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Honokiol Enhances Paclitaxel Efficacy in Multi-Drug Resistant Human Cancer Model through the Induction of Apoptosis

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Abstract

Resistance to chemotherapy remains a major obstacle in cancer therapy. This study aimed to evaluate the molecular mechanism and efficacy of honokiol in inducing apoptosis and enhancing paclitaxel chemotherapy in pre-clinical multi-drug resistant (MDR) cancer models, including lineage-derived human MDR (KB-8-5, KB-C1, KB-V1) and their parental drug sensitive KB-3-1 cell lines. In vitro analyses demonstrated that honokiol effectively inhibited proliferation in KB-3-1 cells and the MDR derivatives (IC₅₀ ranging 3.35±0.13 µg/ml to 2.77±0.22 µg/ml), despite their significant differences in response to paclitaxel (IC₅₀ ranging 1.66±0.09 ng/ml to 6560.9±439.52 ng/ml). Honokiol induced mitochondria-dependent and death receptor-mediated apoptosis in MDR KB cells, which was associated with inhibition of EGRF-STAT3 signaling and downregulation of STAT3 target genes. Combined treatment with honokiol and paclitaxel synergistically augmented cytotoxicity in MDR KB cells, compared with treatment with either agent alone in vitro. Importantly, the combined treatment significantly inhibited in vivo growth of KB-8-5 tumors in a subcutaneous model. Tumor tissues from the combination group displayed a significant inhibition of Ki-67 expression and an increase in TUNEL-positive cells compared with the control group. These results suggest that targeting multidrug resistance using honokiol in combination with chemotherapy drugs may provide novel therapeutic opportunities.

Honokiol is a natural component isolated from the bark of magnolia tree [2,3], which has been traditionally used to treat anxiety-related disorders and digestive complaints [2,4]. Interestingly, honokiol also exhibited potent anticancer activities [5,6,7] and further enhanced conventional chemotherapies in a variety of preclinical models of human cancer, including chronic lymphocytic leukemia [3], prostate cancer [8] and multiple myeloma [9]. These relatively wide-ranging anticancer capabilities and favorable safety profile make honokiol an attractive adjunct therapy to enhance conventional chemotherapy in clinical settings.

Overexpression of anti-apoptotic proteins is an underlying mechanism that contributes to the acquisition of therapeutic resistance, recurrence and metastasis. A growing body of evidence suggests that three anti-apoptotic proteins, i.e., survivin, Mcl-1, and Bcl-2, may be directly related to drug resistance in cancer [10,11]. Inhibition of these crucial survival factors has been shown to trigger apoptosis and sensitize cancer cells to drug treatment [5–
Figure 1. Honokiol inhibits growth and induces apoptosis in multidrug-sensitive and -resistant cells. (a, b) Growth inhibition effect of paclitaxel and p-gp expression in KB-3-1, KB-8-5, KB-C1 and KB-V1 cells. The IC50 values of paclitaxel in drug resistant KB-8-5, KB-C1 and KB-V1 cells.
were increased by 16-, 117-, and 4000-fold respectively when compared to parental KB-3-1 cells. (c, d) Growth inhibition effect of honokiol in KB-3-1, KB-8-5, KB-C1 and KB-V1 cells. The IC50 values of honokiol in drug resistant KB-8-5, KB-C1 and KB-V1 cells were similar to that in parental KB-3-1 cells. (e) Honokiol induces apoptosis in KB cells. KB-3-1 and KB-8-5 cells were treated with 5, 10, 15 μg/ml of honokiol for 24, 48, and 72 h. KB-C1 and KB-V1 cells were treated with honokiol for 48 h. Apoptosis was measured by annexin V-phycocerythrin staining. *indicates statistically significant p values (*,versus control, p<0.05; **, versus 5 μg/ml, p<0.05).
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Honokiol Reduces Chemoresistance

Thiry microgram protein from whole-cell extracts or cytoplasmic and mitochondrial fractions were quantified, separated on SDS-PAGE gels and transferred to nitrocellulose membranes. After being blocked with 5% nonfat dry milk in TBS-T buffer, the membranes were incubated with specific antibodies overnight at 4°C. Mouse anti-β-actin antibody (Trevigen, Gaithersburg, MD) was used as a sample loading control. Immunostained protein bands were detected with an enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK). The experiments were repeated 3 times.

