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Original Article

Honokiol inhibits the growth of hormone-resistant breast cancer cells: its promising effect in combination with metformin

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Abstract

Background and purpose: Primary and metastatic breast cancers still represent an unmet clinical need for improved chemotherapy and hormone therapy. Considerable attention has been paid to natural anticancer compounds, especially lignans. The study aimed to evaluate the activity of several lignans against breast cancer cells and assess the effect of leading lignans on signaling pathways in combination with metformin.

Experimental approach: Human breast cancer cell lines MCF7 (hormone-dependent), MDA-MB-231, and SKBR3 (hormone-independent) were used. A hormone-resistant MCF7/hydroxytamoxifen (HT) subline was obtained by long-term cultivation of the MCF7 line with hydroxytamoxifen. Antiproliferative activity was assessed by the MTT test; the expression of signaling pathway proteins was evaluated by immunoblotting analysis.

Findings/Results: We evaluated the antiproliferative activity of lignans in breast cancer cells with different levels of hormone dependence and determined the relevant IC_{50} values. Honokiol was chosen as the leading compound, and its IC_{50} ranged from 12 to 20 μM, whereas for other tested lignans, the IC_{50} exceeded 50 μM. The accumulation of cleaved PARP and a decrease in the expression of Bcl-2 and ERα in MCF7/HT were induced following the combination of honokiol with metformin.

Conclusions and implications: Honokiol demonstrated significant antiproliferative activity against both hormone-dependent breast cancer cells and lines with primary and acquired hormone resistance. The combination of honokiol with metformin is considered an effective approach to induce death in hormone-resistant cells. Honokiol is of interest as a natural compound with antiproliferative activity against breast cancers, including resistant tumors.

Keywords: Breast cancer cells; Honokiol; Lignans; Hormone resistance; MCF7.

INTRODUCTION

Cancer is one of the most frightful diseases that humanity has been fighting for many decades. Most therapeutic methods, such as chemotherapy, radiotherapy, photodynamic therapy, and active immunotherapy, are only partially successful because of their damaging effects on normal cells, the development of tumor resistance, severe side effects, and immunosuppression (1-4).

The first anticancer drug, embichine (mustargen), was registered in the USA in 1946. Thus, the history of chemotherapy is only a few decades old, whereas the empirical experience of traditional medicine, including antitumor medicine, is much older. There is a strong belief that natural compounds occurring in plants play an important role in cancer treatment (5-8). Much attention has been paid to traditional Chinese medicine, which uses a rich source of biologically active agents such as phenolic acids, coumarins, flavonoids, stilbenes, tannins, and lignans.Lignans and neolignans are biosynthesized in plants through the oxidative coupling of phenylpropanoids (9).

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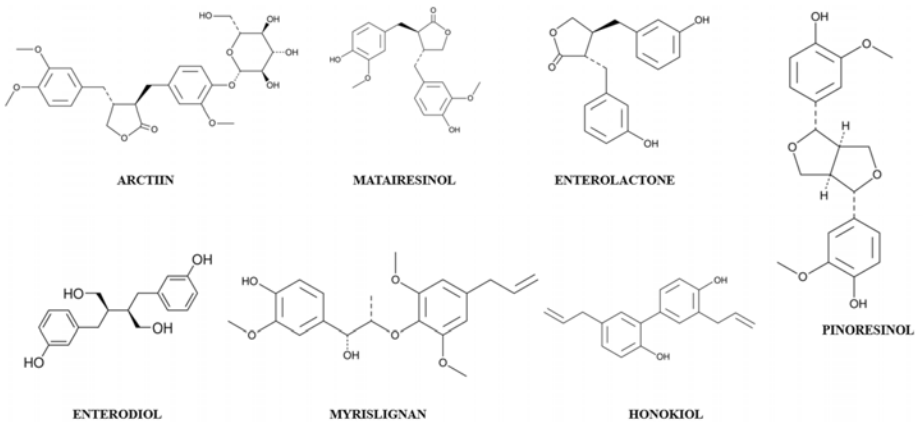
They can be found mainly in cereals but also in vegetables, medicinal plants, and flaxseed. In mammalian intestines, plant lignans are metabolized by bacteria to enterodiol and enterolactone. Many plant lignans have demonstrated antiestrogenic, antiviral, antioxidant, and antimitotic effects (10). Some experimental data on the antiproliferative effects of lignans have been accumulated, and efforts in this field are still of interest.

