Review

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Targeting apoptosis pathways in cancer with magnolol and honokiol, bioactive constituents of the bark of *Magnolia officinalis*

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ABSTRACT: Magnolol and honokiol, main active compounds from the bark of *Magnolia officinalis*, have been found to have various pharmacological actions, including anti-oxidative, anti-inflammatory, anti-tumor, and anti-microbial properties, without appreciable toxicity. Recently, the anti-tumor activity of magnolol and honokiol has been extensively investigated. Magnolol and honokiol were found to possess anti-tumor activity by targeting the apoptosis pathways, which have been considered as targets for cancer therapies. This review will focus on the mechanisms by which magnolol and honokiol act on apoptosis pathways in cancer that have been characterized thus far, including the death receptor-mediated pathway, mitochondria-mediated pathway, caspase-mediated common pathway, and regulation of apoptosis-related proteins. These breakthrough findings may have important implications for targeted cancer therapy and modern applications of traditional Chinese medicine.

Keywords: Anti-tumor activity, apoptosis pathways, honokiol, magnolol, *Magnolia officinalis*

1. Introduction

Apoptosis is a normal physiological process that plays an important role in many normal functions ranging from embryonic development to adult tissue homeostasis (*1*). Defects in apoptosis are common phenomena in many types of cancer and are also the critical step in tumorigenesis and resistance to therapy (*2*). Thus, apoptotic pathways have been considered as targets for cancer therapies (*3*).

Traditional Chinese medicine (TCM) has held and still holds an important position in primary health care in China and has recently been recognized by Western countries as a fertile source of novel lead molecules as part of modern drug discovery. Although TCM has been used for thousands of years in China, its mechanisms of healing at the molecular level are still largely unknown. To better understand the therapeutic action of TCM, considerable efforts have been made to identify the principal constituents of TCM and to unravel the molecular mechanisms behind the efficacy observed (*4*). Over the last two decades, more and more bioactive compounds have been identified from TCM herbs. Magnolol and honokiol, main active compounds from the bark of *Magnolia officinalis* (Cortex Magnoliae Officinalis), have been found to possess anti-tumor activity by inducing apoptosis in cancer (*5*). This review will focus on the mechanisms by which magnolol and honokiol act on apoptosis pathways in cancer that have been characterized thus far. Due to space limitations, only key studies are cited.

2. Magnolol and honokiol

The Chinese herb *Magnolia officinalis* is widely used as a folk remedy for gastrointestinal disorders, cough, anxiety, and allergic diseases as an oriental medicine in South Korea, China, and Japan (*6*). Magnolia bark is rich in two biphenol compounds, magnolol (5,5'-diallyi-2,2'-dihydroxybiphenyl, $C_{18}H_{18}O_2$) and honokiol (3,5'-diallyl-4,2'-dihydroxybiphenyl, $C_{18}H_{18}O_2$), that have been extensively investigated (*7,8*). The structures of magnolol and honokiol are shown in Figure 1. The magnolol content of magnolia bark is generally in the range of 2-10 percent, while honokiol tends to occur naturally at 1-5 percent in dried magnolia bark (*9*). The potent activity of honokiol and magnolol appears to be due to the presence of hydroxyl and allylic groups on a biphenolic moiety (*10*).

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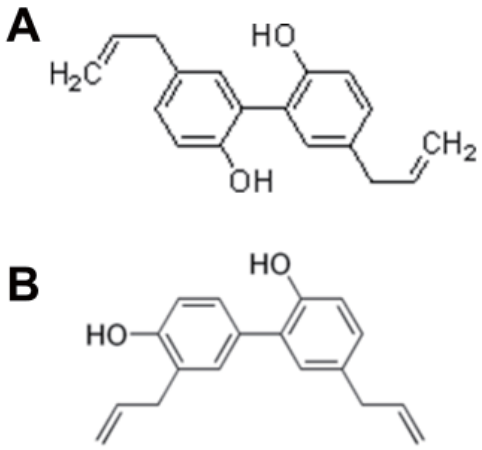

Figure 1. The chemical structures of (A) magnolol and (B) honokiol.

Magnolol, a hydroxylated biphenyl compound isolated from the stem bark of *Magnolia officinalis*, is commonly used to treat acute pain, cough, anxiety, and gastrointestinal disorders in East Asia (*11*). Various pharmacological actions have been reported for magnolol, including anti-inflammatory activity, antimicrobial activity (*12*), antiperoxidative activity (*13*), anti-coagulatory activity, anti-oxidant activity (*14*), neuroprotective activity (*15*), antitumor action (*16-18*), alleviation of inflammatory pain (*19*), and protection of cortical neuronal cells from chemical hypoxia (*20*).

Honokiol, a magnolol isomer, differs in the relative arrangement of one of its hydroxyl groups with respect to allyl groups in the phenolic ring (*21*) and is the most important bioactive constituent within magnolia bark studied thus far. Honokiol has also been found to have a variety of pharmacological action, such as anti-inflammatory action (*22*), antithrombotic activity (*23*), anti-arrhythmic activity (*24*), neuroprotective activity (*15*), antioxidative action (*25*), and anxiolytic action (*26*). Numerous animal studies have also demonstrated that honokiol acts as an anti-stress agent and a potent suppressor of oxidative damage and cancer (*27*).

Recently, magnolol and honokiol have been reported to have antitumor action by inhibiting proliferation, inducing apoptosis and differentiation, countering metastasis, suppressing angiogenesis, and reversing multidrug resistance (*10,28,29*). Numerous signaling pathways have been implicated in the regulation of apoptosis by magnolol and honokiol (*30*).