Immunoblotting

Thirty microgram protein from whole-cell extracts or cytoplasmic and mitochondrial fractions were quantified, separated on SDS-PAGE gels and transferred to nitrocellulose membranes. After being blocked with 5% nonfat dry milk in TBS-T buffer, the membranes were incubated with specific antibodies overnight at 4°C. Mouse anti-β-actin antibody (Trevigen, Gaithersburg, MD) was used as a sample loading control. Immunostained protein bands were detected with an enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK). The experiments were repeated 3 times.

Materials and Methods

Cell Culture

The KB-3-1 and its MDR derivative cell lines were generously provided by Dr. Michael M. Gottesman (NCI, NIH, Bethesda, MD) and have been characterized previously [12]. KB-3-1 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum. KB-8-5 and KB-C1 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum and 0.01 and 1 μg/ml colchicine respectively. KB-V1 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum and 1 μg/ml vinblastine. To eliminate impact of colchicine or vinblastine on experiment result, resistant cells were cultured in drug free medium for one week before any experiment.

Cell Growth Assay

Cells were seeded at a density of 5 × 10^3 cells per well into 96-well plates in quadruplet. Twenty-four hours later, drugs were added in various concentration ranges as single agents or in two-drug combinations and then incubated for 72 h. The range for honokiol was from 0.625 to 20 μg/ml for all four cell lines. For paclitaxel, the concentration range was 0.19 to 97.5 ng/ml, for doxorubicin (Doxo) range was 0.01 and 1 μg/ml, for cisplatin (Cisplatin) range was 0.01 to 1 μg/ml, for colchicine range was 0.12 to 25.0 μg/ml, and for vinblastine 1 μg/ml for KB-3-1, KB-8-5, KB-C1, and KB-V1 cells, respectively. Cell growth inhibition was measured by determining cell density with the sulforhodamine B assay. The percentage of inhibition was determined by comparison of cell density in the drug-treated cells with that of the untreated control cells. All experiments were repeated at least three times.

Apoptosis Analysis

Apoptosis was analyzed in all four cell lines. Cells were treated with honokiol, paclitaxel, or their combination as indicated in the figure legends, trypsinized, and washed in cold 1× PBS. The cells were re-suspended in 1× Annexin binding buffer (BD PharMingen), and then stained with Annexin V-phycocerythrin (Annexin V-PE; BD PharMingen) and 7-AAD (BD PharMingen) for 15 min at room temperature. The stained samples were measured using a fluorescence-activated cell sorting (FACS) caliber bench-top flow cytometer (Becton Dickinson, Franklin Lakes, NJ). FlowJo software (Tree Star, Ashland, OR) was used for apoptosis analysis. The experiments were repeated 3 times independently.

Immunohistochemistry and TUNEL Assay

Immunohistochemical analysis for Ki-67 staining on paraffin-embedded mouse xenograft tissue was performed as previously described [13]. Cells staining positive for Ki-67 were counted and the percentage of positive cells was calculated. An average of the 8 readings was used for statistical analysis.

TUNEL assay was performed by immunofluorescence using the same specimens as above, following the procedure provided by the manufacturer (Promega, Madison, WI). To analyze the assay results, the total cell number and the positive cell number in the same area were counted for five random areas; the result was presented as an average ratio of positive cell number out of the total cell number.