The richest known source of plant lignans is flaxseed. The seeds of *Linum usitatissimum* contain high amounts of plant lignan, secoisolariciresinol diglucoside, and minor amounts of other lignans. The most common plant lignans are mataresinol, secoisolariciresinol diglucoside, lariciresinol, and pinoresinol (11). Their chemical structures and the structures of other common lignans are shown in Scheme 1.

Lignans are recognized as effective anticancer agents. Preclinical *in vivo* studies have shown the ability of lignans to decrease tumor development in tumor induction animal models and to reduce the tumor microenvironment (12,13). Lignans are known to reduce chemically induced mammary and colon tumorigenesis (14). As phytoestrogens, lignans can modulate estrogen receptors (ER), which are important in the case of hormone-dependent cancers because hormones influence cancer cell division, differentiation, survival, and metastasis. Although lignans have low affinity to ER itself (15), the mechanisms of this modulating effect may include reduction of the expression of hormonal and growth factor receptors or their binding affinity (ER, progesterone receptor, epidermal growth factor receptor (EGFR), and insulin-like growth factor 1 receptor) (16), inhibition of aromatase and 17β-hydroxysteroid dehydrogenase and reduction of sex hormone synthesis (17), regulation of plasma sex hormone binding globulin levels or its binding affinity (18), modulation of matrix metalloproteinase (MMP) activity (19), and regulation of expression of cell cycle regulators and signal transductors involved in cell proliferation, survival, and migration (20-22). Moreover, lignans have antiangiogenic properties. They have been shown to inhibit estradiol-induced tumor growth and angiogenesis *in vivo* (23). Lignans can enhance apoptosis through disruption of mitochondrial membrane potential (24) and activation of the intrinsic or extrinsic apoptotic pathways (tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced Bid cleavage) (25), reduction of Bcl-2 and survivin (26), caspase-dependent cell death (24), and death receptor sensitization. Lignans can modulate cancer cell invasion and migration (27). They reduced metastasis in an animal model of melanoma (14). Lignans inhibit MMP responsible for degradation of the extracellular matrix, modulate the phosphorylation of focal adhesion kinase, steroid receptor coactivator (Src), and paxillin, and subsequently modulate their key targets and inhibit organization of the actin cytoskeleton to influence cell motility and clonogenicity (20,28). Because lignans possess multiple effects on cell metabolism, viability, migration, and survival, they are promising candidates for anticancer drugs.



Scheme 1. Chemical structure of some common lignans with established anticancer activity.

Our study aimed to evaluate the antiproliferative effects of several lignans (arctiin, enterodiol, enterolactone, pinoresinol, myrislignan, matairesinol, and honokiol) on human breast cancer cells with different hormone sensitivities and to describe the effects of the lead lignan in combination with metformin on signaling pathways. First, the activity of lignans against breast cancer cells with various levels of hormone sensitivity was analyzed. The experiments were performed on MCF7 cells whose growth is estrogen-dependent. MCF7/HT cells were derived from MCF7 cells by long-term treatment with the antiestrogen HT. MCF7/HT cells have become resistant to this drug. MDA-MB-231 and SKBR3 cells do not express ERα, and their growth is not initially regulated by estrogens. The breast cancer cells were cultured with lignans in a range of concentrations from 3.1 to 50 μM for 72 h. Honokiol was chosen as the leading compound for in-depth study.

MATERIALS AND METHODS

Cell culture and compounds

Lignans (arctiin, enterodiol, enterolactone, pinoresinol, myrislignan, matairesinol, and honokiol) were purchased from Sigma (Sigma-Aldrich, USA) and Cayman Chemical (Cayman Chemical, USA). Human breast cancer cell lines, MCF7, MDA-MB-231, and SKBR3, were purchased from American Type Culture Collection (ATCC, USA) and maintained in high-glucose (4.5 g/L) standard Dulbecco's modified eagle medium (DMEM) medium (PanEco, Russia) supplemented with 10% fetal bovine serum (FBS, HyClone, USA) at 37 °C, 5% CO_2, and 80-85% humidity (NuAire CO_2 incubator). Hydroxytamoxifen (HT) was obtained from Cayman Chemical (Cayman Chemical, USA). The HT-resistant MCF7 subline (MCF7/HT) was established from the parent MCF7 cells by long-term treatment with 5 μM HT (29).

