ARTICLE
Journal of Cellular Biochemistry 116:287–298 (2015)

Journal of Cellular Biochemistry

Honokiol Induces Cell Cycle Arrest and Apoptosis Via Inhibiting Class I Histone Deacetylases in Acute Myeloid Leukemia

Hai-Ying Li,[1] Hai-Ge Ye,[2] Chi-Qi Chen,[1] Li-Hui Yin,[1] Jian-Bo Wu,[1] Li-Cai He,[3] and Shen-Meng Gao[1]*

[1]*Laboratory of Internal Medicine, The First Affiliated Hospital of Wenzhou Medical University, 2 FuXue Road, Wenzhou 325000, China*

[2]*Department of Hematology, The First Affiliated Hospital of Wenzhou Medical University, 2 FuXue Road, Wenzhou 325000, China*

[3]*School of Laboratory Medical and Life Science, Wenzhou Medical University, Chashan Higher Education Park, Wenzhou 325000, China*

ABSTRACT

Honokiol, a constituent of *Magnolia officinalis*, has been reported to possess potent anti-cancer activity through targeting multiple signaling pathways in numerous malignancies including acute myeloid leukemia (AML). However, the underlying mechanisms remain to be defined. Here, we report that honokiol effectively decreased enzyme activity of histone deacetylases (HDACs) and reduced the protein expression of class I HDACs in leukemic cells. Moreover, treatment with proteasome inhibitor MG132 prevented honokiol-induced degradation of class I HDACs. Importantly, honokiol increased the levels of p21/waf1 and Bax via triggering acetylation of histone in the regions of p21/waf1 and Bax promoter. Honokiol induced apoptosis, decreased activity of HDACs, and significantly inhibited the clonogenic activity of hematopoietic progenitors in bone marrow mononuclear cells from patients with AML. However, honokiol did not decrease the activity of HDACs and induce apoptosis in normal hematopoietic progenitors from unbilicial cord blood. Finally, honokiol dramatically reduced tumorigenicity in a xenograft leukemia model. Collectively, our findings demonstrate that honokiol has anti-leukemia activity through inhibiting HDACs. Thus, being a relative non-toxic agent, honokiol may serve as a novel natural agent for cancer prevention and therapy in leukemia. J. Cell. Biochem. 116: 287–298, 2015.

KEY WORDS: HONOKIOL; HISTONE DEACETYLASES; P21/WAF1; BAX

The importance of epigenetic modulation such as DNA methylation and histone acetylation in human cancer development is becoming clear [Chen et al., 2010]. Histone deacetylases (HDACs) have been shown to modulate many important biological processes such as cell cycle progression, differentiation, and development through removing the acetyl group from histones and subsequently inhibiting gene transcription. Overexpression of HDAC causes a global loss of monoacetylation and is associated with leukemia development [Wang et al., 1998; Muller and Kramer, 2010]. In contrast, the expression of HDAC is very low in normal hematopoietic progenitor cells [Wada et al., 2009]. HDACs are broadly classified into four classes according to their sequence homology. Class I HDACs including HDAC1–3 and 8 are frequently overexpressed in various human cancers and this overexpression is associated with drug resistance and poor clinical outcome. Thus, class I HDACs have been considered as the potential targets for cancer [Delcuve et al., 2013].

Inhibition of HDACs has been shown to suppress cancer cell growth and/or induce apoptosis through re-expression of tumor suppressor genes, suggesting HDAC inhibitors possess anti-cancer

The authors of this manuscript have no conflicts of interest to disclose.
Hai-Ying Li and Hai-Ge Ye contributed equally to this work.
Grant sponsor: National Natural Science Foundation of China; Grant number: 81200350; Grant sponsor: Zhejiang Provincial Natural Science Foundation of China; Grant numbers: LY12H08003, Y13H080014, LQ13H080002.
*Corresponding author: Shen-Meng Gao, Laboratory of Internal Medicine, The First Affiliated Hospital of Wenzhou Medical University, 2 FuXue Road, Wenzhou, 325000, China. Email: gaoshenmeng77@126.com
Manuscript Received: 22 August 2014; Manuscript Accepted: 29 August 2014
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 4 September 2014
DOI 10.1002/jcb.24967 • © 2014 Wiley Periodicals, Inc.

activities in solid tumor and hematological malignancies. In fact, several HDAC inhibitors such as vorinostat and romidepsin have been approved by the Food and Drug Administration (FDA) for treating advanced and refractory cutaneous T-cell lymphoma [Marks, 2010]. Aberrant activity of HDACs has been linked with epigenetic silencing of tumor suppressor genes and key oncogenic events. Inhibition of HDACs with vorinostat, valproic acid, and other compounds led to cell cycle arrest and induction of apoptosis by increasing the expressions of cell cycle kinase inhibitor p21/waf1 and pro-apoptotic protein Bax [Huang et al., 2000; Myzak and Dashwood, 2006; Das et al., 2007]. Therefore, p21/waf1 and Bax might play an important role in the anti-cancer activity induced by HDAC inhibitor.

Although HDAC inhibitors show selective toxicities against tumor cells than normal cells, the prolong usage of these compounds in patients also leads to gastrointestinal side effects, fatigue, and severe immune suppression [Prince et al., 2009]. Therefore, it might be advantage to select agents with low toxicities from plant-derived polyphenol, which has the activity of HDAC inhibition. Honokiol, a small molecular weight natural product derived from the stem and bark of the plant *Magnolia officinalis* [Chen et al., 2009], has been reported to possess potential anti-neoplastic and anti-angiogenic properties through targeting multiple signaling pathways including PI3K/Akt, STAT3, nuclear factor-kappa B (NF-kappaB), epidermal growth factor receptor (EGFR), and mammalian target of rapamycin (m-TOR) [Arora et al., 2012]. For example, honokiol induced G1 cell cycle arrest and apoptosis by reducing the expression of cyclin D and surviving in adult T-cell leukemia [Ishikawa et al., 2012]. Recently, it has been reported that plant-derived natural compounds such as green tea polyphenols and curcumin have the ability to modulate gene expression by inhibiting HDAC activity, finally leading to re-expression of some tumor suppressor genes [Thakur et al., 2012b; Chen et al., 2013]. However, whether honokiol can alter gene expression by inhibiting HDAC activity in leukemic cells is not fully determined.

AML consists of heterogeneous subgroups of neoplastic hematopoietic progenitor cells. Current therapeutic regiments, including complete remission induction followed by additional intensive chemotherapy or stem-cell transplantation, have produced limited survival benefits and inevitably led to serious side effects. Therefore, development of less toxic agent is urgently needed. Here, we report that plant-derived honokiol decreased the activity of HDACs and downregulated the expression of HDAC1–3 and 8 in leukemic cells. Moreover, honokiol triggered the mRNA and protein levels of p21/waf1 and Bax via triggering acetylation of histone in the regions of p21/waf1 and Bax promoter. Thus, being a relative non-toxic agent [Liu et al., 2007], honokiol might be a potential therapeutic agent for AML.

MATERIALS AND METHODS

CELL LINES AND PRIMARY AML CELLS

K562 and HEL cells purchased from Shanghai cell bank were employed for the present study. These cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA) in humidified 37°C incubator with 5% CO_2. MS-5 stromal cells were prepared at a concentration of 1×10^5 cells/ml/well in 6-well tissue plate 1–2 days prior to the seeding primary AML cells. Primary AML cells (blasts% > 70%) were obtained from 26 AML patients and normal hematopoietic progenitors were obtained from unbilicial cord blood. Informed consent was given in accordance with the Declaration of Helsinki. All manipulations were approved by the Medical Science Ethic Committee of Wenzhou Medical University. Bone marrow mononuclear cells from AML patients and unbilicial cord blood were isolated by Ficoll density gradient centrifugation (GE Healthcare, Uppsala, Sweden) and were cultured in the same condition as described above. Cell viability was determined by the trypan-blue exclusion assay and growth inhibition rate was calculated according to viable cell numbers of treated cells against numbers of untreated cells. Purified honokiol (Sigma-Aldrich, St Louis, MO) was dissolved in ethyl alcohol. Sodium butyrate (SB, Sigma-Aldrich) was dissolved in distilled water and trichostatin A (TSA, Sigma-Aldrich) was dissolved in ethanol. Proteasome inhibitor MG132 (Calbiochem, Gibbstown, NJ) was dissolved in dimethyl sulfoxide. All these compounds were kept at −20°C until use.

IMMUNOPRECIPITATION (CHIP) ASSAYS

The acetylation levels of genes promoter associated histone H3 and H4 were examined by ChIP. ChIP analysis was performed using the acetyl-histone ChIP assay kit (Upstate Biotechnology, Lake Placid, NY). Briefly, treated and untreated cells were crosslinked with 1% formaldehyde for 10 min. Nuclear extracts were prepared and chromatin was sonicated to generate 200–1000 bp DNA fragments. Protein-DNA complexes were immunoprecipitated with 5 μg of specific antibodies (anti-acetylated histone H3, anti-acetylated histone H4, and nonrelevant rabbit IgG) overnight at 4°C. Cross-linking was reversed by incubating the samples at 65°C for 4 h. Then DNA was purified and resuspended in nuclease-free water. Standard PCR reactions were performed with primer sequences (Table S1) specific for the Bax and p21/waf1 promoter. PCR products were subjected to electrophoresis using 2% agarose gel. Also, immunoprecipitated DNA from leukemic cells were analyzed by quantitative real-time PCR and the amounts of precipitated DNA were calculated as the percentage of the input sample.