Statistical Analysis

The statistical significance of treatment of cells in the in vivo cytotoxicity assay was assessed using the Student’s t-test. For in vivo anti-tumor efficacy assay, a log-linear mixed model with random intercept was used to compare the significance of the mean tumor volumes among each group. The statistical significance of treatment effect on microtubules, apoptosis, and cell proliferation
**Figure 2.** Honokiol induces PARP and caspase-3 cleavage and the release of cytochrome c in the cytoplasm in multidrug-sensitive and -resistant cells. (a) KB-3-1 cells and (b) KB-8-5 cells were treated with 5, 10, 15 μg/ml of honokiol for 24, 48 h. Full-length and cleaved PARP,
cleaved caspase-3 and β-actin as a loading control were detected by immunoblotting using whole cell lysates. (c) KB-C1 and KB-V1 cells were treated with 5, 10, 15 μg/ml of honokiol for 48 h. Cleaved PARP was detected by immunoblotting using whole cell lysates. (d) Upper panel, KB-8-5 cells were treated with 5, 10, 15 μg/ml of honokiol for 48 h. Cytoplasmic and mitochondrial fractions were separated and immunoblotted with cytochrome c (Cyto C) antibody. COX4 (a mitochondrial protein) was used to show efficiency of cell fractionation. Lower panel, KB-8-5 cells were treated with 5, 10, 15 μg/ml of honokiol for 24 and 48 h. DR5 was detected by immunoblotting using whole cell lysates. All experiments were repeated at least three times, and representative data are presented.

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Figure 3. Honokiol inhibits the EGFR signaling pathway and downregulates STAT3 target genes in multidrug-sensitive and -resistant cells. KB-3-1 and KB-8-5 cells were treated with 5 μg/ml of honokiol for short time (0.5, 1, or 2 h) or long time (24, 48, or 72 h). Whole cell lysates were immunoblotted for the indicated proteins. The effect of honokiol at early time points: (a) phosphorylation of EGFR and STAT3; (b) Honokiol decreases phosphorylation of AKT and ERK. The effect of honokiol at late time points: (c) phosphorylation and expression of EGFR and STAT3; (d) phosphorylation and expression of AKT and ERK; (e) expression of survivin, Bcl-2, Mcl-1. (f) Honokiol inhibits the EGFR signaling pathway and downregulates STAT3 target genes in a dose-dependent manner. KB-3-1 and KB-8-5 cells were treated with 5, 10, 15 μg/ml of honokiol for 48 h. Whole cell lysates were immunoblotted for the indicated proteins. (g) Honokiol inhibits the EGFR-STAT3 pathway in KB-C1 and KB-V1 cells. KB-C1 and KB-V1 cells were treated with 5, 10, 15 μg/ml of honokiol for 48 h. All experiments were repeated at least three times, and representative data are presented.

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in xenograft tumor tissues was assessed using the Kruskal-Wallis test (one-way ANOVA). \( P < 0.05 \) was considered statistically significant in all analyses.

**Results**

**Honokiol Reduces Viability and Induces Apoptosis in Human MDR Cancer Cells**

The MDR cancer cell lines KB-8-5, KB-C1 and KB-V1 were derived from their drug-sensitive counterpart KB-3-1 cells, and have been widely used as a clinically relevant model in the study of drug resistance [14,15]. These cells exhibited distinct sensitivities to paclitaxel treatment. As shown in Figure 1a, 1b, the IC\(_{50}\) values in MDR cell lines (KB-8-5:26.73 ± 1.01 ng/ml, KB-C1:195.0 ± 11.11 ng/ml, and KB-V1:6560.0 ± 439.52 ng/ml) were increased by 16-, 117- and 4,000-fold, respectively, when compared to the parental KB-3-1 cell line (1.66 ± 0.09 ng/ml). Consistent with their resistant phenotype, MDR cell lines substantially express the classical MDR marker P-glycoprotein (Fig. 1b). Interestingly, a 72 h-treatment with honokiol effectively reduced the viability of MDR cells (KB-8-5:26.73 ± 1.01 ng/ml, KB-C1:3.04 ± 0.30 μg/ml, KB-V1:2.77 ± 0.22 μg/ml), with IC\(_{50}\) values similar to those in KB-3-1 cells (3.35 ± 0.13 μg/ml) (Fig. 1c, 1d). Apoptosis analysis further showed that honokiol induced apoptosis in a time- and dose-dependent manner (Fig. 1e). 24 h following honokiol treatment, the percentage of apoptotic KB-3-1 cells was 11.9 ± 3.9% (5 μg/ml of honokiol), 32.9 ± 0.9% (10 μg/ml), and 47.1 ± 2.7% (15 μg/ml); the percentage of apoptotic cells increased to 18.4 ± 2.1%, 51.5 ± 0.6%, and 65.3 ± 1.6%, respectively, following a 48 h-treatment, which was further increased at 72 h. Honokiol also induced a similar degree of apoptosis in other MDR cells, including KB-8-5, KB-C1 and KB-V1 (Fig. 1e). For example, a 48 h-treatment with 10 μg/ml of honokiol resulted in apoptosis in 51.5% of KB-3-1 cells, 52.7% of KB-8-5 cells, 52.9% of KB-C1 cells and 67.6% of KB-V1 cells, respectively. These data are consistent with the viability assay (Fig. 1c, 1d), and suggest that honokiol is a potent cytotoxic agent in KB cells regardless of their different paclitaxel sensitivities.