3. Targeting apoptosis pathways in cancer with magnolol and honokiol

Apoptosis occurs through two main pathways (*31,32*). The first, referred to as the extrinsic or death receptor pathway, involves ligation of death receptors (*e.g.* Fas (CD95), tumor necrosis factor receptors (TNFR), and TNF-related apoptosis-inducing ligand (TRAIL) receptors) with their ligands resulting in a sequential activation of caspase-8 and -3. The second pathway is the intrinsic or mitochondrial pathway in which intrinsic death stimuli (*e.g.* reactive oxygen species (ROS), DNA-damaging reagents, and Ca^{2+} mobilizing stimuli), directly or indirectly activate the mitochondrial pathway, resulting in the release of cytochrome *c* and the formation of the apoptosome complex consisting of cytochrome *c*, Apaf-1, and caspase-9 (Figure 2). Caspase-9 is activated at the apoptosome and in turn activates caspase-3. Between the death receptor and the mitochondrial signaling pathways, the pro-apoptotic protein Bid serves as a cross-talker upon cleavage by activated caspase-8 by inducing the translocation of the pro-apoptotic proteins Bax and/or Bak to the mitochondrial membrane (*33*). Both pathways converge to a final common pathway involved in the activation of a cascade of proteases called caspases that cleave regulatory and structural molecules, culminating in the deaths of cells (Figure 2). Although understanding of the detailed signaling pathways that trigger apoptosis is incomplete, this process is controlled by a number of complex proteins that are activated by various triggers and arranged in sequential signaling modules. In the receptor-mediated pathway, FLICE-inhibitory protein (c-FLIP) and XIAP negatively regulate the activity of caspase-8 and caspase-3, respectively (*31,32*). In the mitochondria pathway, apoptosis is largely controlled by the pro-apoptotic proteins, *e.g.* Bax, Bak, Bid, and Smac, and the anti-apoptotic proteins, *e.g.* Bcl-2, Bcl-xL, Mcl-1, and XIAP (*3*).

Many studies have shown that magnolol and honokiol induce the apoptosis of various tumor cells. Magnolol induces the apoptosis of many human cancer cell lines, including lung squamous cancer CH-27 cells, HL-60 cells, colon cancer COLO 205 cells, and liver cancer HepG2 cells (*34-38*), but does not induce the apoptosis of bovine aorta endothelial BAE cells (*38*) or polymorphonuclear and mononuclear leukocytes (*35*). Honokiol induces the apoptosis of human lymphoid leukemia Molt 4B cells, CH27 cells, B-CLL cells, and RKO cells in a time- and dose-dependent manner (*5,34,39,40*). In addition, honokiol has a more obvious apoptosis-inducing effect on B-CLL cells than on normal mononuclear leukocytes (*40*). *In vivo*, honokiol was highly effective against SVR angiosarcoma (*41*) and breast cancer in nude mice (*42*) and in a human A549 lung cancer xenograft model (*43*) with the increased induction of apoptosis.

This review will focus on the mechanisms by which magnolol and honokiol act on apoptosis pathways in cancer that have been characterized thus far, including the death receptor-mediated pathway, mitochondria-mediated pathway, caspase-mediated common pathway, and regulation of apoptosis-related proteins.

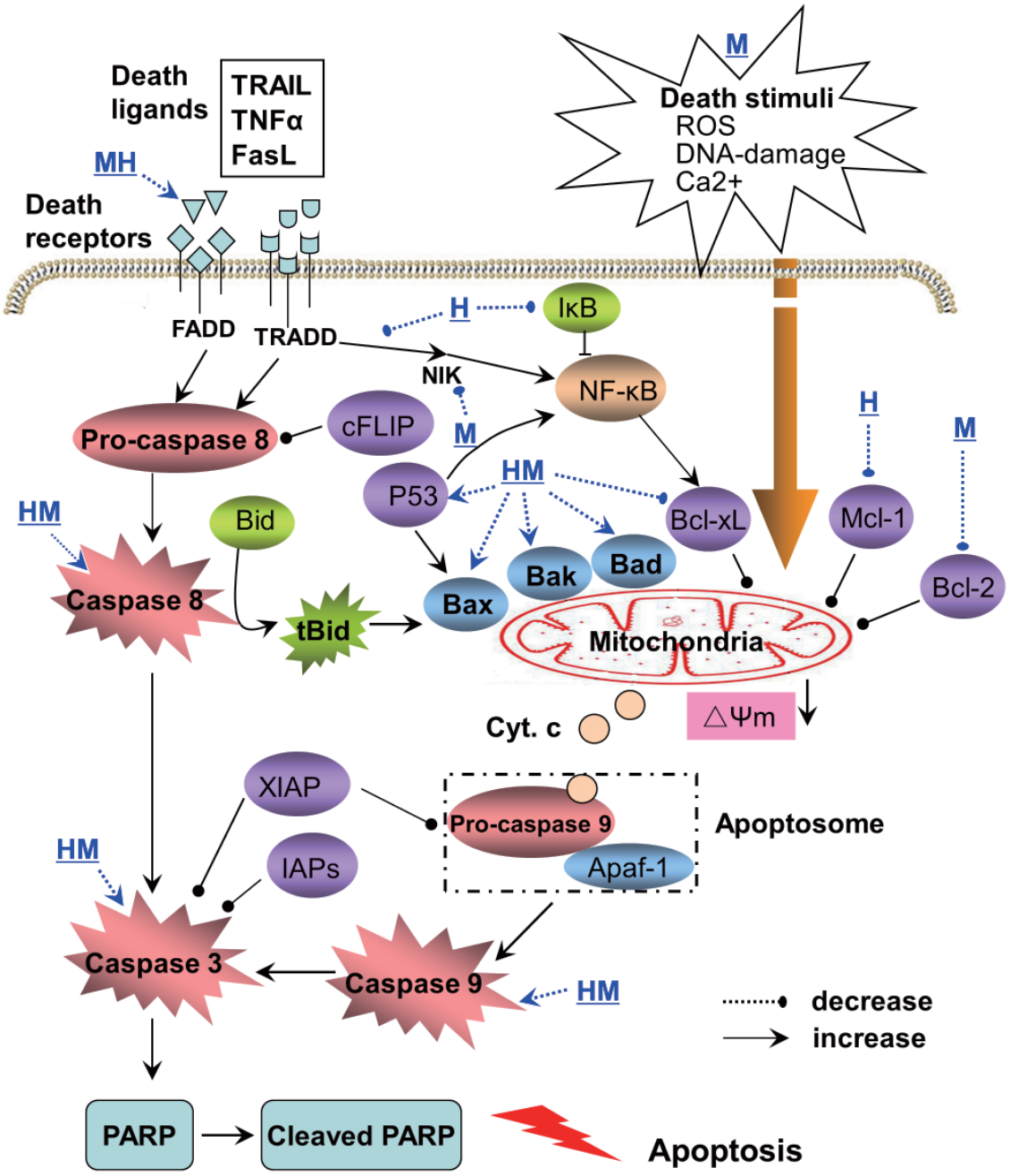

Figure 2. Effects of magnolol (M) and honokiol (H) on extrinsic (also called receptor-mediated) and intrinsic (also called mitochondria-mediated) apoptosis pathways in cancer. The extrinsic pathway involves ligation of death receptors with their ligands, resulting in a sequential activation of caspase-8 and -3. Intrinsic death stimuli, *e.g.* ROS, DNA-damaging reagents, or Ca^{2+} mobilization directly or indirectly activates the mitochondrial pathway by inducing release of cytochrome *c* and formation of the apoptosome, which consists of Apaf-1 and caspase-9. Caspase-9 is activated at the apoptosome and in turn activates pro-caspase-3.