Analysis of the antiproliferative activity of lignans

The antiproliferative activity of lignans was assessed by the MTT test. Briefly, the cells were seeded in 24-well plates (Corning, USA) in 900 μL of the medium. The lignans in 100 μL of medium were added 24 h after seeding at concentrations of 3.1, 6.25, 12.5, 25, and 50 μM. Non-treated cells incubated with dimethylsulfoxide (DMSO) were used as a control. The cells were cultivated for 72 h, then the medium was removed, and the MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) dissolved in the medium was added to each well at the final concentration of 0.2 mg/mL and incubated for 1 h. Then, the medium was removed, and MTT formazan purple crystals were dissolved in DMSO (300 μL per well). Absorbance was measured at 571 nm using a MultiScan reader (ThermoFisher, USA). The half-maximal inhibitory concentrations (IC_{50}) were determined using GraphPad Prism (GraphPad, USA).

Evaluation of the effect of honokiol and metformin on signaling pathway proteins using immunoblotting

Immunoblotting with modifications was performed as described earlier (30). Briefly, the MCF7 and MCF7/HT cells were seeded on Petri dishes (Corning, USA) in 5 mL of standard DMEM medium and treated with 5 μM honokiol, with 2 mM metformin, or with a combination of honokiol and metformin for 72 h. Non-treated MCF7 and MCF7/HT were used as a control. At the 80% monolayer stage, cells were lysed in 150 μL of buffer: 50 mM Tris-HCl at pH 7.4, 1% Igepal CA-630, 150 mM NaCl, 1 mM ethylenediamine tetraacetate, 1 mM dithiothreitol, 1 μg/mL aprotinin, leupeptin, and pepstatin, 1 mM sodium fluoride, and sodium orthovanadate. Samples were then kept on ice for 20 min before centrifugation (10,000 *g*, 10 min, 4 °C). Total protein content was determined by the Bradford method and used to standardize loading.

Cell lysates (40 μg of protein) were separated by 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 10% polyacrylamide gel under reducing conditions and transferred to a nitrocellulose membrane (Santa Cruz, USA). The membranes were treated with 5% nonfat milk (AppliChem, Germany) solution in TBS buffer (20 mM Tris, 500 mM NaCl, pH 7.5)

with 0.1% Tween 20 to prevent non-specific absorption and then incubated with primary antibodies overnight at +4 °C.

Primary antibodies to ERα, phosphorylated S6 kinase (p-S6K), S6K, Bcl-2, cleaved poly (ADP-ribose) polymerase (PARP), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology, USA) were used. The antibodies against GAPDH (Cell Signaling Technology, USA) were used to standardize loading. Detection was performed using secondary antibodies to rabbit Ig conjugated with horseradish peroxidase (Jackson ImmunoResearch, USA), enhanced chemiluminescence (ECL) reagent, and an ImageQuant LAS 4000 imager for chemiluminescence (GE Healthcare, USA), as described in Mruk and Cheng's protocol (31).

Statistical analysis

All experiments were performed in three replicates. Statistical analysis was performed using Microsoft Excel. The results of IC_{50} values are shown as the mean value ± standard deviation (SD). *P*-values less than 0.05 were considered statistically significant.

RESULTS

Antiproliferative activity of lignans toward breast cancer cell lines

The antiproliferative effect of lignans was evaluated by the MTT assay. As shown in Fig. 1A, although, arctiin, matairesinol, pinoresinol, enterolactone, enterodiol, and myrislignan did not cause any significant antiproliferative effects against hormone-dependent MCF7 cells, honokiol considerably decreased cells' viability concentration-dependently. These lignans suppressed MCF7 cell growth by no more than 30%. Honokiol was much more active; at a concentration of approximately 20 μM, it caused a 50% suppression of cell growth (Table 1).