CELL CYCLE ANALYSIS

Leukemic cells were treated with different concentrations of honokiol for 24 h. Then, about 2×10^6 cells were washed, pelleted, and resuspended in 0.04 μg/ml propidium iodide (Invitrogen) and 100 μg/ml RNase in PBS. The samples were incubated at room temperature for 30 min and was performed by FACSCalibur instrument (Becton Dickinson, Mountain View, CA) using the CELLQuest program (Becton Dickinson). Software Modifit was used to generate histograms to determine number of cells in each phase of the cell cycle.

APOPTOSIS DETECTION

Leukemic cells were plated in triplicate at 2×10^5 cells/ml. After being treated with honokiol, apoptosis was detected by annexin V (Invitrogen) in combination with propidium iodide (Invitrogen) and

samples were analyzed by flow cytometry (Becton Dickinson) within 30 min after staining. Experiments were done in duplicate and repeated at least three times.

MEASUREMENT OF REACTIVE OXYGEN SPECIES (ROS) GENERATION

ROS generation in honokiol-treated and untreated AML blasts was measured by flow cytometry, followed by staining with DCFH-DA (Beyotime Institute of Biotechnology, Jiangsu, China). Briefly, AML blasts were seeded in six-well plates (2×10^5 cells per well), and then treated with honokiol. The cells were stained with 10 μM DCFH-DA for 20 min at 37°C, and the fluorescence was measured by flow cytometry according to the manufacturer's protocol.

MRNA EXTRACT AND QUANTITATIVE REAL-TIME PCR

Total RNA from honokiol-treated and untreated cells were extracted by TRIzol (Invitrogen, Carlsbad, CA, USA). RNA concentration and quality were quantified by measuring the absorbance at 260 nm with Beckman DU6400 spectrophotometer (Beckman Counter, Miami, FL, USA) and gel analysis. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method. The primers of Bax and other gene transcripts were indicated in Supplemental Table S1. Glyceraldehyde-3- phosphate dehydrogenase (GAPDH) housekeeping gene was used for normalization.

HDAC ENZYME ACTIVITY

HDAC enzyme activity in cells was detected by the colorimetric HDAC Activity Assay Kit (Upstate Biotechnology). HDAC assay was performed following the manufacturer's instruction measuring the color intensity at 405 nm. In vitro deacetylase assay was performed by Fluor de Lys™ HDAC Fluorescent Activity Kit (Enzo Life Sciences, Farmingdale, New York) according to manufacturer's instruction.

WESTERN BLOTTING

Western blotting analysis was performed using standard techniques. The following antibodies were used: Bax, p21/waf1, bcl-2, cleavaged caspase-3 (Cell Signaling Technology, Beverly, MA); ac-H3, ac-H4 (Upstate Biotechnology); HDAC1, HDAC2, HDAC3, HDAC8, p53, RaR-α, and AML1 (Santa Cruz Biotechnology, Santa Cruz, CA). As necessary, blots were stripped and reprobed with β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) as an internal control. Signals were detected by chemiluminescence reagents (Millipore, Billerica, MA). All experiments were repeated three times with the similar results.

COLONY ASSAY

Bone marrow (BM) was obtained from six patients with AML. Mononuclear cells were separated by Ficoll-Paque liquid and suspended in IMDM (Gibco). Cells diluted 1:10 in IMDM containing 10% fetal bovine serum (Invitrogen) with or without 40 μM honokiol were plated in 10 cm dishes with Methocult H4434 Classic (Stem Cell Technologies, Vancouver, BC, Canada) for 14 days of incubation at 37°C with 5% CO_2. Colony (>40 cells) was counted as one colony. Granulocyte macrophage colony-forming unit (CFU-GM) and erythroid burstforming unit (BFU-E) were counted according to the manufacturer's protocol. These experiments were approved by the Medical Science Ethic Committee of Wenzhou Medical University.

IN VIVO STUDIES

Female athymic nude mice (5 weeks old) were purchased from SLAC (Shanghai SLAC Laboratory Animal, Shanghai, China) and were housed in a pathogen-free animal facility. Animal studies were performed according to Wenzhou Medical University Institutional Guidelines. A total of 2×10^7 viable K562 cells in 200 μL of PBS were injected subcutaneously into right flank of each nude mouse. After 24 h, mice were randomly divided into two groups. One group of mice was treated with 100 mg honokiol/kg body weight in 100 μL of PBS by oral gavage twice per week. The other group of mice received an equal volume of PBS by gavage and served as a control group. Mice were sacrificed and the tumors from each mouse were harvested when the experiment was terminated at 10 weeks after tumor cell inoculation. The wet weights and volumes of tumors were recorded. Tumor volumes were measured using the equation V (in mm^3) = A $\times$ $B^2/2$, where A is the largest diameter and B is the perpendicular diameter. Also, tumor lysates were prepared for western blotting analysis and HDAC activity assay.

STATISTICAL ANALYSIS

The significance of the difference between groups was determined by Student's *t*-test. A *P*-value of less than .05 was considered statistically significant. All statistical analyses were performed with SPSS software (Chicago, IL).

RESULTS

HONOKIOL CAUSES CELL CYCLE ARREST AND INDUCES APOPTOSIS IN LEUKEMIC CELLS

To explore the potential anti-cancer effects of honokiol in leukemic cells, we analyzed cell proliferation, cell cycle, and apoptosis in K562 and HEL cells treated with honokiol. As shown in Fig. 1B, treatment with different concentrations of honokiol in K562 and HEL cells significantly inhibited cell proliferation in a time- and concentration-dependent manner, which paralleled to the reduced viability (Fig. 1C). At 48 h of treatment, 5 μM honokiol led to 7.5% and 11% cell death in K562 and HEL cells, respectively, while 40 μM honokiol almost caused cell death in K562 and HEL cells (Figs. 1B and C). Next, we investigate whether honokiol induced cytotoxicity by way of apoptosis in leukemic cells. Annexin V-FITC/PI double staining was performed in K562 and HEL cells treated with different concentrations of honokiol for 24 h. As compared with untreated cells, a high percentage of annexin V^+ labeling was detected in cells treated with honokiol (Figs. 1D and E). Notably, the sensitivity of K562 cells in honokiol-induced apoptosis was lower than that of HEL cells.

To determine whether honokiol-inhibited cell proliferation is due to cell cycle arrest, K562 and HEL cells were treated with 5, 10, and 20 μM honokiol for 24 h. Analysis of DNA content by flow cytometry indicated that honokiol led to a marked increase in the percentage of G0/G1 phase. Honokiol treatment in K562 cells caused an arrest of 33.2% in G0/G1 phase at 5 μM, which further increased to 40.2% at 10 μM and 48.5% at 20 μM, compared with an arrest of