3.1. *Targeting cancer cells by death receptor-mediated apoptosis*

Fas, TRAIL, and TNF receptors are highly specific physiological mediators of the extrinsic signaling pathway of apoptosis. Cross-linking of death receptors either with their natural ligands (*e.g.* FasL, TRAIL, and TNF-α) or with agonistic antibodies (such as anti-APO-1) induces a sequential activation of caspase-8 and -3, which cleaves target proteins and leads to apoptosis (*44*). Activation of the death receptor-mediated apoptotic pathway is primarily inhibited by cellular c-FLIP, which inhibits caspase-8 activation by preventing recruitment of caspase-8 to the death-inducing signaling complex. In some cells, the activation of caspase 8 may be the only requirement for death to ensue, while in other cell types caspase 8 interacts with the intrinsic apoptotic pathway by cleaving Bid (a proapoptotic member of the Bcl-2 family), leading to the subsequent release of cytochrome *c* (*45*).

Studies have shown that activation of the Fas-mediated pathway did not always result in magnolol-induced apoptosis. In response to magnolol administration, Fas was activated and cytochrome *c* was translocated from mitochondria to the cytoplasm through elevation of the cytosolic free Ca^{2+} concentration and downregulation of Bcl-2. Caspase-8 was activated by Fas activation, whereas caspase-9 was activated by cytochrome *c* release (*46*). Pretreating cells with ZB4 (which disrupts the Fas response mechanism) also decreased subsequent magnolol-induced caspase-8 activation and reduced the occurrence of apoptosis (*46*). Whether magnolol activates Fas directly or it promotes the action of a Fas ligand that in turn activates Fas remains to be determined.

Honokiol down-regulated c-FLIP in cancer cells, resulting in sensitization of cancer cells to both TRAIL-mediated and Fas ligand-mediated apoptosis (*47*). Honokiol alone moderately inhibited the growth of human lung cancer cells; when combined with TRAIL, however, honokiol had a greater impact on decreasing cell survival and inducing apoptosis than did TRAIL alone, indicating that honokiol cooperates with TRAIL to enhance apoptosis. This was also true for Fas-induced apoptosis when it was combined with a Fas ligand or an agonistic anti-Fas antibody. Of several apoptosis-associated proteins tested, c-FLIP was the only one that was rapidly down-regulated by honokiol in all of the cell lines tested (*47*). These results indicate that c-FLIP down-regulation is a key step in honokiol's modulation of death receptor-induced apoptosis.

3.2. *Targeting cancer cells by mitochondria-mediated apoptosis*

The effects of magnolol on the intrinsic pathway of apoptosis have been examined in many cell lines, including human leukemia U937 cells (*48*), human hepatoma Hep G2 and colon cancer COLO 205 cells (*46*), and rat vascular smooth muscle cells (VSMCs) (*49*). Magnolol increased caspase-3 and caspase-9 activity significantly and reduced the mitochondrial potential ($\Delta\Psi m$) in these cells. Treatment with magnolol was found to partly inhibit growth by inducing apoptosis in cultured human leukemia U937 cells and apoptosis was found to be induced *via* the sequential ordering of molecular events. Thus, magnolol-induced apoptosis is mediated *via* the intrinsic pathway with release of AIF from mitochondria in U937 cells (*48*). Lin SY, *et al*. showed that treatment with magnolol induced apoptosis by increasing translocation of cytochrome *c* from mitochondria to cytosol and activation of caspase-3, -8, and -9 in cultured Hep G2 and COLO 205 cell lines (*46*). In addition, Huang SH, *et al*. showed that magnolol initiated apoptosis *via* cytochrome *c*/caspase-3/PARP/AIF and PTEN/Akt/caspase-9/PARP pathways and necrosis *via* PARP activation (*50*).

Similar results were found in honokiol-treated cells. Honokiol treatment caused the release of mitochondrial cytochrome *c* to cytosol and sequential activation of caspases in human squamous lung cancer CH27 cells (*51*). Honokiol also induced release of mitochondrial proapoptotic protein AIF to the cytosol in human multiple myeloma (MM) cells (*52*). The current review has mainly focused on induction of mitochondria-mediated apoptosis *via* reactive oxygen species (ROS)-mediated and Ca^{2+}-mediated mechanisms of magnolol and honokiol.

3.2.1. *ROS-mediated mechanisms*

ROS, including free radicals such as superoxide ($\bullet O_2^-$), hydroxyl radicals ($\bullet$OH), and the non-radical H_2O_2, are generated through multiple sources in the cells (*53*). Tumors, and particularly those in advanced stages, produce elevated levels of ROS and have an altered redox status. ROS and mitochondria play an important role in apoptosis induction under both physiological and pathological conditions (*54*). Interestingly, mitochondria are both sources and targets of ROS. Cytochrome *c* release from mitochondria, which triggers caspase activation, appears to be largely mediated by direct or indirect ROS action (*55*). High levels of ROS may cause the oxidative damage of various cellular components and finally result in cell apoptosis.

Magnolol has been shown to attenuate oxidized low-density lipoprotein (oxLDL)-induced ROS generation, subsequently reducing nuclear factor-kappaB (NF-κB) activation (*56*). Magnolol also inhibited UV-induced mutations by scavenging $\bullet$OH generated by UV irradiation (*57*). The attenuation of ROS by magnolol has been proposed as a reason for its inhibitory effect on neutrophil adherence to the extracellular matrix during injury (*58*).

Honokiol was also found to be a potent scavenger of hydroxyl radicals, which is likely due to its allyl groups (*22,59*). The ortho allyl group may potentially form a six-member ring after absorption of a hydroxyl group. This may account for its superior antioxidant activity when compared to magnolol, which has two allyl groups with hydroxyl groups in the *para* position and thus cannot form a six-member ring. Honokiol-induced apoptosis has been closely associated with ROS production. Inhibition of reactive oxygen-driven tumors by honokiol is due to its involvement in the NADPH oxidase (NOX) pathway (*22*). This inhibition was first demonstrated in neutrophils (*22*) and later in hepatocytes (*60*) and human umbilical vein endothelial cells (HUVECs) (*22*). A possible chemical mechanism for this involves a peroxide intermediate followed by the phenolic hydroxyl group attacking the peroxide carbon chain, yielding a pentose or hexose ring and water.

