells (PC-3). An induction of apoptosis by natural agents is significantly attenuated by antioxidants because of reduced activation of multi domain Bcl-2 family member Bax. Hahm et al. [48] also found that treatment of honokiol induced apoptosis in PC-3 and LNCaP cells, which was associated with induction of Bax and Bak, and claimed that honokiol may be effective in prevention and therapy of prostate cancer.

11.12 Honokiol Inhibits Metastatic Potential and Tumor Growth of Gastric Cancer

Gastric cancer is also a leading cause of cancer-related death worldwide, and the majority of patients exhibit a high incidence of lymph node metastases. Emerging evidence suggests that EMT leads to increased tumor formation, tissue invasiveness,

and tumor dissemination [81]. Recent studies have shown a close relation between EMT and gastric cancer progression. A number of signaling pathways, including developmental transcriptional factors, are involved in regulating the motile-invasion phenotype of tumor cells [82]. The decreased expression of epithelial marker E-cadherin in gastric cancer has established the potential role of EMT in gastric cancer metastasis [83, 84]. Tumor progression locus 2 (Tpl2) is a serine-threonine kinase, regulates the activation of the mitogen-activated protein kinase, and critically involved in inflammation, oncogenic events, and tumor progression [85, 86]. Pan et al. [87] have shown that honokiol treatment regulates Tpl2 mediated mesenchymal markers and significantly down regulates Snail, vimentin, N-cadherin expression, and upregulates cytokeratin-18 and E-cadherin expression. Tumor growth in gastric cancer has been associated with Tpl2 [88, 89]. Pan et al. [87] also reported that honokiol treatment reduces growth of gastric tumor through an inhibition of Tpl2 expression. Honokiol significantly inhibited tumor angiogenesis as indicated by reduced microvessel density in tumor mass [87]. Cell cycle regulation is an important regulatory mechanism to control cell growth, and activation of p53, a tumor suppressor protein, is involved in the regulation of cell cycle arrest and apoptosis. The phosphorylation of cell cycle regulatory proteins is involved in arresting effect of gastric carcinoma cells on the cell cycle at G2/M phase [90, 91]. Yen et al. [92] investigated the anticancer mechanism of honokiol in human gastric carcinoma using MGC-803 cells and investigated that honokiol induces apoptosis in gastric carcinoma cells, and the underlying mechanism was mediated through downregulation of CDC2/cdc25C and upregulation of p53. These studies provide evidence of anticancer activity of honokiol against human gastric carcinoma.

11.13 Honokiol Inhibits Migratory Potential of Lung Cancer Cells

Lung cancer is a major cause of cancer-related deaths in the United States as well as worldwide each year and thus have a tremendous impact on human health and health care expenditures. Non-small-cell lung cancer (NSCLC) accounts for approximately 80 % of all types of lung cancer. COX-2 is frequently over express in lung cancer and associated with excessive production of PGE_2, which promotes tumor cell survival, invasion and metastasis [93–96]. Therefore, COX-2 inhibitors have shown potential in treatment of lung cancer. Singh and Katiyar [97] reported the use of honokiol as an inhibitor of COX-2 expression to inhibit migratory potential of lung cancer cells and supported their findings by the evidence that treatment of the NSCLC cells with celecoxib, a potent COX-2 inhibitor, resulted in a reduction in cell migration. The β-catenin signaling is downstream target of

COX-2 and played important roles in tumor progression as well as cell migration. β-catenin forms a dynamic link between E-cadherin and cytoskeleton [98, 99], and this cell-to-cell adhesion may prevent the migration of tumor cells. In contrast, the breaking of cell-to-cell adhesion due to activation of β-catenin and its nuclear accumulation may increase the migration potential of tumor cells. Singh and Katiyar [97] also reported that honokiol induced degradation of β-catenin or reduces nuclear accumulation, which leads to inhibition of lung cancer cell migration.