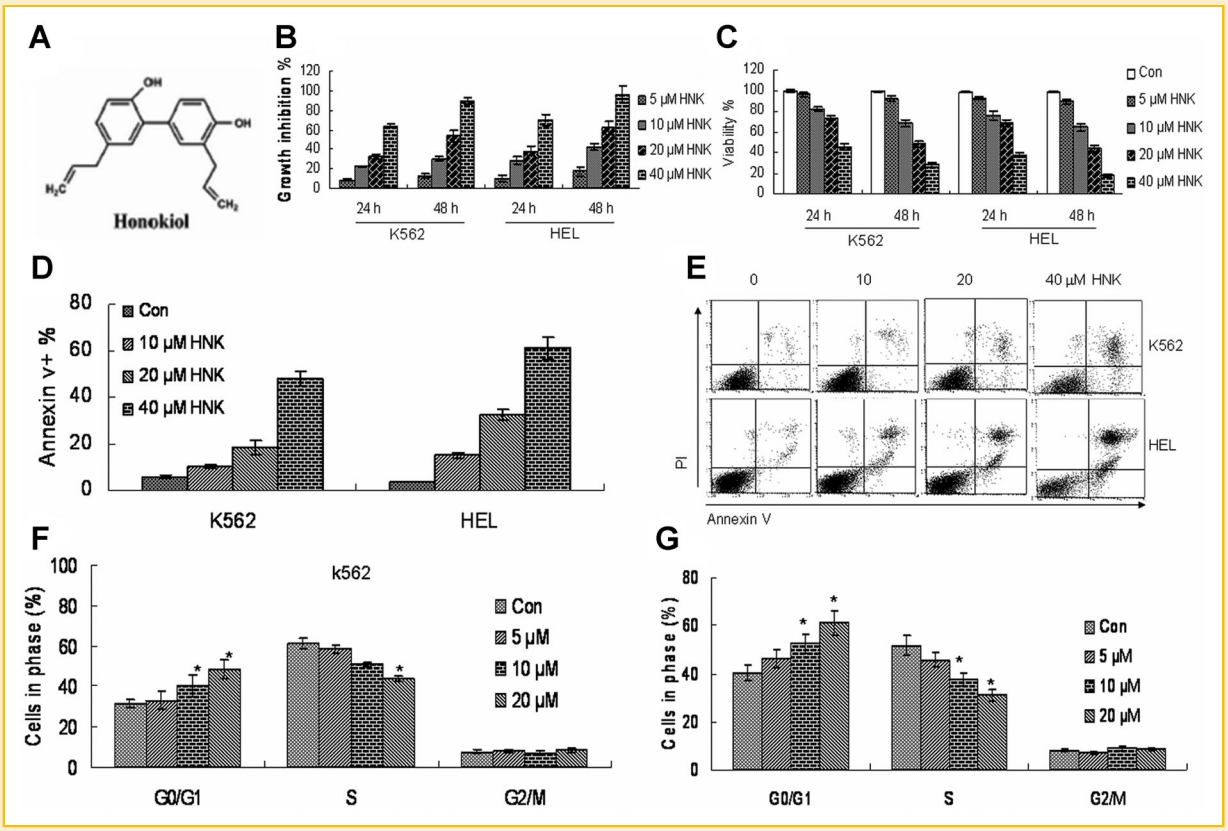

Fig. 1. Effects of honokiol on cell growth, apoptosis, and cell cycle in leukemic cell lines. (A) Chemical structure of honokiol. (B–C) Cell growth inhibition and viability were counted by trypan-blue exclusion assay in K562 and HEL cells treated with different concentrations of honokiol (10–40 μM) for 24 and 48 h. (D–E) K562 and HEL cells were treated with different concentrations of honokiol (10–40 μM) for 24 h. The number of cells undergoing apoptosis were determined using Annexin V staining. (F–G) Distribution of cells were recorded in different stages of cell cycle analyzed using fluorescence activated cell sorting analysis in K562 and HEL cells treated with different concentrations of honokiol (5–20 μM) for 24 h. Data represent the mean ± SD of three different assays.

31% in G0/G1 phase in control (Fig. 1F). Similarly, honokiol caused an arrest of 46.2% at 5 μM, 52.4% at 10 μM and 61% at 20 μM, respectively, compared with 40.3% in untreated HEL cells (Fig. 1G). This increase in G0/G1 cell population was accompanied with a concomitant decrease of cell number in S phase (Figs. 1F–G).

To address whether HDAC inhibitors present anti-leukemia activity, cell proliferation and cell apoptosis were detected in leukemic cells treated with two well known class I and II HDAC inhibitor trichostatin A (TSA) and class I HDAC inhibitor sodium butyrate (SB). As indicated in Figure S1A–B, 1 μM TSA and 1 mM SB significantly inhibited cell proliferation and reduced cell viability in K562 and HEL cells. Meanwhile, apoptosis was analyzed in K562 and HEL cells treated with TSA and SB for 24 h. TSA and SB obviously induced apoptosis both in K562 and HEL cells (Fig. S1C–D).

HONOKIOL REDUCES ENZYME ACTIVITY OF HDACS AND INHIBITS THE PROTEIN LEVELS OF HDAC1–3 AND 8 VIA ENHANCED PROTEASOME-MEDIATED PROTEIN DEGRADATION OF HDACS IN LEUKEMIC CELLS

To address whether honokiol could inhibit HDAC activity, K562 and HEL cells were treated with different concentrations of honokiol and 1 μM TSA for 24 h and HDAC enzyme activity was detected. We found treatment of 20 and 40 μM honokiol resulted in a significant decrease of about 20–60% HDAC activity in K562 and HEL cells (Fig. 2A). TSA, serving as positive control, reduced the HDAC activity by approximately 80% in leukemic cells. Thus, honokiol treatment causes dose-dependent inhibition of HDAC enzyme activity. To determine whether honokiol is a direct inhibitor of HDAC, cell lysates of K562 and HEL were incubated with honokiol in vitro for 6 h, followed by the measurement of HDAC activity. We found only 40 μM honokiol led to significant decrease of HDAC activity, but 10 and 20 μM honokiol failed to inhibit HDAC activity in cell lysates (Fig. 2B). Although honokiol effectively decreased the activity of HDAC, it is unknown whether honokiol affected the expression of class I HDACs including HDAC1–3 and HDAC8. As indicated in Figure 2E, honokiol significantly downregulated the protein levels of HDAC1–3 and HDAC8 in K562 and HEL cells. Intriguingly, honokiol did not alter the mRNA levels of HDAC1–3 and HDAC8 (Figs. 2C–D).

Although our data suggest honokiol reduced activity of HDAC enzyme and inhibited the protein levels of HDAC1–3 and 8, the underlying mechanism was unknown. Next, we asked whether proteasomal degradation pathway mediated honokiol-induced inhibition of class I HDACs. K562 and HEL cells were treated with 40 μM honokiol for 18 h followed by addition of 10 and 20 μM MG132, an

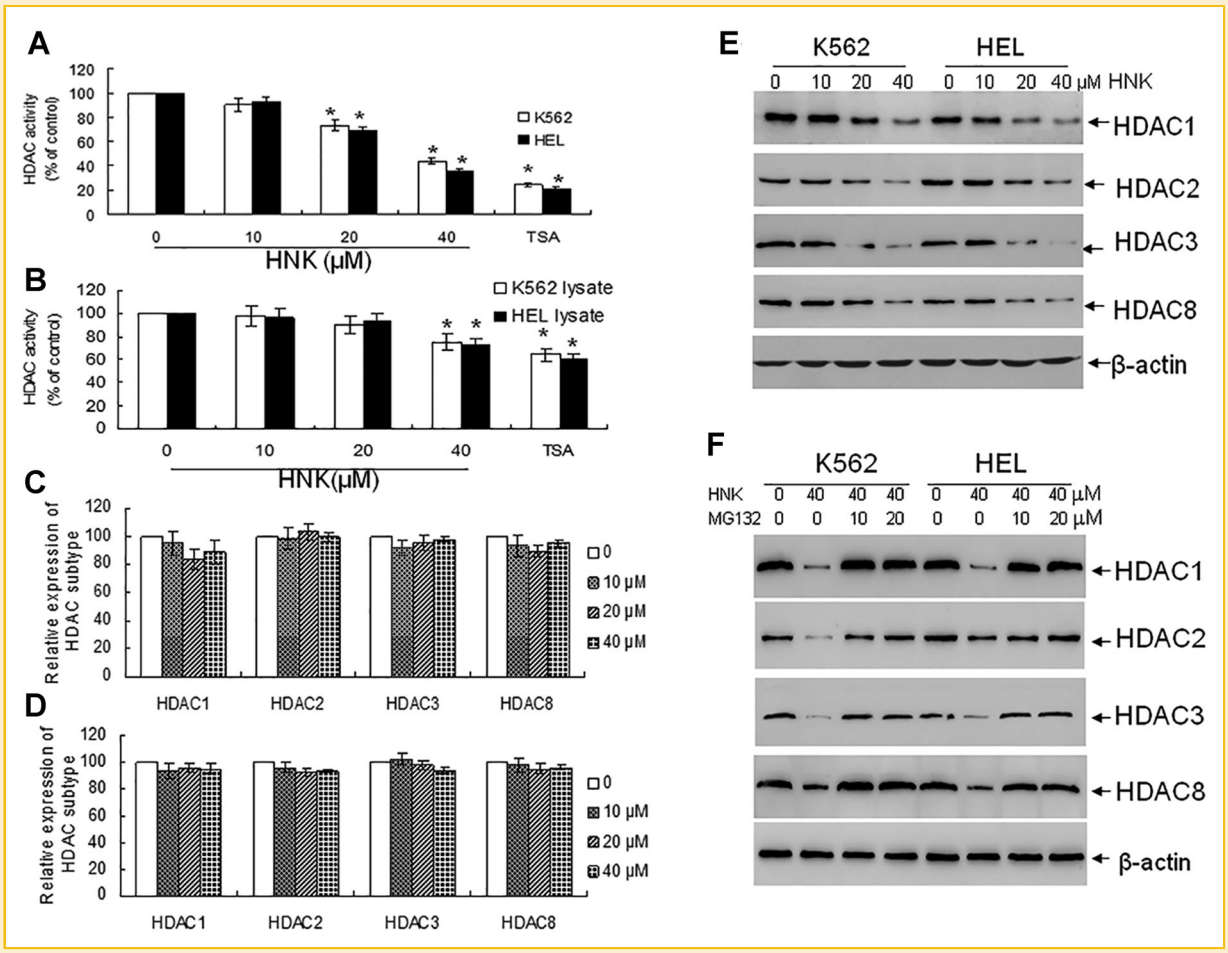

Fig. 2. Effects of honokiol on HDAC enzyme activities and protein levels of class I HDACs in leukemic cells. (A) HDAC activity was measured by colorimetric HDAC Activity Assay Kit in K562 and HEL cells treated with indicated concentrations of honokiol and 1 μM TSA for 24 h. *$P < 0.01$ versus untreated cells. (B) Cell lysates from K562 and HEL cell were incubated with different concentrations of honokiol and 1 μM TSA for 6 h. HDAC activity was measured by the Fluor de Lys™ HDAC Fluorescent Activity Kit. *$P < 0.01$ versus untreated cells. (C–E) The mRNA and protein levels of HDAC1–3 and 8 were measured in K562 and HEL cells treated with indicated concentrations of honokiol for 24 h by quantitative real-time PCR (C–D) and western blotting (E). (F) K562 and HEL cells were cultured with 40 μM honokiol for 18 h, followed by treatment with or without 10 and 20 μM MG132 for additional 6 h. The protein levels of HDAC1–3 and 8 were detected by western blotting. β-actin antibody served as an internal control.

inhibitor of proteasomal degradation, for additional 6 h. As indicated in Figure 2F, the levels of class I HDAC proteins were significantly higher in the cells treated with honokiol plus MG132 compared with honokiol alone, suggesting that proteasome-mediated degradation of HDACs is a possible mechanism through which honokiol decreases the levels of class I HDACs in leukemic cells.