**Honokiol Induces Mitochondria- and Death Receptor (DR)-dependent Apoptosis in Human Cancer Cells**

We investigated the mechanism of honokiol-induced apoptosis in KB cells. As shown in Figure 2a-c, honokiol treatment induced the cleavage of PARP and caspase-3 in a dose- and time-dependent manner in all the tested KB cells, indicating the
Western blot analyses found that honokiol not only induced the release of cytochrome c in the cytoplasm in a dose-dependent manner (Fig. 2d), but also increased the expression of DR5 (Fig. 2d), a surface receptor for activation of apoptotic signals. Western blot analyses found that honokiol not only induced the release of cytochrome c in the

cytosol in a dose-dependent manner (Fig. 2d), but also increased the expression of DR5 (Fig. 2d), a surface receptor for

Figure 5. Honokiol enhancement of paclitaxel induced apoptosis involves the inhibition of the EGFR-STAT3 signaling pathway. (a) Honokiol enhances paclitaxel induced PARP and caspase-3 cleavage in KB-8-5 cells. The cells were treated with 5 μg/ml of honokiol, 15 ng/ml of paclitaxel, or the combination for 24, 48, and 72 h. Full-length and cleaved PARP, cleaved caspase-3 and β-actin as a loading control were detected by immunoblotting using whole cell lysates. (b) Honokiol enhances paclitaxel induced release of cytochrome c in the cytoplasm in KB-8-5 cells. The cells were treated with 5 μg/ml of honokiol, 15 ng/ml of paclitaxel, or the combination for 48 h. Cytoplasmic and mitochondrial fractions were separated and immunoblotted with cytochrome c (Cyto C) antibody. COX4 (a mitochondrial protein) was used to show efficiency of cell fractionation. (c) Honokiol inhibits the EGFR-STAT3 signaling pathway and downregulates STAT3 target gene expression in KB-8-5 cells. The cells were treated with 5 μg/ml of honokiol, 15 ng/ml of paclitaxel, or the combination for 48 h. Whole cell lysates were immunoblotted for the indicated proteins. (d) Paclitaxel induces microtubule polymerization in KB-8-5 cells. Drug-induced stabilization of microtubules as evidenced by an increase in microtubule polymer mass resulting in bundling was observed upon treatment with both paclitaxel and the combination; however, honokiol alone was not effective in microtubule stabilization (magnification 400x). The cells were treated with 5 μg/ml of honokiol, 15 ng/ml of paclitaxel, or the combination for 24 h. All experiments were repeated at least three times, and representative data are presented.

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Figure 6. Inhibition of tumor growth by the combination of honokiol and paclitaxel in a multidrug-resistant xenograft model. (a) The tumor growth of KB-8-5 xenografts was significantly inhibited in the combination-treated group compared with the control (p<0.0001), honokiol
Honokiol Reduces Chemoresistance