Next, we analyzed the effect of lignans on hormone-resistant cells (Fig. 1B). Analysis of MCF7/HT cell growth revealed similar trends; arctiin, matairesinol, pinoresinol, enterolactone, enterodiol, and myrislignan caused no significant effects, and the most active compound (myrislignan) inhibited cell growth within 40%. Honokiol retained its activity against MCF7/HT cells, and 50% of surviving cells were detected at a concentration of approximately 18 μM (Table 1). The antiproliferative effects of arctiin, matairesinol, pinoresinol, enterolactone, enterodiol, and myrislignan against SKBR3 cells were insignificant, more than 50% of cells were viable after incubation with indicated lignans (Fig. 2A). The activity of honokiol was higher against SKBR3 cells compared to that against MCF7 cells.

The effects of the lignans against MDA-MB-231 triple-negative cancer cells were insignificant (IC_{50} values < 50 μM), except for honokiol, which suppressed the cellular growth with an IC_{50} of approximately 17 μM (Fig. 2B, Table 1). Thus, honokiol showed the highest activity against breast cancer cells. Its antiproliferative effects were significant on hormone-dependent cancer cells (MCF7) and cells with acquired (MCF7/HT) and intrinsic (SKBR3, MDA-MB-231) hormone resistance. Cells with acquired hormonal resistance were selected for an in-depth study.

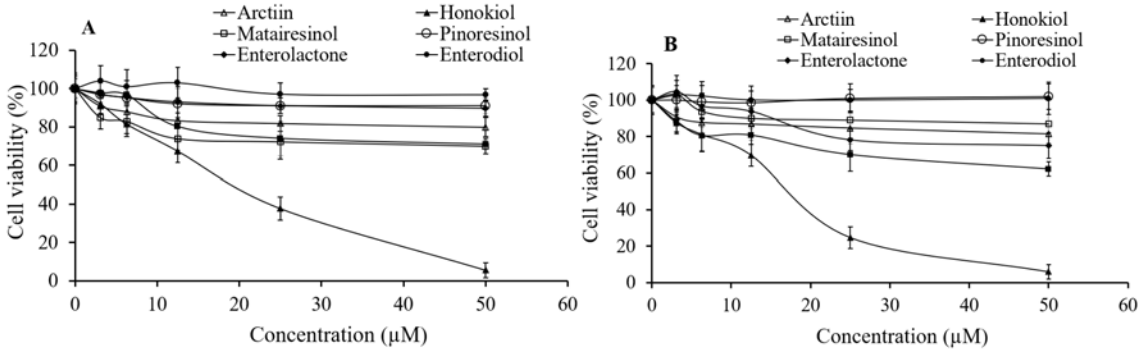


Fig. 1. Antiproliferative activity of lignans towards (A) MCF7 and (B) MCF7/HT cells. Cell viability was assessed using the MTT assay. The data represent mean ± SD, n = 3.

Table 1. The IC_{50} values of lignans in breast cancer cells. *$P < 0.05$ Indicates significant differences versus other tested lignans.

Cell lines	IC_{50} values (μM)						
	Arctiin	**Honokiol**	**Matairesinol**	**Pinoresinol**	**Enterolactone**	**Enterodiol**	**Myrislignan**
MCF7	> 50	19.7 ± 2.0 *	> 50	> 50	> 50	> 50	> 50
MCF7/HT	> 50	17.9 ± 1.8 *	> 50	> 50	> 50	> 50	> 50
SKBR3	> 50	12.1 ± 1.3 *	> 50	> 50	> 50	> 50	> 50
MDA-MB-231	> 50	17.1 ± 1.6 *	> 50	> 50	> 50	> 50	> 50