11.14 Honokiol Inhibits Pancreatic Cancer Growth

Pancreatic cancer is one of the most lethal malignancies with increasing incidence in the United States [33]. Due to its asymptomatic progression, pancreatic cancer is diagnosed at later stage, when it has already metastasized or locally advanced. The NF-κB is constitutively activated in a variety of hematologic and solid malignancies, including pancreatic cancer and controls the expression of an array of genes involved in cell proliferation and survival through direct and indirect mechanisms [100]. Arora et al. [101, 102] examined the effect of honokiol against pancreatic cancer and reported that honokiol showed growth inhibitory potential for pancreatic cancer lines (such as, Miapaca and PANC-1), which may result of cell cycle arrest and induction of apoptosis. Furthermore, to explore the underlying mechanism, these authors have tested the effect of honokiol on NF-κB signaling in this system. Treatment of honokiol to pancreatic cancer cells inhibited transcriptional activity of NF-κB, and decrease protein expression in the nuclear fraction and suppresses constitutive activation of NF-κB in pancreatic cancer cells and thus induce cell death.

11.15 Conclusion and Future Prospects

Honokiol, a small molecule phytochemical, has been shown to have significant chemopreventive and therapeutic effects against cancers of various organs in vitro and in vivo animal models. Honokiol targets distinct signaling pathways, molecular and cellular targets and leads to inhibition of growth and progression of tumors of various organs (Fig. 11.3). Importantly, development of cancers are considered as chronic disease. Therefore, it has significant potential to serve as a novel agent for prevention and therapy of chronic diseases, like cancers. This small molecule from *Magnolia spp*. may be of significant interest for attenuation of the adverse effects of environmental factors, such as solar UV radiation, on human skin. The use of honokiol in combination with already available cancer therapeutic drugs may offer an enhanced ability to attack other cancer-related targets, reduce toxicity and resistance to the cancer drugs and may improve the therapeutic efficacy of the existing cancer drugs.

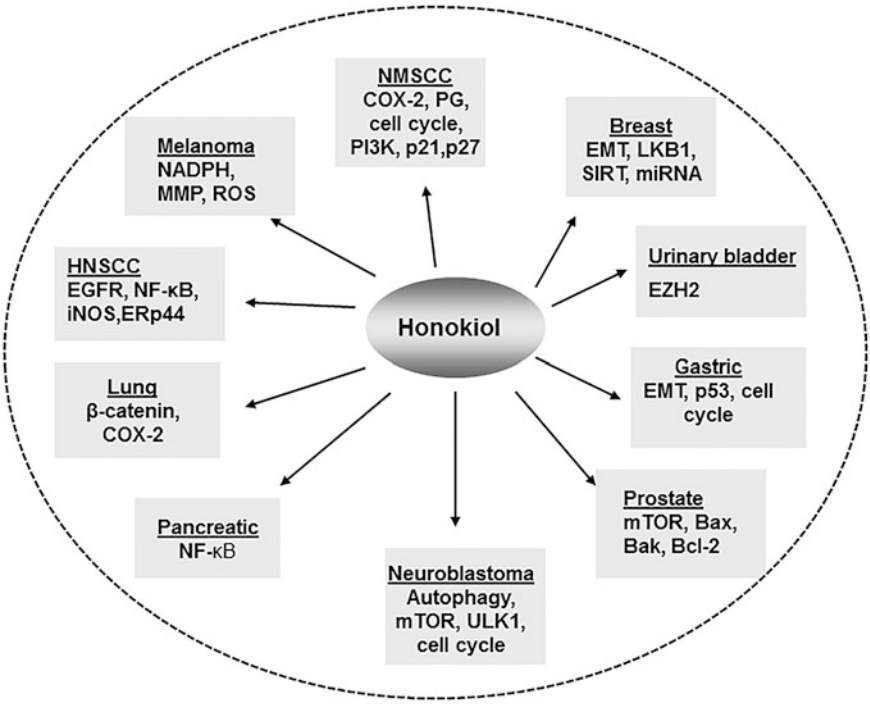

Fig. 11.3 The schematic diagram reflects the preventative and therapeutic effect of honokiol on cancers of different organs. Various molecular and cellular targets in cancers of different organs are affected by the treatment of honokiol in vitro and in vivo models. Inflammation and inflammatory mediators are the main targets of honokiol in prevention or treatment of cancers. *Underline words* indicate the name of organ-specific cancer

Acknowledgments The work reported from Dr. Katiyar's research laboratory was financially supported from the funds from National Cancer Institute/NIH (CA183869) and Veterans Administration Merit Review Award (1I01BX001410). The content of this communication does not necessarily reflect the views or policies of the funding agencies.

Conflict of interest The author has declared no conflict of interest.

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