In order to determine the expression of class I HDACs in AML blasts, we measured the mRNA levels of HDAC1–3 and 8 in 26 leukemic blasts and 10 normal hematopoietic progenitors obtained from unbilicial cord blood (UCB). Because UCB includes plenty of normal hematopoietic progenitors and the amounts of hematopoietic progenitors are sufficient to complete the following series of tests, hematopoietic progenitors obtained from UCB were commonly used for the normal control. The expression of HDAC1 and HDAC2 were significantly higher in leukemic blasts compared with those in UCB (Fig. S2A-B). However, no significant differences of HDAC3 and HDAC8 levels were found between leukemic blasts and UCB (Figs. S2C-D). Finally, we found HDAC activity was significantly increased in leukemic blasts compared with that in UCB (Fig. S2E).

HONOKIOL TRIGGERS THE MRNA AND PROTEIN EXPRESSION OF P21/WAF1 AND BAX IN LEUKEMIC CELLS

HDAC inhibitors have been reported to induce cell cycle arrest by increasing p21/waf1 expression and activate apoptosis by upregulating Bax expression in various cancer cells [Huang et al., 2000; Myzak and Dashwood, 2006]. Next, we explored whether honokiol-induced inhibition of HDACs also resulted in the upregulation of p21/waf1 and Bax. As demonstrated in Figure 3A, exposure of honokiol in leukemic cells led to the increased protein levels of p21/waf1 and Bax in a concentration-dependent manner. Also, treatment of honokiol significantly increased the mRNA expression of p21/waf1 and Bax in a concentration-dependent manner in both K562 and HEL cells (Fig. 3B). A 2.8–5.0-fold increase and 2.5–4.1-fold

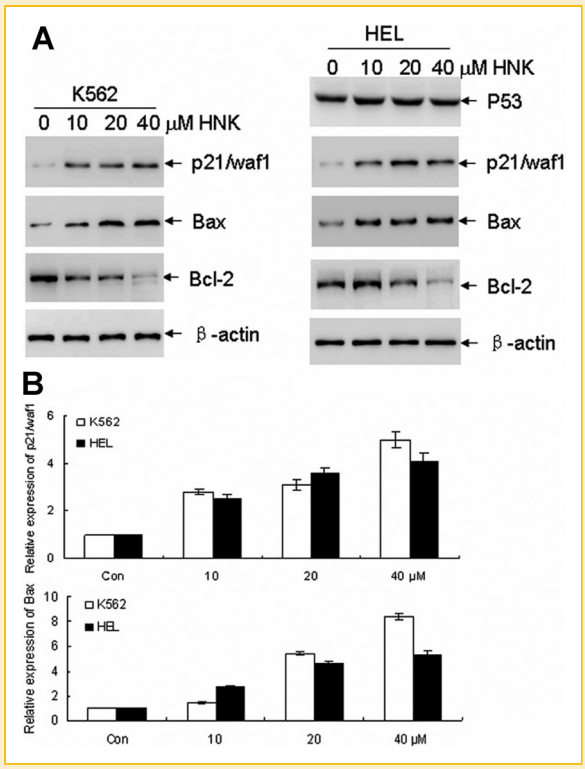

Fig. 3. Honokiol increased the protein and mRNA levels of p21/waf1 and Bax in leukemic cells. (A) K562 and HEL cells were treated with various concentrations of honokiol (10–40 μM) for 24 h. The protein levels of p21/waf1, Bax, p53, and Bcl-2 were measured by western blotting. β-actin served as an internal control. (B) Dose-dependent increase in the mRNA levels of p21/waf1 and Bax in K562 and HEL cells as determined by quantitative real-time PCR. Data represent the mean ± SD of three different assays. $^*P < 0.01$ versus control.

increase of p21/waf1 were found in K562 and HEL cells, respectively. Meanwhile, a 1.4–8.4-fold increase and 2.7–5.3-fold increase of Bax were found in K562 and HEL cells, respectively. Finally, we found honokiol significantly decreased the protein levels of Bcl-2 in leukemic cells (Fig. 3A).

Because previous studies clearly established that p21/waf1 and Bax were direct downstream targets of p53 tumor suppressor gene [Zeng and Berger, 2006; Kizildag and Ates, 2010], we then asked whether konokiol affects p53 expression in K562 and HEL cells. As indicated in Figure 3A, honokiol failed to modulate the levels of p53 in HEL cells. However, the expression of p53 is lacking in K562 cells.

HONOKIOL INCREASES BINDING OF ACETYLATED H3 (AC-H3) AND ACETYLATED H4 (AC-H4) TO THE PROMOTER REGIONS OF P21/WAF1 AND BAX GENES

Low levels of acetylation on histones H3 and H4 (ac-H3 and ac-H4) are linked to HDACs- mediated gene silence. We then determined whether honokiol could increase the levels of ac-H3 and ac-H4 in K562 and HEL cells. As indicated in Figure 4A, the levels of ac-H3 and ac-H4 were markedly increased in honokiol-treated K562 and HEL cells than untreated cells. Although honokiol effectively blocked HDAC activity (Figs. 2A–B) and increased the levels of ac-H3 and ac-H4, it was not determined whether honokiol modulated the levels of histone acetylation in p21/waf1 and Bax promoter region. To test it, we performed ChIP to detect the levels of histone acetylation in p21/waf1 and Bax promoter regions. As indicated in Figure 4C–D, the band densities of p21/waf1- and Bax-associated acetylated histone proteins were obviously higher in chromatin that was extracted from honokiol-treated cells than in chromatin extracted from untreated cells. Moreover, immunoprecipitated DNA from leukemic cells was analyzed by quantitative real-time PCR. The levels of ac-H3 and ac-H4 in p21/waf1 and Bax promoter regions were increased by 4–8-fold in honokiol-treated cells compared with untreated cells (Figs. 4E–F).

THE EFFECTS OF HONOKIOL ON PRIMARY BLASTS FROM AML PATIENTS AND NORMAL HEMATOPOIETIC PROGENITORS FROM UNBILICIAL CORD BLOOD

To explore if honokiol has anti-cancer activity in primary leukemic blasts, bone marrow mononuclear cells from AML patients were separated and treated with honokiol or not for 24 h. Firstly, apoptosis was detected by annexin V/PI staining. As indicated in Table I, 40 μM honokiol led to an increase of percentage of apoptotic cells from 5.1% to 68.3% in mononuclear cells from 26 AML patients. Totally, honokiol induced apoptosis in 20 of 26 (77%) AML blasts irrespective of FAB status and karyotype. As previous data reported that honokiol induced caspase-dependent apoptosis [Ishikawa et al., 2012], we then determine whether honokiol induced caspase-3 activation in AML blasts. We found caspase-3 was cleavaged in three of four honokiol-treated blasts (Fig. S3A), which were induced apoptosis by honokiol (Fig. S3B). Meanwhile, substantial increases of ROS levels were also observed in three of four honokiol-treated blasts (Fig. S3C), which were induced apoptosis by honokiol. Since bone marrow stromal cells are essential components of the leukemic microenvironment in promoting leukemic cell survival and chemoresistance, we then determine whether stromal cells affected honokiol-induced apoptosis in AML blasts. Honokiol also induced apoptosis in AML blasts in the presence of stromal cells (Fig. S3D), but the co-treatment with stromal cells obviously decreased the rate of apoptotic cells in #1 and #4, but not in #2.

Although honokiol inhibited the activity of HDACs in K562 and HEL cells, it is still unknown whether honokiol suppressed the activity of HDACs in primary leukemic cells. Next, we measured the relative activities of HDACs in bone marrow mononuclear cells treated with 40 μM honokiol or not. We found honokiol decreased the activities of HDACs in 21 of 26 (80%) AML blasts (Fig. 5A). Furthermore, we analyzed the relative mRNA levels of p21/waf1 and Bax in fresh AML blasts treated with 40 μM honokiol for 24 h. P21/waf1 and Bax were increased in 22 of 26 (84.6%) AML blasts and 17 of 26 (65%) AML blasts (Figs. 5B and C), respectively. Finally, we determined the effects of honokiol on the clonogenic activity of fresh bone marrow from six patients with AML. As shown in Figure 5D–F, honokiol obviously decreased the number of CFU-GM and BFU-E in six of six AML patients.