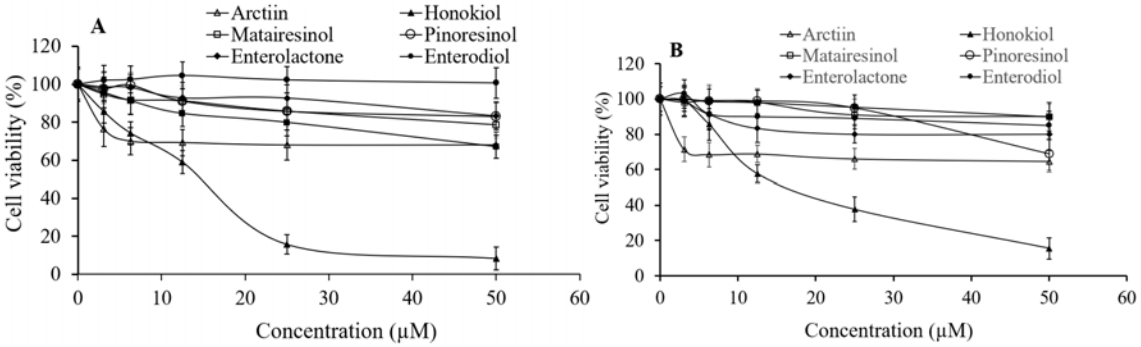


Fig. 2. Antiproliferative activity of lignans towards hormone-independent breast cancer cell lines (A) SKBR3 and (B) MDA-MB-231. Cell viability was assessed using the MTT assay. The data represent mean ± SD, n = 3.

The activity of honokiol in combination with metformin

As shown earlier (32), honokiol inhibits mitochondrial complex I. We assumed that the combination of honokiol with another inhibitor of complex I would be effective. We selected metformin as the complex I inhibitor (33). The anti-tumor effects of metformin have been extensively studied (34-36). Metformin inhibits complex I and causes significant AMPK activation in tumor cells, which results in its antiproliferative effect against several tumors (37). We previously determined that the IC_{50} value against MCF7 cells was 5.8 mM for metformin (38). In this experiment, cells were treated with compounds at concentrations below IC_{50}, and then protein expression was analyzed by immunoblotting.

Metformin at high concentrations could inhibit S6K activity. S6K is considered a potential target of honokiol. We analyzed the effect that each drug and its combination had. In MCF7 cells, S6K and p-S6K expression did not change in samples treated with honokiol, metformin, or their combination compared to control samples (Fig. 3). In MCF7/HT cells, we found a decrease in S6K and p-S6K when exposed to a combination of metformin and honokiol. ERα is considered the main driver of hormone-dependent cancer growth (15,16).

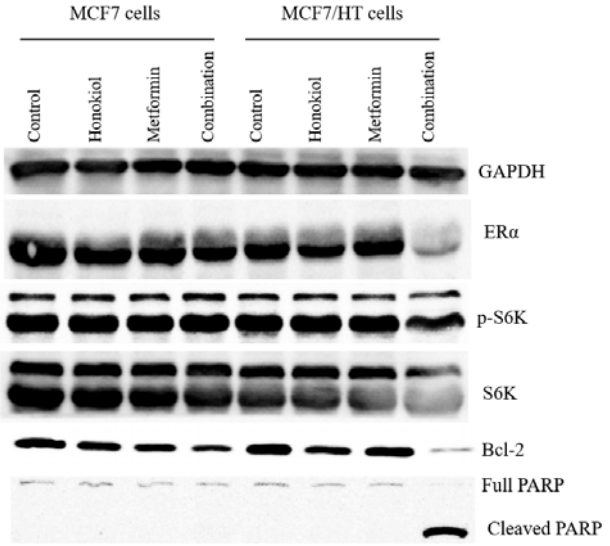


Fig. 3. The effect of honokiol, metformin and their combination on ERα, p-S6K, S6K, and Bcl-2 and cleaved PARP expression in MCF7 and MCF7/HT cells. The cells were treated with 5 μM honokiol, 2 mM metformin, or their combination for 72 h. Antibodies to GAPDH were used to normalize and control the loading of samples into the gel. GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; ERα, estrogen receptor alpha; S6K, S6 kinase; p-S6K, phosphorylated S6 kinase; Bcl-2, B-cell lymphoma 2; PARP, poly (ADP-ribose) polymerase.