We evaluated the antitumor efficacy of honokiol, paclitaxel, and their combination in treating MDR cancer in the KB-8-5 xenograft model. The treatment was continued for 4 weeks. As shown in **Fig. 6a**, at a dose of 20 mg/kg once per week, a 4-week injection of paclitaxel alone did not significantly inhibit the growth of KB-8-5 subcutaneous tumors, when compared to the vehicle control \( (p = 0.917) \). Treatment with honokiol alone (50 mg/kg, 3 times per week, via i.p.) slightly suppressed tumor growth, although the difference was not significant \( (p = 0.194) \) (**Fig. 6a**). In contrast, the combination treatment dramatically inhibited the growth of KB-8-5 tumors, when compared with all other groups \( (vs. \text{control}: p = 0.0001; \text{vs. \text{paclitaxel:} } p = 0.036; \text{vs. \text{paclitaxel:}} p = 0.004) \). The average tumor volume in each treatment group at the endpoint was 2785.4 ± 510.0 mm^3 (control), 1810.2 ± 483.2 mm^3 (honokiol), 2591.3 ± 726.2 mm^3 (paclitaxel), and 573.9 ± 146.1 mm^3 (combination). Representative mice from
each group are shown in (Fig. 6c). These results indicate that honokiol could significantly potentiate the antitumor activity of paclitaxel in MDR cancer xenograft tumors.

We evaluated the systemic toxicity of honokiol, paclitaxel and the combination treatment in the KB-8-5 xenograft model. Compared with the control group, the body weights of mice in all three treatment groups were similar, indicating a negligible toxicity under the tested conditions (Fig. 6b). Consistently, histopathologic analyses did not find any considerable tissue damage in the major organs (including liver, spleen, kidney, heart and lung) collected from any treatment group, including the combination treatment (Fig. 6d).

We further performed IHC analysis to examine the in vivo effect of each treatment on the expression of general tumor biomarkers. The combined treatment significantly reduced the tissue level of Ki-67 in KB-8-5 tumors (percentage of Ki-67 positive cells: 3.84 ± 1.10, when compared to that in the control group (11.96 ± 2.86; \(p < 0.001\)), honokiol group (7.18 ± 2.07; \(p = 0.027\), or paclitaxel group (11.68 ± 1.82; \(p < 0.001\)) (Fig. 6e). Consistently, TUNEL assay revealed a significant increase in apoptotic tumor cells in the tumor tissues from the combination group (7.07 ± 2.11) when compared with that in the control group (1.64 ± 0.17; \(p < 0.001\)), honokiol group (5.45 ± 1.19; \(p = 0.05\)) and paclitaxel group (3.97 ± 3.58; \(p = 0.08\)) (Fig. 6e). These results indicate that honokiol could sensitize MDR cancer cells to paclitaxel through the induction of apoptosis.

Discussion

Despite some success in transiently controlling the clinical symptoms of cancer with chemotherapy, a significant percentage of patients develop “multidrug resistance”, or MDR, and inevitably progress with no cure. It is urgent to develop new strategies to overcome MDR and improve the efficacy of chemotherapy. In this study, we investigated the potential utility of the natural compound honokiol in the treatment of chemoresistant cancer using preclinical models. We demonstrated that honokiol could effectively induce cell death in cancer cells regardless of their resistance to the chemotherapy drug paclitaxel. Significantly, honokiol markedly increased the in vivo efficacy of paclitaxel in inhibiting the growth of MDR cancer in a xenograft model. We further provided molecular evidence supporting that honokiol induced apoptosis and enhanced chemotherapy through the inhibition of EGFR-STAT3 signaling and downregulation of several survival factors, including survivin, Bcl-2 and Mcl-1. These observations indicate that honokiol could be promising in sensitizing cancer cells to chemotherapy and improving paclitaxel efficacy in clinical settings.