To determine the effects of honokiol on normal human hematopoietic progenitors, mononuclear cells were freshly isolated

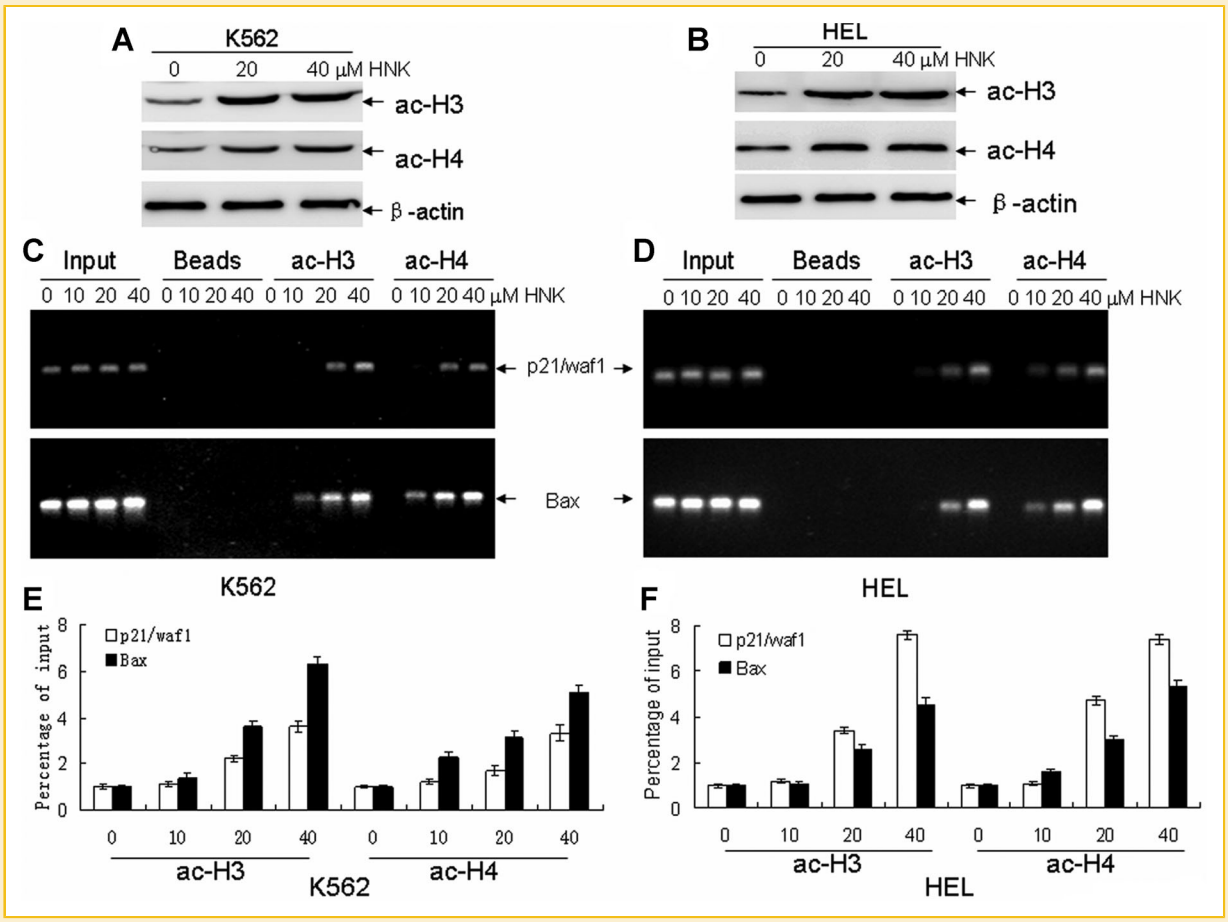

Fig. 4. Honokiol contributes to the binding of ac-H3 and ac-H4 to the promoter regions of p21/waf1 and Bax genes. (A–B) Total levels of ac-H3 and ac-H4 were measured by western blotting in K562 and HEL cells treated with different concentrations of honokiol for 24 h. (C–D) Soluble chromatin from K562 and HEL cells treated with or without honokiol were immunoprecipitated with anti-ac-H3 and anti-ac-H4 antibodies. Precipitated DNAs were analyzed by PCR and then were analyzed by agarose gel electrophoresis. (E–F) Precipitated DNAs from leukemic cells were qualified by quantitative real-time PCR. The amounts of precipitated DNA were calculated as the percentage of the input sample. *$P < 0.01$ versus untreated cells.

from six UCB. We firstly measured the activities of HDAC enzyme in hematopoietic progenitors cultured with or without 40 μM honokiol for 24 h. No significant change of HDAC activities was found in honokiol-treated hematopoietic progenitors compared with untreated cells (Fig. 5G). We also found honokiol did not decrease HDAC activities in cell lysates of hematopoietic progenitors from UCB in vitro (Fig. S4A). Furthermore, honokiol failed to increase the levels of ac-H3 and ac-H4 in hematopoietic progenitors from four UCB (Fig. S4B). No significant cell apoptosis or death was observed in honokiol-treated normal hematopoietic progenitors than untreated cells (Fig. 5H). Finally, we detected the mRNA levels of p21/waf1 and Bax. Honokiol failed to affect the transcript levels of p21/waf1 and Bax in honokiol-treated hematopoietic progenitors compared with untreated cells (data not shown).

EFFECTS OF HONOKIOL IN A K562 XENOGRAFT MODEL

Next, we determined whether honokiol could reduce the tumorigenicity in a xenograft model. About 2×10^7 viable K562 cells were injected subcutaneously into right flank of each nude mice. After 24 h, honokiol was administered by oral gavage twice a week. All the mice that were treated with honokiol did not indicate any abnormal behavior or visible sign of toxicity, suggesting its safety. All mice were sacrificed and the tumor from each mouse was harvested to measure tumor volume and tumor weight. Tumors in honokiol-treated mice were significantly smaller than those in PBS-treated mice (Fig. 6A). The average tumor volume in administration of honokiol was reduced by 46.3% compared with those in administration of PBS (Fig. 6B). Similarly, honokiol resulted in 44.8% decrease in average tumor weight (Fig. 6C).

To further explore whether the reduction of tumor xenograft by honokiol was linked with the inhibition of HDAC activity, HDAC activity was detected in tumor lysates. The average HDAC activity in honokiol-treated mice was reduced by 60.6% than that in PBS-treated mice (Fig. 6D). Finally, the levels of class I HDACs, p21/waf1, and Bax were detected in honokiol-treated mice and control mice. As indicated in Figure 6E, the protein levels of HDAC1–3 and HDAC8 were significantly decreased, while the levels of p21/waf1 and Bax

TABLE I. Effects of Honokiol on Blasts of Individuals With AML

No.	Sex	Age (year)	FAB subtype	Karyotyoe	Blasts %	Annexin V^+ %	
						Ethyl alcohol	Honokiol
1	M	53	M1	46, XY	83	5.4 ± 0.2	30.4 ± 4.5*
2	F	62	M1	47, XX, +8	75	12.4 ± 1.8	13.1 ± 2.0
3	M	57	M1	46, XY	88	3.6 ± 0.6	35.2 ± 4.3*
4	F	52	M1	47, XX,+13	90	11.7 ± 0.9	12.3 ± 0.8
5	M	64	M2	46, XY	72	7.3 ± 0.9	45.6 ± 5.7*
6	F	48	M2	ND	89	5.8 ± 0.3	18.5 ± 3.2*
7	M	42	M2	46, XY	92	6.2 ± 0.4	37.2 ± 3.6*
8	F	38	M2	46, XX, t(8;21)	72	6.4 ± 0.5	53.5 ± 3.2*
9	M	35	M2	48, XY, +8, +11	78	7.3 ± 0.8	37.9 ± 3.1*
10	F	52	M2	46, XX	76	4.2 ± 0.4	35.2 ± 3.0*
11	M	49	M3	46, XY, t(15;17)	84	8.1 ± 0.6	9.8 ± 1.4
12	M	52	M3	46, XY, t(15;17)	88	7.8 ± 0.7	40.2 ± 3.7*
13	F	48	M3	46, XY, t(15;17)	71	9.6 ± 0.6	11.5 ± 2.1
14	F	51	M4	47, XX,+8, Inv (16) (p13q22)	93	8.3 ± 0.9	68.3 ± 8.3*
15	M	55	M4	46, XY	85	5.7 ± 0.3	52.8 ± 6.7*
16	M	64	M4	46, XY, 7q-	82	7.9 ± 0.6	26.8 ± 3.4*
17	F	57	M4	46, XX	80	6.6 ± 0.5	42.9 ± 3.9*
18	F	56	M4	46, XX, t(9;11) (p22;q23)	79	9.3 ± 0.8	36.2 ± 4.5*
19	F	46	M5	46, XX	94	6.6 ± 0.4	28.7 ± 1.9*
20	M	67	M5	ND	86	4.9 ± 0.5	48.2 ± 3.8*
21	M	35	M5	47, XY, +11	82	5.2 ± 0.2	32.2 ± 4.3*
22	M	46	M5	43, XY, −3, −17, −18	74	4.7 ± 0.4	5.1 ± 0.4
23	F	54	M5	46, XX	91	6.8 ± 0.6	24.5 ± 2.8*
24	F	58	M5	46,XX, t(6;11) (q27;q23)	79	7.3 ± 0.7	9.2 ± 1.1
25	M	62	M5	46, XY	85	5.3 ± 0.3	48.6 ± 3.7*
26	F	68	M5	46,XX, t(9;11) (p22;q23)	80	7.3 ± 0.5	34.4 ± 2.4*

ND, not determined.
*$P <$ 0.01 verse Ethyl alcohol.

were obviously increased in the tumors obtained from honokiol-treated mice as compared with control mice.