Cells with acquired hormone resistance retained expression of this protein. Honokiol, metformin, and their combination had no significant effect on ERα expression in MCF7 cells. The combination of honokiol with metformin significantly reduced the expression of ERα in MCF7/HT cells. The combination of honokiol with metformin demonstrated more pronounced effects on the hormone-resistant subline compared to the parent cell line (Fig. 3).

DISCUSSION

In recent years, it has become apparent that natural compounds are a promising "basis" for the development of effective drugs (39-41). High anti-carcinogenic and anti-tumor activities for several natural products have been reported (42), and among them, lignans are of particular interest for in-depth analysis (43).

A large volume of data has been collected on different lignans, including honokiol. Honokiol is a vital bioactive biphenolic compound that belongs to the neolignan class and occurs in *Magnolia officinalis, M. obovate*, and *M. grandiflora* (44). Honokiol has a wide range of biological activities including antioxidative, anti-arrhythmic, anti-inflammatory, anticancer, neuroprotective, anti-angiogenic, anti-thrombocytic, anxiolytic, anti-nociceptive, antidepressant, anti-spasmodic activities, and others (44). Considering this, honokiol has multiple molecular targets, in particular those that are involved in carcinogenesis and chemoresistance.

The ability of honokiol to inhibit tumor cell growth has been well-studied (45-49). According to the published data, honokiol can induce death of various types of tumor cells. Most researchers have mentioned that honokiol induces cell death at sufficiently high concentrations. For example, the effect of honokiol on cell viability and colony formation in ovarian cancer cell lines was described by Lee *et al*. They found that honokiol induced a dose-dependent decrease in ovarian cancer cell growth, with IC_{50} values of approximately 50 μM for SKOV3 and Caov-3 cells (50). In our experiments, we revealed that none of the obtained IC_{50} values of honokiol for breast cancer cells exceeded 20 μM. Nevertheless, we believe that to develop effective antiproliferative agents, it is necessary to find approaches to reduce applied doses. That is why the combination of honokiol with the low-toxicity drug metformin was used. A significant decrease in the expression of ERα and the anti-apoptotic protein Bcl-2 was found in resistant cells treated with the combination of metformin and honokiol. Interestingly, approaches to synthesize honokiol derivatives with 1,3,5-triazine of metformin cyclization have been described (51); the obtained derivatives were active against tumor cells. Compound 2 (3,5′-diallyl-2′- ((4-amino-6- (dimethylamino)-1,3,5-triazin-2-yl) methoxy)- [1,1′-biphenyl]-4-ol) demonstrated a promising antiproliferative effect with IC_{50} values ranging from 5.6 to 8.7 μM, and it significantly decreased caspase-3 and Bcl-2 expression in HepG2 cells. Thus, the combination of honokiol with metformin and synthesized honokiol-metformin hybrids could significantly change apoptosis pathways and reduce the expression of Bcl-2.

During the second half of the 20th century, quite a lot of experience has been accumulated in the development of products based on natural compounds. IC_{50} values, which are determined *in vitro*, vary greatly. However, most authors are of the opinion that a crude plant extract is considered to have *in vitro* antiproliferative activity if the IC_{50} value is less than 20 μg/mL (52-54). Thus, we did not test compounds at concentrations above 50 μM (ranging from 13 to 27 μg/mL for various lignans) because evaluation at such high concentrations is not interesting from a pharmacological point of view. Lignan honokiol turned out to be the most active. The other lignans, which did not show significant effects in the performed tests, may be interesting as sensitizers of chemotherapeutic drugs.

It has been shown that honokiol induces apoptosis by increasing the expression of pro-apoptotic proteins (such as Bax and Bak) and decreasing the expression of anti-apoptotic proteins (such as Bcl-2 and Bcl-x_L). Honokiol also induces the release of mitochondrial cytochrome c to the cytosol and activation of caspase cascades that cause apoptotic cancer cell death and PARP cleavage (44). The ability of honokiol to induce apoptosis has been shown previously (55); specifically, honokiol was shown to sensitize different cancer cell types to

TRAIL-induced apoptosis and downregulate survival and c-FLIP through STAMBPL1. In another study (56), honokiol increased the activity of caspase-9, the level of cleaved caspase-3, and the activities of caspase-3 and caspase-6 in glioblastoma cells that are resistant to temozolomide. These data are in line with our results concerning the antiproliferative effect of honokiol and induction of apoptosis.