3.2.2. *Ca^{2+}-mediated mechanisms*

Ca^{2+} signals are known to play an important role in the regulation of cell death and survival (*61*). One known Ca^{2+}-regulated Bcl-2-associated pro-apoptotic protein is Bad. In non-apoptotic cells, Bad is phosphorylated and sequestered by the cytosolic protein 14-3-3, avoiding its hetero-dimerization with Bcl-2 and Bcl-xL at the mitochondrial membrane. In the presence of an apoptotic stimulus (*e.g.* Ca^{2+}), Bad is dephosphorylated by Ca^{2+}/calmodulin-dependent phosphatase calcineurin, leading to dissociation from its inhibitor 14-3-3 and promoting apoptosis (*62*). Since mitochondria are the major organelles that take up Ca^{2+}, Ca^{2+} over-loading of the mitochondria may also directly lead to release of cytochrome *c* as part of a stress response.

Lin SY, *et al*. showed that treatment with 100 μM of magnolol increased cytosolic free Ca^{2+}, resulting in induced apoptosis in cultured human Hep G2 and COLO 205 cell lines but not in human untransformed gingival fibroblasts and human umbilical vein endothelial cells (*46*). In rat neutrophils, magnolol increased $[Ca^{2+}]_i$ by stimulating Ca^{2+} release from internal stores and Ca^{2+} influx across the plasma membrane in a concentration-dependent manner *via* the inositol trisphosphate signalling pathway (*63*). Magnolol relaxed vascular smooth muscle by releasing endothelium-derived relaxing factor (EDRF) and by inhibiting calcium influx through voltage-gated calcium channels (*64*). Magnolol also increased the probability of these channels opening in a concentration-dependent manner, independent of internal Ca^{2+}, in tracheal smooth muscle cells (*65*).

3.3. *Targeting cancer cells by caspase-mediated apoptosis*

The caspases are a family of proteins that are one of the main executors of the apoptotic process. As of November 2009, twelve caspases have been identified in humans (*66*). There are two types of apoptotic caspases: initiator (apical) caspases (caspase-2, -8, -9, and -10) and effector (executioner) caspases (caspase-3, -6, and -7). Initiator caspases cleave inactive pro-forms of effector caspases, thereby activating them. Effector caspases in turn cleave other protein substrates within the cell, triggering the apoptotic process.

Activation of caspase-3, -8, -9, and -2, and the proteolytic cleavage of poly(adenosine diphosphate-ribose) polymerase (PARP) were noted during apoptosis induced by magnolol (*46*). Pretreatment with Z-Val-Ala-Asp-fluoromethyl ketone (Z-VAD-FMK), a pan-caspase inhibitor, markedly inhibited magnolol-induced cell death but did not prevent cytosolic cytochrome *c* accumulation. Apoptosis may be partially attenuated by caspase-3 and -2 inhibitors. These results indicate that magnolol-induced apoptotic signaling was carried out through mitochondrial alterations to caspase-9 and that the downstream effector caspases were then activated sequentially (*35*).

Honokiol-induced apoptosis is characterized by the activation of caspase-3, -8, and -9 and cleavage of PARP (*48*). Honokiol induced caspase-dependent cell death in all of the B-CLL cells examined and was more toxic toward B-CLL cells than to normal mononuclear cells, suggesting that malignant cells were more susceptible. Although activation of caspase-3, -8, and -9 is triggered by honokiol, the pan-caspase inhibitor Z-VAD-FMK does not abrogate honokiol-induced apoptosis (*52*). Importantly, honokiol treatment induces the release of an executioner of caspase-independent apoptosis, AIF, from mitochondria. Honokiol also induced apoptosis in the SU-DHL4 cell line, which has low levels of caspase-3 and -8 (*52*). These results suggest that honokiol induced apoptosis *via* both caspase-dependent and -independent pathways.

3.4. *Targeting cancer cells by regulating apoptosis-related proteins*

Treatment with magnolol significantly increased the expression of Bad and Bcl-x(S) proteins, whereas it decreased the expression of Bcl-x(L) (*67*). Magnolol treatment also caused a decrease in Ser(136) phosphorylation of Bad, which is a downstream target of Akt, and translocation of Bax to the mitochondrial membrane. Similar results were observed in the human colon cancer HCT116Bax(+/-) cell line but not in the HCT116Bax(-/-) cell line. In addition, apoptotic cell death due to magnolol was found to be associated with significant inhibition of pEGFR, pPI3K, and pAkt (*67*).

Honokiol-induced apoptosis correlated with induction of Bax, Bak, and Bad and a decrease in Bcl-xL and Mcl-1 protein levels. Transient transfection of PC-3 cells with Bak- and Bax-targeted siRNAs and Bcl-xL plasmid conferred partial yet significant protection against honokiol-induced apoptosis (*68*). Another study showed that honokiol caused cleavage of Mcl-1 and downregulation of XIAP while Bad was markedly upregulated; Bid, p-Bad, Bak, Bax, Bcl-2, and Bcl-xL were unchanged (*52*). Honokiol also induced release of mitochondrial proapoptotic protein AIF to the cytosol and prevented phosphorylation of Akt, Stat-3, and Erk2, again implying an upstream target of action (*52,69*).

The effects of magnolol and honokiol on two important apoptosis-related proteins, p53 and NF-κB, have been summarized below.

3.4.1. *p53*

Loss of function of the *p53* tumor suppressor gene is a frequent and important event in the genesis or progression of many human malignancies. In many tumor cells, wild-type p53 is thought to participate

in apoptosis in response to DNA damage (*70*). P53 may transactivate apoptotic regulators, such as Bcl-2 (*49,71-74*) and Bax (*75-77*). Recent studies have shown that p53 plays a role in apoptosis by the mitochondria-mediated apoptotic pathway (*74,78*). Activation of p53 upregulates Bax, increases the ratio of Bax:Bcl-2, and releases cytochrome *c* and other polypeptides from the intermembrane space of mitochondria into the cytoplasm (*76*). p53-dependent apoptosis was activated by the Bax/mitochondrial/caspase-9 pathway.