DISCUSSION

Post-translational modifications of histone that affect chromatin structure might play important roles in epigenetic alterations. Acetylation and deacetylation are the two main histone modifications that are clinically identified as predictors of cancer initiation and progression. In our present study, we find plant-derived natural compound honokiol possesses the ability of inhibiting the HDAC activity and decreases the expression of class I HDACs, which is mediated by the enhanced proteasomal degradation. Furthermore, honokiol increases the expression of p21/waf1 and Bax through triggering ac-H3 and ac-H4 in the promoter regions of p21/waf1 and Bax, finally leading to cell cycle arrest and apoptosis in leukemic cells.

Recently, emerging data have demonstrated that HDAC inhibitors induce cell cycle arrest, differentiation, and apoptosis in leukemia cells through induction of pro-apoptotic proteins and cell cycle kinase inhibitors. For example, HDAC inhibitors vorinostat and MS275 induced both p21 and tumor necrosis factor-related apoptosis-inducing ligand expression via directly triggering the promoters [Nebbioso et al., 2005]. TSA restored the function of apoptosome component Apaf-1, whose functional activity was impaired in ovarian cancer cells, to enhanced cisplatin-induced apoptosis [Tan et al., 2011]. Consistent with the previous reports [Battle et al., 2005; Ishikawa et al., 2012], honokiol significantly reduced the proliferation and viability of human leukemic cell lines and primary AML blasts but failed to cause cell death in normal hematopoietic progenitors. The parallel concentration- and time-dependence suggest that the inhibition of cell growth by honokiol may be mediated, at least in part, through the downregulation of class I HDAC proteins and inhibition of HDAC activity in these leukemic cells. Further support for this possibility was demonstrated by the effects of HDAC inhibitors which also reduced cell viability and induced cell apoptosis in leukemic cell lines. All these data suggest that the action of honokiol against leukemic cells is partly similar to that of these synthetic HDAC inhibitors.

Plant-derived compounds, which have the ability to inhibit the activity of HDAC, are minimally toxic compared with chemical derived-compounds. Toxicity of honokiol was evaluated in Sprague-Dawley rats by intravenous administration with honokiol. No significant differences were observed in hematological values, serum biochemical values, body weight, and others between honokiol-treated rats and vehicle-treated rats [Wang et al., 2011]. The potential genotoxic effects of Magnolia bark extract were also assessed in Chinese hamster lung cells and Chinese hamster ovary cells by chromosomal aberration assays. No mutagenic activity was observed [Zhang et al., 2008]. These results suggest that honokiol either alone or as a part of magnolia bark extract does not induce toxicity in animal models and thus will be clinically safe. Green tea polyphenols (GTPs) reactivated epigenetically silenced genes through inhibiting HDAC activity and reducing class I HDACs expression in prostate cancer cells [Thakur et al., 2012a]. Honokiol was reported to induce apoptosis and inhibit growth via decreasing class I HDACs expression in non-small cell lung cancer [Singh et al., 2012]. Therefore, without severe side

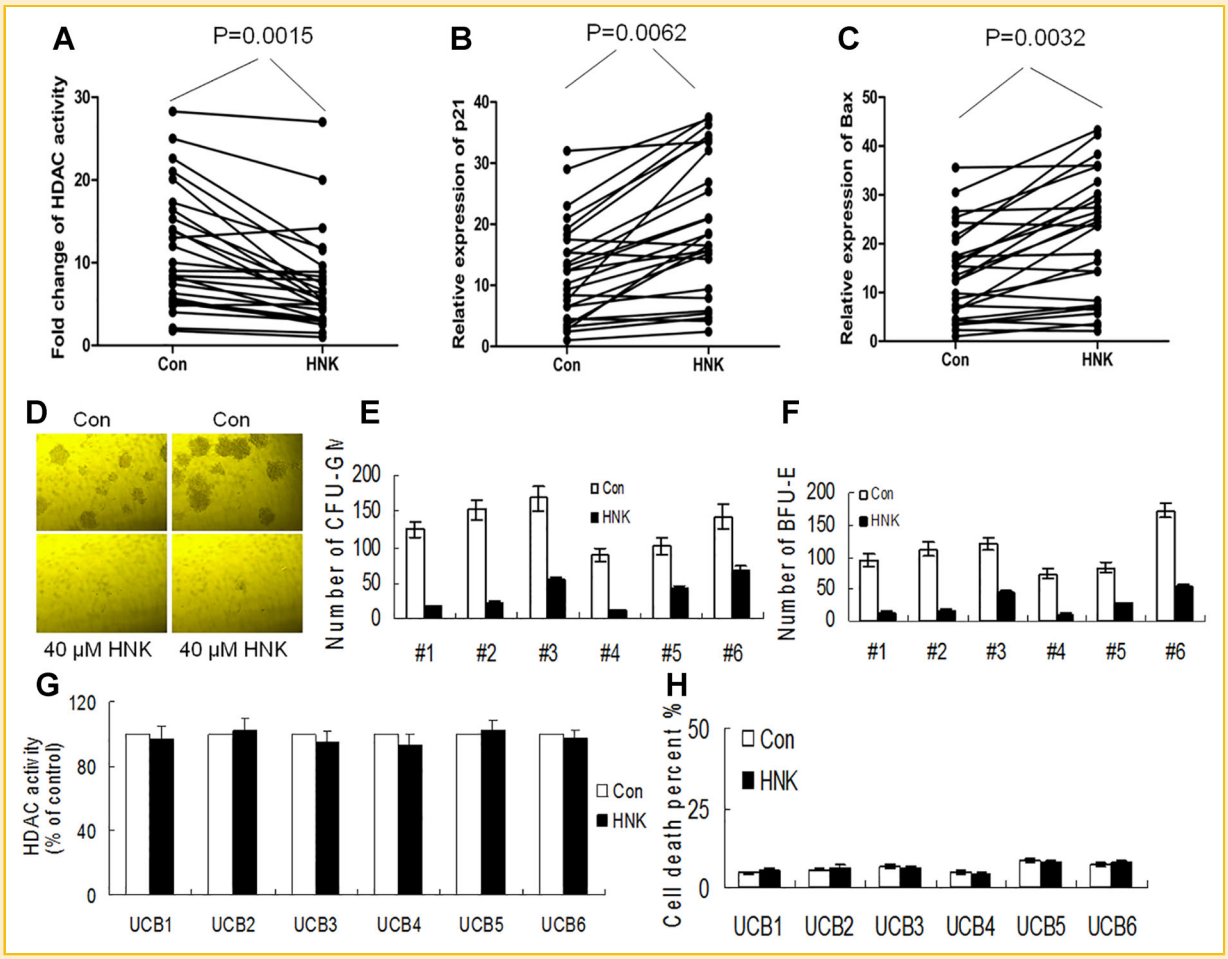

Fig. 5. The effects of honokiol on primary AML blasts and normal human hematopoietic progenitors from unbilicial cord blood. (A–C) Bone marrow mononuclear cells from 26 AML patients were treated with 40 μM honokiol or not for 24 h. HDAC activities were measured by colorimetric HDAC Activity Assay (A). The mRNA levels of p21/waf1 and Bax were detected by quantitative real-time PCR, respectively (B–C). (D–F) Bone marrow mononuclear cells from six AML patients (1 M1, 2 M2, and 3 M5) were cultured with 40 μM honokiol for colony assay as described in Material and methods. Representative images of colony for the case 1 patient (D). Images were visualized with a LEICA DMIRB (LEICA, Wetzlar, Germany) microscope equipped with a × 40/0.5 numerical aperture objective lens (LEICA) and were captured through Canon digital camera. The numbers of CFU-GM (E) and BFU-E (F) colonies containing >40 cells in each dish was counted. All experiments were done twice using triplicate plates per experimental point. (G–H) Mononuclear cells were freshly isolated from six unbilicial cord blood and then were treated with 40 μM honokiol or not for 24 h. HDAC activities were measured by colorimetric HDAC Activity Assay (G). The numbers of cells undergoing apoptosis were determined using Annexin V/PI staining (H).

effects, these compounds isolated from plant with the inhibition of HDAC activity can be utilized in the prevention and treatment for different tumors including hematological malignancies. The levels of HDAC activity can be altered through modulation of HDAC protein levels or direct inhibition of HDAC enzyme activity. HDAC inhibitors vorinostat and TSA were reported to inhibit HDAC enzymatic activity via directly interacting with catalytic sites of HDAC protein [Finnin et al., 1999]. Although honokiol reduced class I HDACs expression in leukemic cells, further studies are required to determine whether honokiol inhibits HDAC enzymatic activity via directly interacting with catalytic sites of HDAC protein.