The amount of data concerning the anticancer effects of honokiol and its mechanism is significant. Honokiol inhibits EGFR expression and its phosphorylation (48,57), while it is known that in many types of tumors, there are mutations or overexpression of EGFR. In several studies, honokiol has been shown to downregulate the expression of signal transducer and activator of transcription 3 (STAT3), which are transcription factors affecting the expression of different genes involved in cell differentiation, development, metabolism, and carcinogenesis (58,59). It has also been found that honokiol inhibits the activation of mammalian target of rapamycin (mTOR) by deregulating the extracellular signal-regulated kinase (ERK) pathway, suppresses the mTOR signaling mediators 4E-BP1 and p70 S6 kinase by enhancing the expression of phosphatase and tensin homolog (PTEN), and reduces the immunoresistance of breast cancer, glioblastoma, and prostate cancer cells by downregulating the phosphoinositide 3-kinases (PI3K/Akt)/mTOR pathway (60).

One of the mechanisms of honokiol action is its effect on the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Honokiol inhibits the activation of inhibitor kinase IκB, which does not allow further phosphorylation of NF-κB and results in a reduction of IκBα degradation. Moreover, honokiol inhibits NF-κB activation by suppressing the Akt signaling pathway. Modulation of NF-κB under honokiol influence causes apoptosis and inhibition of invasion and osteoclastogenesis (49). Honokiol also exhibits effects on cell cycle proteins (61). It suppressed the expression of cyclin-B1, cell division cycle protein 2 (CDC2) and CDC25C, upregulated the expression of p-CDC2 and p-CDC25 in human gastric carcinoma and human neuroglioma cells (62), downregulated cyclin-dependent kinase (CDK)-2 and CDK-4, upregulated the cell cycle suppressors, p21 and p27, which caused cell cycle arrest at the G1 stage in human oral squamous cell carcinoma cells (46), downregulated the expression of c-Myc, and induced cell cycle arrest at the G0-G1 phase in prostate cancer cells (63).

In a bioinformatic study, the potential targets and molecular mechanisms of honokiol in breast cancer stem cells were revealed (64). Honokiol inhibits the cell cycle *via* the PI3K/Akt/mTOR pathway by upregulating PTEN and P21 and suppressing p-Akt, cyclin D/CDK4, c-Myc, Rac1, and aurora kinase B. Honokiol can effectively block vascular endothelial growth factor receptor (VEGFR) 2 and suppress angiogenesis. It was also found that honokiol reduces hypoxia-inducible factor (HIF)-induced VEGFR/VEGF activation and inhibits MMP activity and cell migration (64). Also, honokiol can induce apoptosis by upregulating BAD (the BCL2-associated agonist of cell death), caspase-9, caspase-3, and caspase-8. Honokiol is also considered an immunotherapeutic agent for mBCSCs because of its ability to modulate the tumor's immune environment (64).

The efficacy of the combination of honokiol with other compounds has been described in several studies, so far. For example, the combination of the mTOR inhibitor rapamycin and honokiol induced toxicity and autophagy, prolonged allograft survival, significantly inhibited post-transplantation renal tumor growth, reduced tumor expression of rubicon (a negative regulator of autophagy), and downregulated the co-inhibitory programmed death-1 ligand (65). Yi *et al.* showed that honokiol enhanced doxorubicin sensitivity by downregulating miR-188-5p in doxorubicin-resistant breast cancer cells (MCF7/ADR). It also induced apoptosis in MCF7/ADR and MDA-MB-231/ADR cells (66).

Another study indicated the synergistic antitumor action of honokiol and paclitaxel in a non-small cell lung cancer cell line (67). The abovementioned study showed that the combination of paclitaxel and honokiol had a cytotoxic effect on this cell line (sensitive to paclitaxel) and its subline, which is resistant to paclitaxel, and that this cytotoxic action was mediated by cytoplasmic vacuolation. Moreover, this effect was observed not only *in*

vitro but also *in vivo* in paclitaxel-resistant xenograft tumors. This combination induces paraptosis through ER dilation and disruption of Ca^{2+} homeostasis, which causes mitochondrial dysfunction (67).