Honokiol has been found to prevent the growth of MDA-MB-231 breast cancer cells in murine xenografts (*79*). An interesting finding is that MDA-MB-231 cells display mutant p53 and mutant K-ras, which is preferentially observed in the triple-negative breast cancer phenotype (*79,80*). In the same study, honokiol had less activity on the MCF7 breast cancer cell line, which exhibited wild-type p53 and loss of p16ink4a. Given that SVR cells have defects in p53 signaling because of expression of SV40 large T and that MDA-MB-231 cells also express mutant p53, tumors that have defects in p53 signaling may be targets of honokiol. Similarly, honokiol caused apoptosis in other solid-tumor cell lines that feature mutant p53 and ras activation, including lung and bladder cell lines (*81*). Thus, honokiol appears to have distinct activity against tumors with mutant p53, through its inhibition of ras-phospholipase D activation, and tumors with wild-type p53, through its induction of cyclophilin D. However, Wang T, *et al*. showed that honokiol induced RKO cell apoptosis by activating the caspase cascade *via* a p53-indepenent pathway (*82*). Hahm ER, *et al*. also showed that exposure of human prostate cancer cells (PC-3, LNCaP, and C4-2) to honokiol resulted in apoptotic DNA fragmentation in a concentration- and time-dependent manner, irrespective of their androgen responsiveness or p53 status (*68*).

3.4.2. *NF-κB*

The NF-κB pathway is one of the most important cellular signal transduction pathways involved in immunity, inflammation, proliferation, and defense against apoptosis (*83*). NF-κB is generally considered to be a survival factor that activates expression of various anti-apoptotic genes, *e.g*. Bcl-2, Bcl-xL, Mcl-1 and c-FLIP, that block apoptosis (*83,84*). The classic form of NF-κB is the p65/p50 heterodimer that contains the transcriptional activation domain and is sequestered in the cytoplasm as an inactive complex by IκB. Acute stimuli such as TNF-α, LPS or PMA lead to the activation of IκB kinases (IKK), which in turn phosphorylate Ser32 and Ser36 within the *N*-terminal response domain of IκB. Phosphorylated IκB undergoes ubiquitination-dependent proteolysis and the release of IκB unmasks the nuclear localization signal and results in the translocation of NF-κB to the nucleus, followed by the activation of specific target genes.

Both magnolol and honokiol have been shown to inhibit the NF-κB signaling pathway. In a previous study, magnolol was shown to reduce the nuclear NF-κB content in TNF-α-stimulated endothelial cells (*85*). However, their mechanisms of action are poorly understood. Chen YH, *et al*. demonstrated that magnolol suppressed IKK activity, stabilized cytoplasmic IκBα, and subsequently reduced the nuclear translocation and phosphorylation of the p65 subunit of NF-κB (*86*). Magnolol also inhibited NF-κB-dependent reporter gene expression induced by TNF-α and it inhibited over-expression of NIK, IKK, and the p65 subunit while it enhanced TNF-α-mediated apoptosis. In human U937 promonocytes cells, magnolol inhibited the TNF-α-stimulated phosphorylation and degradation of the cytosolic NF-κB inhibitor IκBα and did so in a dose-dependent manner (*87*). In addition, magnolol differentially down-regulated the expression of NF-κB-regulated inflammatory gene products, *e.g*. MMP-9, IL-8, MCP-1, MIP-1α, and TNF-α. The involvement of IKK was further verified in a HeLa cell NF-κB-dependent luciferase reporter system (*87*).

Honokiol affected NF-κB signaling, but not through a direct effect on NF-κB DNA binding (*88*). Honokiol inhibited TNF-induced NF-κB activation and IkBα phosphorylation and degradation through its inhibition of the activation of IκBα kinase and Akt. Honokiol also inhibited NF-κB-dependent reporter gene expression induced by TNFR1, TRADD, TRAF, NIK, and IKKβ (*88*). Consistent with honokiol's effect on NF-κB, honokiol decreased levels of NF-κB target genes, including IAP1, IAP2, Bcl-xL, Bcl-2, cFLIP, TRAF1, and survivin (*88*). NF-κB and NF-κB-regulated gene expression inhibited by honokiol can thus enhance apoptosis. Honokiol also down-regulated NF-κB activation in an *in vivo* mouse dorsal skin model. Another study showed that honokiol blocked the production of TNF-α, MCP-1, interleukin-8, and ICAM-1 and was found to act at the level of IKK or upstream of IKK, indicating a possible mechanism of its anti-tumor action (*89*).

4. Summary

In recent years, various biologically active constituents have been isolated from TCM and have been found to have varied activity in experimental studies. Honokiol and magnolol have been found to have anti-oxidative, anti-inflammatory, anti-tumor, and anti-microbial properties in preclinical models. Their safety during long-term administration, combined with their cost and future therapeutic potential, makes them ideal therapeutic agents (*90*). In addition, magnolol and honokiol are small molecular weight natural products that are orally bioavailable and able to cross the blood-brain barrier. Clinical trials are needed to fully realize

the potential of honokiol and magnolol as effective antitumor drugs. Honokiol and magnolol analogues with improved pharmacokinetic and pharmacodynamics will also encourage further advances.

Many studies have shown that both magnolol and honokiol induce apoptosis of many types of cancer cells, though those studies describe different mechanisms of action. Moreover, investigation of how they specifically induce apoptosis in cancers and spare normal cells will provide new clues to help identify more efficient drugs and to develop apoptosis-targeting therapies.