Most cellular proteins are degraded by ubiquitin-proteasome system. Several reports showed that HDAC1–3 were degraded through ubiquitin-proteasome system [Gopal and Van, 2006; Zhao et al., 2010; Zhang et al., 2011]. Although HDAC inhibitor valproic acid selectively led to proteasomal degradation of HDAC2 protein through inducing the E2 ubiquitin conjugase Ubc8 and the E3 ubiquitin ligase RLIM expressions, TSA failed to cause HDAC2 protein degradation because TSA rapidly decreases RLIM protein level in addition to inducing Ubc8. Further, an anticancer agent 3, 3′-Diindolylmethane (DIM) selectively induced proteasome-mediated degradation of HDAC in human colon cancer cells through inducing the formation of the ubiquitin-labeled HDAC, but not inducing RLIM expression [Li et al., 2010]. Therefore, HDAC inhibitors induced proteasomal degradation of HDAC through a quite complicated mechanism. Considering that honokiol inhibited all class I HDACs and MG132 prevented honokiol-caused downregulation of HDACs, we speculate that honokiol may enhance the ubiquitin-labeled HDAC1–3 and 8 expressions to promote degradation in leukemic cells. Leukemogenic proteins such as AML1-ETO and PML-RARα were also degraded through the Ubc8-and E3-ligase SIAH-1-dependent ubiquitin-proteasome system. Furthermore, HDAC inhibitor resulted in

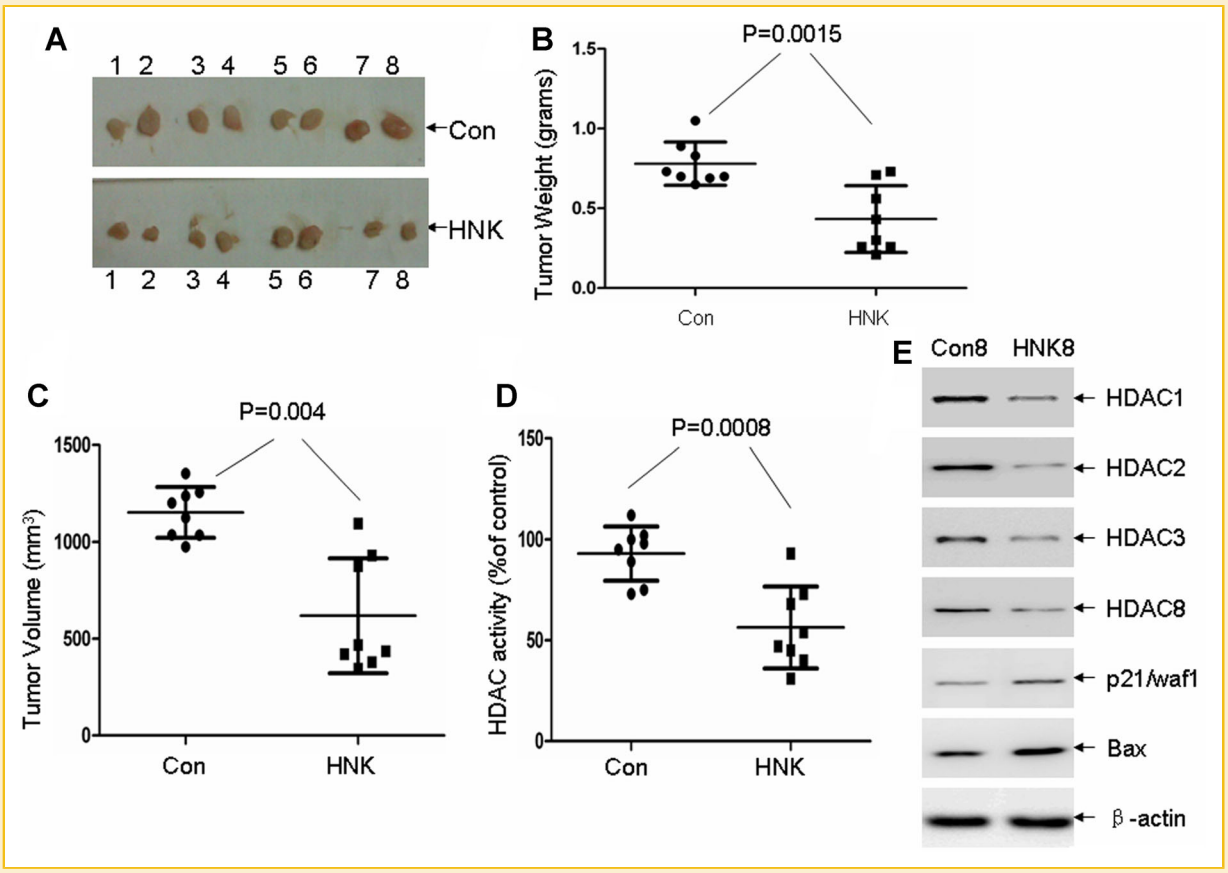

Fig. 6. The anti-tumor effect of honokiol in K562 tumor xenograft. About 2×10^7 viable K562 cells were injected subcutaneously into right flank of each nude mouse. After 24 h, honokiol was administered by oral gavage (100 mg/kg body weight of mice) twice a week. All mice were sacrificed and the tumors from each mouse were harvested at 10 weeks. (A) A photograph of tumors in honokiol-treated mice and control mice. (B–C) All tumors were harvested at the termination of experiment to measure net weight (B) and net volume (C). (D) Tumor lysates from honokiol-treated mice and control mice were prepared for HDAC activity assay. (E) The protein levels of class I HDACs, p21/waf1, and Bax were detected in tumor lysates from one honokiol-treated mouse and one control mouse.

AML1-ETO and PML-RARα degradation through inducing Ubc8 expression [Kramer et al., 2008]. In order to determine whether honokiol degraded AML1-ETO and PML-RARα fusion proteins, Kasumi and NB4 cells, which carry AML1-ETO and PML-RARα fusion proteins, respectively, were treated with honokiol [Muller et al., 1998; Markova et al., 2012]. Our data showed treatment with honokiol decreased AML1-ETO protein expression in Kasumi cells, but failed to reduce PML-RARα protein expression in NB4 cells (Figs. S5A–B).

Increased activity of histone deacetylases was demonstrated in childhood acute leukemia and philadelphia-negative chronic myeloproliferative neoplasms [Skov et al., 2012; Sonnemann et al., 2012]. HDAC2, HDAC3, HDAC6, and HDAC8 were overexpressed in childhood acute lymphoblastic leukemia (ALL) compared with normal bone marrow samples. Moreover, higher expressions of HDAC7 and HDAC9 were associated with poorer prognosis in childhood ALL, respectively [Moreno et al., 2010]. Therefore, inhibition of these overexpressed individual HDACs might contribute to the clinical treatment. In addition to inhibiting class I HDAC expressions, honokiol also decreased class II HDACs such as HDAC4, 5, and 6 in K562 and HEL cells (Figs. S6A–B), suggesting that honokiol may be a pan-HDAC inhibitor in leukemic cells.

Previous data reported that HDAC inhibitors increased acetylation of p53 and enhanced its DNA-binding activity to promote to the expression of its downstream targets, such as p21/waf1 and Bax [Oh et al., 2012]. Our data showed honokiol increased the expression of p21/waf1 and Bax in K562 cells, in which wide-type p53 expression was lacking [Feinstein et al., 1992], suggesting honokiol-induced upregulation of p21/waf1 and Bax might be independent of p53. Although our data indicated that honokiol failed to affect the protein levels of p53 in HEL cells, it still remains to elucidate that whether honokiol might cause the acetylation of wild-type p53, which finally enhances binding to the p21/waf1 and Bax promoter to increase their expression. Intriguingly, in addition to activating transcription of genes, HDAC inhibitors were reported to regulate genes at multiple levels. For example, HDAC inhibitors increased p21/waf1 expression via inducing p21/waf1 mRNA stabilization [Hirsch and Bonham, 2004]. Therefore, further studies are still needed to explore whether honokiol can induce p21/waf1 and Bax mRNA stabilization to increase their expression.