EGFR overexpression has been closely associated with tumor progression, therapeutic resistance and poor clinical outcome in head and neck cancer and other cancer types [16,17,18,19]. The EGFR signaling pathway, including its multiple downstream pathways, such as PI3K/AKT, ERK1/2, JAK/STAT3 and mTOR/NF-κB, plays an important role in the regulation of proliferation, survival, migration and chemoresistance in cancer cells. EGFR signaling, therefore, is being actively pursued as a promising target to develop therapeutics for resistant and recurrent head and neck cancer and other cancer type using small molecule inhibitors and antibodies [20,21]. In this study, we demonstrated that honokiol decreased both the phosphorylation and expression of EGFR, suggesting an inhibition of EGFR signaling that is consistent with previous studies [5]. As expected, the expression and activity of several important components of EGFR signaling, including AKT, ERK and STAT3, were also inhibited in chemoresistant KB cells upon honokiol treatment. A recent study reported that honokiol downregulates heat shock protein (HSP) 90 in breast cancer cells [22]. HSP90 and other chaperone proteins such as HSP70 and HSP40 regulate EGFR degradation. Interruption of the HSP70/HSP90-folding cycle leads to ubiquitinylation and degradation of EGFR [23] [24]. Therefore, it is possible that honokiol promotes EGFR degradation and inhibits EGFR signaling through an HSP90-dependent mechanism.

Several STAT3 target genes, such as survivin, Bcl-2 and Mcl-1, have central roles in the regulation of survival in cancer cells, and their overexpression has been often linked to resistance to therapy (radiation or chemotherapy), aggressive tumor behavior and shortened survival in many types of cancers [5–11]. Moreover, earlier studies reported that paclitaxel induces STAT3 activation and stimulates antiapoptotic protein expression in several cell types, which could be an underlying mechanism for the acquired chemoresistance. Consistently, inhibition of STAT3 and its target genes could enhance the therapeutic efficacy of paclitaxel [25,26]. In this report, we observed that honokiol inhibits the STAT3 signaling pathway and downregulates the expression of survivin, Bcl-2 and Mcl-1. Since these proteins are involved in the regulation of both intrinsic and extrinsic apoptotic signaling, honokiol suppression of their expression could partially explain the potent cytotoxicity of honokiol in both paclitaxel-sensitive and -resistant KB cells. Further, downregulation of these critical antiapoptotic proteins may provide a molecular explanation for the chemosensitization capability of honokiol in these cells.

The MDR cancer cell lines used in this study, including KB-8-5, KB-C1, and KB-V1, have been found to substantially overexpress the classical MDR gene product P-glycoprotein (P-gp) [14,27,28]. P-gp functions as an energy-dependent efflux pump for which paclitaxel is a substrate, whose overexpression has been correlated with MDR in many cancer types. At the transcriptional level, MDR-1 expression is regulated by NF-κB in different cell types, and several studies reported that honokiol inhibits NF-κB activation and downregulates NF-κB targeting genes including P-gp [1,3,29]. In our study, we also found that pre-incubation of resistant cell lines with 10 μM/ml of honokiol for 48 h downregulated P-gp expression and enhanced the intracellular accumulation of paclitaxel (data not shown); thus, downregulation of P-gp by honokiol may also contribute to the synergistic effect of honokiol and paclitaxel in the resistant KB cell lines.

In conclusion, we have shown that honokiol can inhibit cell proliferation and induce apoptosis in multi-drug resistant cell lines. Our study also demonstrated that honokiol can synergistically augment the cytotoxicity of paclitaxel by inhibiting the EGFR-STAT3 signaling pathway and downregulating multiple antiapoptotic proteins. Honokiol, therefore, is a promising agent in the development of novel treatments for drug-resistant cancer, particularly in combination with conventional therapeutic drugs. Bcl-2 and Mcl-1 are Bcl-2 family member protein.

Supporting Information

Figure S1 Honokiol treatment significantly decreases the levels of survivin mRNA. KB-8-5 cells were treated with 5 μg/ml of honokiol for the indicated times. Total RNA was isolated from each sample and relative quantification of survivin gene expression was performed with reference to GAPDH RNA as an internal standard. (DOCX)

Table S1 Combination index (CI) assay confirmed the synergistic effect of combined treatment with honokiol
and paclitaxel in reducing the viability of MDR cancer cells.

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Methods S1

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Acknowledgments

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References


Author Contributions

Conceived and designed the experiments: XW DMS. Performed the experiments: XW HW MJP. WH LK SN ARA MB. Analyzed the data: XW HCS. Contributed reagents/materials/analysis tools: ZG JLA. Wrote the paper: XW JJB DMS.