References

1. Kasibhatla S, Tseng B. Why target apoptosis in cancer treatment. Mol Cancer Ther. 2003; 2:573-580.
2. Kaufmann SH, Earnshaw WC. Induction of apoptosis by cancer chemotherapy. Exp Cell Res. 2000; 256:42-49.
3. Ghobrial IM, Witzig TE, Adjei AA. Targeting apoptosis pathways in cancer therapy. C.A. Cancer J Clin. 2005; 55:178-194.
4. Hsiao WL, Liu L. The role of traditional Chinese herbal medicines in cancer therapy – from TCM theory to mechanistic insights. Planta Med. 2010; 76:1118-1131.
5. Chen F, Wang T, Wu YF, Gu Y, Xu XL, Zheng S, Hu X. Honokiol: A potent chemotherapy candidate for human colorectal carcinoma. World J Gastroenterol. 2004; 10:3459-3463.
6. Maruyama Y, Kuribara H, Morita M, Yuzurihara M, Weintraub ST. Identification of magnolol and honokiol as anxiolytic agents in extracts of saiboku-to, an oriental herbal medicine. J Nat Prod. 1998; 61:135-138.
7. Shen CC, Ni CL, Shen YC, Huang YL, Kuo CH, Wu TS, Chen CC. Phenolic constituents from the stem bark of Magnolia officinalis. J Nat Prod. 2009; 72:168-171.
8. Watanabe K, Watanabe H, GotoY, Yamaguchi M, Yamamoto N, Hagino K. Pharmacological properties of magnolol and honokiol extracted from Magnolia officinalis: Central depressant effects. Planta Med. 1983; 49:103-108.
9. Cheng DC, Liu JW. Quantitative analysis of magnolol and honokiol in the bark of Magnolia officinalis Rehd et Wils and Magnolia rostrata WW Smith. Acta Pharmaceutica Sinica. 1982; 17:360-364.
10. Amblard F, Govindarajan B, Lefkove B, Rapp KL, Detorio M, Arbiser JL, Schinazi RF. Synthesis, cytotoxicity, and antiviral activities of new neolignans related to honokiol and magnolol. Bioorg Med Chem Lett. 2007; 17:4428-4431.
11. Jeong SI, Kim YS, Lee MY, Kang JK, Lee S, Choi BK, Jung KY. Regulation of contractile activity by magnolol in the rat isolated gastrointestinal tracts. Pharmacol Res. 2009; 59:183-188.
12. Park J, Lee J, Jung E, Kim K, Park B, Jung K, Park E, Kim J, Park D. *In vitro* antibacterial and anti-inflammatory effects of honokiol and magnolol against Propionibacterium sp. Eur J Pharmacol. 2004; 496:189-195.
13. Haraguchi H, Ishikawa H, Shirataki N, Fukuda A. Antiperoxidative activity of neolignans from Magnolia obovata. J Pharm Pharmacol. 1997; 49:209-212.
14. Tsai YC, Cheng PY, Kung CW, Peng YJ, Ke TH, Wang JJ, Yen MH. Beneficial effects of magnolol in a rodent model of endotoxin shock. Eur J Pharmacol. 2010; 641:67-73.
15. Lin YR, Chen HH, Ko CH, Chan MH. Neuroprotective activity of honokiol and magnolol in cerebellar granule cell damage. Eur J Pharmacol. 2006; 537:64-69.
16. Kuo DH, Lai YS, Lo CY, Cheng AC, Wu H, Pan MH. Inhibitory effect of magnolol on TPA-induced skin inflammation and tumor promotion in mice. J Agric Food Chem. 2010; 58:5777-5783.
17. Konoshima T, Kozuka M, Tokuda H, Nishino H, Iwashima A, Haruna M, Ito K, Tanabe M. Studies on inhibitors of skin tumor promotion, IX. Neolignans from Magnolia officinalis. J Nat Prod. 1991; 54:816-822.
18. Lee SJ, Cho YH, Park K, Kim EJ, Jung KH, Park SS, Kim WJ, Moon SK. Magnolol elicits activation of the extracellular signal-regulated kinase pathway by inducing p27KIP1-mediated G2/M-phase cell cycle arrest in human urinary bladder cancer 5637 cells. Biochem Pharmacol. 2008; 75:2289-2300.
19. Lin YR, Chen HH, Ko CH, Chan MH. Effects of honokiol and magnolol on acute and inflammatory pain models in mice. Life Sci. 2007; 81:1071-1078.
20. Lee MM, Hseih MT, Kuo JS, Yeh FT, Huang HM. Magnolol protects cortical neuronal cells from chemical hypoxia in rats. Neuroreport. 1998; 9:3451-3456.
21. Li H, Wang X, Hu Y. Distinct photoacidity of honokiol from magnolol. J Fluoresc. 2011; 21:265-273.
22. Liou KT, Shen YC, Chen CF, Tsao CM, Tsai SK. The anti-inflammatory effect of honokiol on neutrophils: Mechanisms in the inhibition of reactive oxygen species production. Eur J Pharmacol. 2003; 475:19-27.
23. Teng CM, Chen CC, Ko FN, Lee LG, Huang TF, Chen YP, Hsu HY. Two antiplatelet agents from Magnolia officinalis. Thromb Res. 1988; 50:757-765.
24. Liou KT, Lin SM, Huang SS, Chih CL, Tsai SK. Honokiol ameliorates cerebral infarction from ischemia-reperfusion injury in rats. Planta Med. 2003; 69:130-134.
25. Lo YC, Teng CM, Chen CF, Chen CC, Hong CY. Magnolol and honokiol isolated from Magnolia officinalis protect rat heart mitochondria against lipid peroxidation. Biochem Pharmacol. 1994; 47:549-553.
26. Kuribara H, Kishi E, Hattori N, Yuzurihara M, Maruyama Y. Application of the elevated plus-maze test in mice for evaluation of the content of honokiol in water extracts of magnolia. Phytother Res. 1999; 13:593-596.
27. Kim BH, Cho JY. Anti-inflammatory effect of honokiol is mediated by PI3K/Akt pathway suppression. Acta Pharmacol Sin. 2008; 29:113-122.
28. Hwang ES, Park KK. Magnolol suppresses metastasis *via* inhibition of invasion, migration, and matrix metalloproteinase-2/-9 activities in PC-3 human prostate carcinoma cells. Biosci Biotechnol Biochem. 2010; 74:961-967.
29. Luo H, Zhong Q, Chen LJ, Qi XR, Fu AF, Yang HS, Yang F, Lin HG, Wei YQ, Zhao X. Liposomal honokiol, a promising agent for treatment of cisplatin-resistant human ovarian cancer. J Cancer Res Clin Oncol. 2008; 134:937-945.
30. You Q, Li M, Jiao G. Magnolol induces apoptosis *via* activation of both mitochondrial and death receptor pathways in A375-S2 cells. Arch Pharm Res. 2009; 32:1789-1794.
31. Johnstone RW, Frew AJ, Smyth MJ. The TRAIL apoptotic pathway in cancer onset, progression and