Here, we report that honokiol decreases HDAC enzyme activity and reduces the expression of class I HDACs in vitro and in vivo, but fails to alter HDAC activity in normal hematopoietic progenitors,

suggesting that honokiol presents selective toxicity against leukemic cells. Further, the decrease in HDAC activity by honokiol is associated with increased global ac-H3 and ac-H4 as well as increased acetylation of histone in the promoter regions of p21/waf1 and Bax, finally leading to the increased expression of p21/waf1 and Bax. Collectively, the anti-cancer effects of honokiol might be due, in part, to HDAC inhibition. Therefore, in combination with other modes of therapy or not, honokiol has the potential to develop into a therapeutic agent for leukemia without severe side effects.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (81200350 to Shen-meng Gao), Zhejiang Provincial Natural Science Foundation of China (LY12H08003 to Shen-meng Gao; Y13H080014 to Hai-ge Ye; LQ13H080002 to Li-cai He).

REFERENCES

Arora S, Singh S, Piazza GA, Contreras CM, Panyam J, Singh AP. 2012. Honokiol: A novel natural agent for cancer prevention and therapy. Curr Mol Med 12:1244–1252.

Battle TE, Arbiser J, Frank DA. 2005. The natural product honokiol induces caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells. Blood 106:690–697.

Chen G, Izzo J, Demizu Y, Wang F, Guha S, Wu X, Hung MC, Ajani JA, Huang P. 2009. Different redox states in malignant and nonmalignant esophageal epithelial cells and differential cytotoxic responses to bile acid and honokiol. Antioxid Redox Signal 11:1083–1095.

Chen J, Odenike O, Rowley JD. 2010. Leukaemogenesis: More than mutant genes. Nat Rev Cancer 10:23–36.

Chen CQ, Yu K, Yan QX, Xing CY, Chen Y, Yan Z, Shi YF, Zhao KW, Gao SM. 2013. Pure curcumin increases the expression of SOCS1 and SOCS3 in myeloproliferative neoplasms through suppressing class I histone deacetylases. Carcinogenesis 34:1442–1449.

Das CM, Aguilera D, Vasquez H, Prasad P, Zhang M, Wolff JE, Gopalakrishnan V. 2007. Valproic acid induces p21 and topoisomerase-II (alpha/beta) expression and synergistically enhances etoposide cytotoxicity in human glioblastoma cell lines. J Neurooncol 85:159–170.

Delcuve GP, Khan DH, Davie JR. 2013. Targeting class I histone deacetylases in cancer therapy. Expert Opin Ther Targets 17:29–41.

Feinstein E, Gale RP, Reed J, Canaani E. 1992. Expression of the normal p53 gene induces differentiation of K562 cells. Oncogene 7:1853–1857.

Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP. 1999. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature 401:188–193.

Gopal YN, Van Dyke MW. 2006. Depletion of histone deacetylase protein: A common consequence of inflammatory cytokine signaling?. Cell Cycle 5:2738–2743.

Hirsch CL, Bonham K. 2004. Histone deacetylase inhibitors regulate p21WAF1 gene expression at the post-transcriptional level in HepG2 cells. FEBS Lett 570:37–40.

Huang L, Sowa Y, Sakai T, Pardee AB. 2000. Activation of the p21WAF1/CIP1 promoter independent of p53 by the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) through the Sp1 sites. Oncogene 19:5712–5719.

Ishikawa C, Arbiser JL, Mori N. 2012. Honokiol induces cell cycle arrest and apoptosis via inhibition of survival signals in adult T-cell leukemia. Biochim Biophys Acta 1820:879–887.

Kizildag S, Ates H. 2010. Treatment of K562 cells with 1,25-dihydroxyvitamin D3 induces distinct alterations in the expression of apoptosis-related genes BCL2, BAX, BCLXL, and p21. Ann Hematol 89:1–7.

Kramer OH, Muller S, Buchwald M, Reichardt S, Heinzel T. 2008. Mechanism for ubiquitylation of the leukemia fusion proteins AML1-ETO and PML-RARalpha. FASEB J 22:1369–1379.

Li Y, Li X, Guo B. 2010. Chemopreventive agent 3,3′-diindolylmethane selectively induces proteasomal degradation of class I histone deacetylases. Cancer Res 70:646–654.

Liu Z, Zhang X, Cui W, Li N, Chen J, Wong AW, Roberts A. 2007. Evaluation of short-term and subchronic toxicity of magnolia bark extract in rats. Regul Toxicol Pharmacol 49:160–171.

Markova EN, Kantidze OL, Razin SV. 2012. Transcription of the AML1/ETO chimera is guided by the P2 promoter of the AML1 gene in the Kasumi-1 cell line. Gene 510:142–146.

Marks PA. 2010. The clinical development of histone deacetylase inhibitors as targeted anticancer drugs. Expert Opin Investig Drugs 19:1049–1066.

Moreno DA, Scrideli CA, Cortez MA, de Paula Queiroz, Valera R, da Silva ET, Silveira V, Yunes JA, Brandalise SR, Tone LG. 2010. Differential expression of HDAC3, HDAC7 and HDAC9 is associated with prognosis and survival in childhood acute lymphoblastic leukaemia. Br J Haematol 150:665–673.

Muller S, Kramer OH. 2010. Inhibitors of HDACs–effective drugs against cancer? Curr Cancer Drug Targets 10:210–228.

Muller S, Miller WH, Jr., Dejean A. 1998. Trivalent antimonials induce degradation of the PML-RAR oncoprotein and reorganization of the promyelocytic leukemia nuclear bodies in acute promyelocytic leukemia NB4 cells. Blood 92:4308–4316.

Myzak MC, Dashwood RH. 2006. Histone deacetylases as targets for dietary cancer preventive agents: lessons learned with butyrate, diallyl disulfide, and sulforaphane. Curr Drug Targets 7:443–452.

Nebbioso A, Clarke N, Voltz E, Germain E, Ambrosino C, Bontempo P, Alvarez R, Schiavone EM, Ferrara F, Bresciani F, Weisz A, de Lera AR, Gronemeyer H, Altucci L. 2005. Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells. Nat Med 11:77–84.

Oh ET, Park MT, Choi BH, Ro S, Choi EK, Jeong SY, Park HJ. 2012. Novel histone deacetylase inhibitor CG200745 induces clonogenic cell death by modulating acetylation of p53 in cancer cells. Invest New Drugs 30:435–442.

Prince HM, Bishton MJ, Harrison SJ. 2009. Clinical studies of histone deacetylase inhibitors. Clin Cancer Res 15:3958–3969.

Singh T, Prasad R, Katiyar SK. 2012. 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 8.

Skov V, Larsen TS, Thomassen M, Riley CH, Jensen MK, Bjerrum OW, Kruse TA, Hasselbalch HC. 2012. Increased gene expression of histone deacetylases in patients with Philadelphia-negative chronic myeloproliferative neoplasms. Leuk Lymphoma 53:123–129.

Sonnemann J, Gruhn B, Wittig S, Becker S, Beck JF. 2012. Increased activity of histone deacetylases in childhood acute lymphoblastic leukaemia and acute myeloid leukaemia: Support for histone deacetylase inhibitors as antileukaemic agents. Br J Haematol 158:664–666.

Tan L, Kwok RP, Shukla A, Kshirsagar M, Zhao L, Opipari AW, Jr., Liu JR. 2011. Trichostatin A restores Apaf-1 function in chemoresistant ovarian cancer cells. Cancer 117:784–794.

Thakur VS, Gupta K, Gupta S. 2012a. Green tea polyphenols causes cell cycle arrest and apoptosis in prostate cancer cells by suppressing class I histone deacetylases. Carcinogenesis 33:377–384.

Thakur VS, Gupta K, Gupta S. 2012b. Green tea polyphenols increase p53 transcriptional activity and acetylation by suppressing class I histone deacetylases. Int J Oncol 41:353–361.

Wada T, Kikuchi J, Nishimura N, Shimizu R, Kitamura T, Furukawa Y. 2009. Expression levels of histone deacetylases determine the cell fate of hematopoietic progenitors. J Biol Chem 284:30673–30683.

Wang J, Hoshino T, Redner RL, Kajigaya S, Liu JM. 1998. ETO, fusion partner in t(8;21) acute myeloid leukemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. Proc Natl Acad Sci U S A 95:10860–10865.

Wang X, Duan X, Yang G, Zhang X, Deng L, Zheng H, Deng C, Wen J, Wang N, Peng C, Zhao X, Wei Y, Chen L. 2011. Honokiol crosses BBB and BCSFB, and inhibits brain tumor growth in rat 9L intracerebral gliosarcoma model and human U251 xenograft glioma model. PLoS One 6:e18490.

Zeng PY, Berger SL. 2006. LKB1 is recruited to the p21/WAF1 promoter by p53 to mediate transcriptional activation. Cancer Res 66:10701–10708.

Zhang J, Kan S, Huang B, Hao Z, Mak TW, Zhong Q. 2011. Mule determines the apoptotic response to HDAC inhibitors by targeted ubiquitination and destruction of HDAC2. Genes Dev 25:2610–2618.

Zhang B, Maniatis T, Song Y, Zhang W, Zhang X, Li N, Chen J, Wong AW, Roberts A. 2008. Evaluation of magnolia bark extract in chromosomal aberration assays. Mutat Res 654:133–137.

Zhao HL, Ueki N, Hayman MJ. 2010. The Ski protein negatively regulates Siah2-mediated HDAC3 degradation. Biochem Biophys Res Commun 399:623–628.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.