The idea of overcoming resistance to chemotherapy with honokiol has been addressed in several studies (64,68,69). It has been indicated that honokiol in combination with osimertinib exerted antiproliferative action towards osimertinib-resistant cell lines and induced apoptosis through enhanced myeloid cell leukemia-1 reduction.

In another study, it was shown that honokiol significantly decreased the function of breast cancer resistance protein (BCRP), was verified as a substrate of BCRP, reduced the expression of BCRP, and inhibited the phosphorylation of EGFR and PI3K, which makes honokiol a promising candidate for reversing the multidrug resistance of chemotherapy (70). In another study, the researchers described an intravenous injection of honokiol at a dose of 10 mg/kg in patients with drug-resistant tumors. Honokiol was well-tolerated by the patients and gave a positive clinical response, including improved symptoms and quality of life. Honokiol was used in combination with chemotherapy (metformin in one case and gemcitabine and carboplatin in another case) (71).

As described above, the volume of evidence concerning the effective use of honokiol with different anticancer agents is considerable. In our study, we have demonstrated that honokiol in combination with metformin effectively induced apoptosis in breast cancer cells with acquired hormone resistance (MCF/HT), induced accumulation of a cleaved form of PARP, and decreased the expression of Bcl-2 and ERα compared to that in parent MCF7 cells.

The clinical significance of honokiol in the treatment and prevention of cancer has been supported by multiple studies. In a previous study, the authors have shown that honokiol inhibits urinary bladder cancer cell proliferation, survival, migration, and invasion; honokiol reduces the expression of MMP-9, CD44, Sox2, and the enhancer of zeste homolog 2, and induces the expression of tumor suppressor miR-143 (56). The same effects were observed in the animal model with T24 xenografts in which honokiol also suppressed tumor growth and stemness. Moreover, honokiol inhibited metastasis and epithelial-mesenchymal transition in these cells by downregulation of expression of SRC-3, MMP-2, Twist1, and N-cadherin, and upregulation of E-cadherin (72). Honokiol has potent anticancer effects on different colon cancer cell lines *in vitro* and *in vivo* by inducing apoptosis (73), activating caspase-independent of p53, inhibiting HIF-1α, which leads to a reduction in tumor growth (45), promoting endoplasmic reticulum stress, downregulating calreticulin causing regression of tumor progression and metastasis (74), inhibiting the Notch signaling pathway (75), decreasing the concentration of survivin, and increasing the expression of p53 (76). Honokiol has also demonstrated activity towards gastric cancer cell lines and xenograft mice models by inhibiting peritoneal dissemination and angiogenesis, dephosphorylating STAT-3, and reducing its DNA binding efficacy (77), inducing apoptosis by cleavage of glucose-regulated protein-94 by m-calpain (78), and deregulating proteins involved in tumor growth by affecting the expression of 15-lipoxygenase-1. Honokiol has shown anticancer activity *in vitro* and *in vivo* against glioblastoma (79,80), head and neck squamous cell carcinoma (48,81), renal cell carcinoma (47,82), and leukemia cell lines (83). As described above, honokiol is of scientific and clinical significance, and more studies on its effects are of utmost importance.

CONCLUSION

In this study, hormone-dependent breast cancer cell line MCF7 and cell lines with primary (SKBR3, MDA-MB-231) or acquired hormone resistance (MCF7/HT) were more sensitive to honokiol of all the tested lignans: honokiol has demonstrated significant antiproliferative activity. In combination with metformin, honokiol effectively induced apoptosis in breast cancer cells with acquired hormone resistance. Thus, honokiol is of interest as a natural compound with antiproliferative activity against breast cancers, including resistant tumors.

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Conflict of interest statements

The authors declared no conflict of interest in this study.

Authors' contributions

E.I. Mikhaevich contributed to experimental studies, data acquisition and analysis, and wrote and revised the manuscript; D. Sorokin contributed to experimental studies and data acquisition and analysis; and A.M. Scherbakov designed and supervised the study and reviewed and revised the manuscript. The finalized article was approved by all authors.

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