therapy. Nat Rev Cancer. 2008; 8:782-798.
32. Li-Weber M. Targeting apoptosis pathways in cancer by Chinese medicine. Cancer Lett. 2010.
33. Billen LP, Shamas-Din A, Andrews DW. Bid: A Bax-like BH3 protein. Oncogene. 2008; 27:S93-104.
34. Yang SE, Hsieh MT, Tsai TH, Hsu SL. Effector mechanism of magnolol-induced apoptosis in human lung squamous carcinoma CH27 cells. Br J Pharmacol. 2003; 138:193-201.
35. Zhong WB, Wang CY, Ho KJ, Lu FJ, Chang TC, Lee WS. Magnolol induces apoptosis in human leukemia cells *via* cytochrome *c* release and caspase activation. Anticancer Drugs. 2003; 14:211-217.
36. Lin SY, Liu JD, Chang HC, Yeh SD, Lin CH, Lee WS. Magnolol suppresses proliferation of cultured human colon and liver cancer cells by inhibiting DNA synthesis and activating apoptosis. J Cell Biochem. 2002; 84:532-544.
37. Syu WJ, Shen CC, Lu JJ, Lee GH, Sun CM. Antimicrobial and cytotoxic activities of neolignans from Magnolia officinalis. Chem Biodivers. 2004; 1:530-537.
38. Ikeda K, Nagase H. Magnolol has the ability to induce apoptosis in tumor cells. Biol Pharm Bull. 2002; 25:1546-1549.
39. Hibasami H, Achiwa Y, Katsuzaki H, Imai K, Yoshioka K, Nakanishi K, Ishii Y, Hasegawa M, Komiya T. Honokiol induces apoptosis in human lymphoid leukemia Molt 4B cells. Int J Mol Med. 1998; 2:671-673.
40. Battle TE, Arbiser J, Frank DA. The natural product honokiol induces caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells. Blood. 2005; 106:690-697.
41. Bai X, Cerimele F, Ushio-Fukai M, *et al*. Honokiol, a small molecular weight natural product, inhibits angiogenesis *in vitro* and tumor growth *in vivo*. J Biol Chem. 2003; 278:35501-35507.
42. Wolf I, O'Kelly J, Wakimoto N, Nguyen A, Amblard F, Karlan BY, Arbiser JL, Koeffler HP. Honokiol, a natural biphenyl, inhibits *in vitro* and *in vivo* growth of breast cancer through induction of apoptosis and cell cycle arrest. Int J Oncol. 2007; 30:1529-1537.
43. Jiang QQ, Fan LY, Yang GL, Guo WH, Hou WL, Chen LJ, Wei YQ. Improved therapeutic effectiveness by combining liposomal honokiol with cisplatin in lung cancer model. BMC Cancer. 2008; 8:242.
44. Krammer PH. CD95's deadly mission in the immune system. Nature. 2000; 407:789-795.
45. Wajant H. The Fas signaling pathway: more than a paradigm. Science. 2002; 296:1635-1636.
46. Lin SY, Chang YT, Liu JD, Yu CH, Ho YS, Lee YH, Lee WS. Molecular mechanisms of apoptosis induced by magnolol in colon and liver cancer cells. Mol Carcinog. 2001; 32:73-83.
47. Raja SM, Chen S, Yue P, Acker TM, Lefkove B, Arbiser JL, Khuri FR, Sun SY. The natural product honokiol preferentially inhibits cellular FLICE-inhibitory protein and augments death receptor-induced apoptosis. Mol Cancer Ther. 2008; 7:2212-2223.
48. Ikai T, Akao Y, Nakagawa Y, Ohguchi K, Sakai Y, Nozawa Y. Magnolol-induced apoptosis is mediated *via* the intrinsic pathway with release of AIF from mitochondria in U937 cells. Biol Pharm Bull. 2006; 29:2498-2501.
49. Chen JH, Wu CC, Hsiao G, Yen MH. Magnolol induces apoptosis in vascular smooth muscle. Naunyn Schmiedebergs Arch Pharmacol. 2003; 368:127-133.
50. Huang SH, Chen Y, Tung PY, Wu JC, Chen KH, Wu JM, Wang SM. Mechanisms for the magnolol-induced cell death of CGTH W-2 thyroid carcinoma cells. J Cell Biochem. 2007; 101:1011-1022.
51. Yang SE, Hsieh MT, Tsai TH, Hsu SL. 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.
52. Ishitsuka K, Hideshima T, Hamasaki M, *et al*. Honokiol overcomes conventional drug resistance in human multiple myeloma by induction of caspase-dependent and -independent apoptosis. Blood. 2005; 106:1794-1800.
53. Schumacker PT. Reactive oxygen species in cancer cells: Live by the sword, die by the sword. Cancer Cell. 2006; 10:175-176.
54. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol. 2003; 552:335-344.
55. Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis. 2000; 5:415-418.
56. Ou HC, Chou FP, Sheu WH, Hsu SL, Lee WJ. Protective effects of magnolol against oxidized LDL-induced apoptosis in endothelial cells. Arch Toxicol. 2007; 81:421-432.
57. Fujita S, Taira J. Biphenyl compounds are hydroxyl radical scavengers: Their effective inhibition for UV-induced mutation in Salmonella typhimurium TA102. Free Radic Biol Med. 1994; 17:273-277.
58. Shen YC, Sung YJ, Chen CF. Magnolol inhibits Mac-1 (CD11b/CD18)-dependent neutrophil adhesion: Relationship with its antioxidant effect. Eur J Pharmacol. 1998; 343:79-86.
59. Park EJ, Zhao YZ, Na M, Bae K, Kim YH, Lee BH, Sohn DH. 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.
60. Park EJ, Kim SY, Zhao YZ, Sohn DH. Honokiol reduces oxidative stress, c-jun-NH_2-terminal kinase phosphorylation and protects against glycochenodeoxycholic acid-induced apoptosis in primary cultured rat hepatocytes. Planta Med. 2006; 72:661-664.
61. Roderick HL, Cook SJ. Ca^{2+} signalling checkpoints in cancer: Remodelling Ca^{2+} for cancer cell proliferation and survival. Nat Rev Cancer. 2008; 8:361-375.
62. Wang HG, Pathan N, Ethell IM, *et al*. Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD. Science. 1999; 284:339-343.
63. Wang JP, Chen CC. Magnolol induces cytosolic-free Ca^{2+} elevation in rat neutrophils primarily *via* inositol trisphosphate signalling pathway. Eur J Pharmacol. 1998; 352:329-334.
64. Teng CM, Yu SM, Chen CC, Huang YL, Huang TF. EDRF-release and Ca^{+}(+)-channel blockade by magnolol, an antiplatelet agent isolated from Chinese herb Magnolia officinalis, in rat thoracic aorta. Life Sci. 1990; 47:1153-1161.
65. Wu SN, Chen CC, Li HF, Lo YK, Chen SA, Chiang HT. Stimulation of the BK(Ca) channel in cultured smooth muscle cells of human trachea by magnolol. Thorax. 2002; 57:67-74.
66. Yeretssian G, Doiron K, Shao W, Leavitt BR, Hayden MR, Nicholson DW, Saleh M. Gender differences

in expression of the human caspase-12 long variant determines susceptibility to Listeria monocytogenes infection. Proc Natl Acad Sci U S A. 2009; 106:9016-9020.
67. Lee DH, Szczepanski MJ, Lee YJ. Magnolol induces apoptosis *via* inhibiting the EGFR/PI3K/Akt signaling pathway in human prostate cancer cells. J Cell Biochem. 2009; 106:1113-1122.
68. Hahm ER, Arlotti JA, Marynowski SW, Singh SV. Honokiol, a constituent of oriental medicinal herb magnolia officinalis, inhibits growth of PC-3 xenografts *in vivo* in association with apoptosis induction. Clin Cancer Res. 2008; 14:1248-1257.
69. Funa NS, Reddy K, Bhandarkar S, Kurenova EV, Yang L, Cance WG, Welsh M, Arbiser JL. Shb gene knockdown increases the susceptibility of SVR endothelial tumor cells to apoptotic stimuli *in vitro* and *in vivo*. J Invest Dermatol. 2008; 128:710-716.
70. Bellamy CO. p53 and apoptosis. Br Med Bull. 1997; 53:522-538.
71. Haldar S, Negrini M, Monne M, Sabbioni S, Croce CM. Down-regulation of bcl-2 by p53 in breast cancer cells. Cancer Res. 1994; 54:2095-2097.
72. Beham A, Marin MC, Fernandez A, Herrmann J, Brisbay S, Tari AM, Lopez-Berestein G, Lozano G, Sarkiss M, McDonnell TJ. Bcl-2 inhibits p53 nuclear import following DNA damage. Oncogene. 1997; 15:2767-2772.
73. Marin MC, Hsu B, Meyn RE, Donehower LA, el-Naggar AK, McDonnell TJ. Evidence that p53 and bcl-2 are regulators of a common cell death pathway important for *in vivo* lymphomagenesis. Oncogene. 1994; 9:3107-3112.
74. Walia V, Kakar S, Elble R. Micromanagement of the mitochondrial apoptotic pathway by p53. Front Biosci. 2011; 16:749-758.
75. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffman B, Reed JC. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression *in vitro* and *in vivo*. Oncogene. 1994; 9:1799-1805.
76. Selvakumaran M, Lin HK, Miyashita T, Wang HG, Krajewski S, Reed JC, Hoffman B, Liebermann D. Immediate early up-regulation of bax expression by p53 but not TGF beta 1: A paradigm for distinct apoptotic pathways. Oncogene. 1994; 9:1791-1798.
77. Qiu P, Guan H, Dong P, Li S, Ho CT, Pan MH, McClements DJ, Xiao H. The p53-, Bax- and p21-dependent inhibition of colon cancer cell growth by 5-hydroxy polymethoxyflavones. Mol Nutr Food Res. 2011; 55: 613-622.
78. Trinh DL, Elwi AN, Kim SW. Direct interaction between p53 and Tid1 proteins affects p53 mitochondrial localization and apoptosisy as a target for cancer therapy. Oncotarget. 2010; 1:396-404.
79. Hui L, Zheng Y, Yan Y, Bargonetti J, Foster DA. Mutant p53 in MDA-MB-231 breast cancer cells is stabilized by elevated phospholipase D activity and contributes to survival signals generated by phospholipase D. Oncogene. 2006; 25:7305-7310.
80. Zhong M, Shen Y, Zheng Y, Joseph T, Jackson D, Foster DA. Phospholipase D prevents apoptosis in v-Src-transformed rat fibroblasts and MDA-MB-231 breast cancer cells. Biochem Biophys Res Commun. 2003; 302:615-619.
81. Garcia A, Zheng Y, Zhao C, Toschi A, Fan J, Shraibman N, Brown HA, Bar-Sagi D, Foster DA, Arbiser JL. Honokiol suppresses survival signals mediated by Ras-dependent phospholipase D activity in human cancer cells. Clin Cancer Res. 2008; 14:4267-4274.
82. Wang T, Chen F, Chen Z, Wu YF, Xu XL, Zheng S, Hu X. Honokiol induces apoptosis through p53-independent pathway in human colorectal cell line RKO. World J Gastroenterol. 2004; 10:2205-2208.
83. Dutta J, Fan Y, Gupta N, Fan G, Gelinas, C. Current insights into the regulation of programmed cell death by NF-kappaB. Oncogene. 2006; 25:6800-6816.
84. Jost PJ, Ruland J. Aberrant NF-kappaB signaling in lymphoma: Mechanisms, consequences, and therapeutic implications. Blood. 2007; 109:2700-2707.
85. Chen YL, Lin KF, Shiao MS, Chen YT, Hong CY, Lin SJ. Magnolol, a potent antioxidant from Magnolia officinalis, attenuates intimal thickening and MCP-1 expression after balloon injury of the aorta in cholesterol-fed rabbits. Basic Res Cardiol. 2001; 96:353-363.
86. Chen YH, Lin SJ, Chen JW, Ku HH, Chen YL. Magnolol attenuates VCAM-1 expression *in vitro* in TNF-alpha-treated human aortic endothelial cells and *in vivo* in the aorta of cholesterol-fed rabbits. Br J Pharmacol. 2002; 135:37-47.
87. Tse AK, Wan CK, Zhu GY, Shen XL, Cheung HY, Yang M, Fong WF. Magnolol suppresses NF-kappaB activation and NF-kappaB regulated gene expression through inhibition of IkappaB kinase activation. Mol Immunol. 2007; 4:2647-2658.
88. Ahn KS, Sethi G, Shishodia S, Sung B, Arbiser JL, Aggarwal BB. Honokiol potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through modulation of nuclear factor-kappaB activation pathway. Mol Cancer Res. 2006; 4:621-633.
89. Tse AK, Wan CK, Shen XL, Yang M, Fong WF. Honokiol inhibits TNF-alpha-stimulated NF-kappaB activation and NF-kappaB-regulated gene expression through suppression of IKK activation. Biochem Pharmacol. 2005; 70:1443-1457.
90. Shen JL, Man KM, Huang PH, Chen WC, Chen DC, Cheng YW, Liu PL, Chou MC, Chen YH. Honokiol and magnolol as multifunctional antioxidative molecules for dermatologic disorders. Molecules. 2010; 15:6452-6465.

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