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ORIGINAL ARTICLE

Honokiol antagonizes doxorubicin resistance in human breast cancer via miR-188-5p/FBXW7/c-Myc pathway

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Abstract

Background Honokiol, a natural phenolic compound derived from Magnolia plants, is a promising anti-tumor compound that exerts a wide range of anti-cancer effects. Herein, we investigated the effect of honokiol on doxorubicin resistance in breast cancer.

Methods Doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines were treated with doxorubicin in the absence or presence of honokiol; then, the following tests were performed: flow cytometry for cell apoptosis, WST-1 assay for cell viability, qPCR and western blot for the expression of miR-188-5p, FBXW7, and c-Myc. MiR-188-5p mimic, miR-188-5p inhibitor, siFBXW7, and c-Myc plasmids were transfected into cancer cells to evaluate whether miR-188-5p and FBXW7/c-Myc signaling are involved in the effect of honokiol on doxorubicin resistance in breast cancer. A dual luciferase reporter system was used to study the direct interaction between miR-188-5p and FBXW7.

Results Honokiol sensitized doxorubicin-resistant breast cancer cells to doxorubicin-induced apoptosis. Mechanically, upregulation of miR-188-5p was associated with doxorubicin resistance, and honokiol enhanced doxorubicin sensitivity by downregulating miR-188-5p. *FBXW7* was confirmed to be a direct target gene of miR-188-5p. FBXW7/c-Myc signaling was involved in the chemosensitization effect of honokiol. Honokiol induced apoptosis in MCF-7/ADR and MDA-MB-231/ADR cells. However, FBXW7 silencing or c-Myc transfection resulted in resistance to the honokiol-induced apoptotic effect.

Conclusion These findings suggest that downregulation of miR-188-5p by honokiol enhances doxorubicin sensitivity through FBXW7/c-Myc signaling in human breast cancer. Our study finds an important role of miR-188-5p in the development of doxorubicin resistance in breast cancer, and enriches our understanding of the mechanism of action of honokiol in cancer therapy.

Keywords Breast cancer · Chemoresistance · Honokiol · MiR-188-5p · FBXW7 · C-myc

Background

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer-related death in women worldwide [1]. Classic chemotherapy drugs, including doxorubicin, remain the mainstay for treatment of breast cancer, especially triple-negative breast cancer [2]. However, chemotherapy resistance is a significant challenge [3]. Elucidation of the underlying mechanisms responsible for chemoresistance and the development of more effective therapeutic agents are urgently required.

C-Myc, encoded by the proto-oncogene *c-myc* at chromosome 8q24, is an important molecule involved in cell proliferation and apoptosis [4]. As a critical transcription factor, c-Myc controls the proliferation and apoptosis

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of cancer cells by regulating 10–15% of the genes in the human genome. C-Myc is overexpressed in more than 70% of human cancers. A prominent feature of invasive breast cancer is the overexpression of c-Myc in cancer cells, which is highly correlated with advanced progression and chemotherapy resistance [5].

The cellular c-Myc protein level is tightly controlled. FBXW7 (F-box with 7 tandem WD40), a crucial component of the E3 ubiquitin ligase Skp-Cullin1-F-box (SCF) complex, facilitates the ubiquitination and proteasomal degradation of many oncoproteins, including c-Myc, thus regulating cancer cell growth [6–8]. FBXW7 is considered as a potent tumor suppressor. The human FBXW7 gene maps to chromosome 4q32, a region deleted in 30% of cancers. Moreover, the loss of FBXW7 has been implicated in multiple human malignancies, including breast cancer [9–11], and clinical data show the correlation between decreased expression of FBXW7 in tumors and poor prognosis in these cancer patients. Studies have also indicated that the loss of FBXW7 may lead to the overexpression of c-Myc, acceleration of proliferation, cancer progression, and development of chemoresistance [12–14].

MicroRNAs (miRNAs) are a class of endogenous, short-sequence (about 18–25 nucleotides in length) RNA molecules that do not encode proteins. They bind to the 3′ untranslated regions (3′UTR) of target mRNAs and regulate gene expression by inducing mRNA degradation or interfering with mRNA translation [15]. By regulating oncogenes or tumor-suppressor genes, miRNAs play important roles in cancers [16, 17]. An increasing number of studies have analyzed the miRNA expression profiles in different types of cancer and their potential clinical significance [18–20]. As an oncogenic or tumor-suppressive miRNA, miR-188-5p has been reported to be either upregulated or downregulated in various types of cancer [21–25]. However, the function of miR-188-5p has not been thoroughly elucidated. In particular, no study has investigated the role of miR-188-5p in chemotherapy response and the underlying mechanism. In addition, the regulatory relationship between miR-188-5p and FBXW7/c-Myc signaling has not been reported.

Honokiol, a natural phenolic compound derived from Magnolia plants, has anti-inflammatory and anti-oxidative effects. Recent studies have found that honokiol is a promising anti-tumor compound that exerts a wide range of anti-cancer effects in vitro and in vivo. Through various pathways, such as the nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3), epidermal growth factor receptor (EGFR), mammalian target of rapamycin (mTOR), and vascular endothelial growth factor (VEGF) pathways, honokiol has been shown to effectively induce apoptosis and cell cycle arrest and to inhibit cancer cell proliferation, migration, invasion, epithelial-mesenchymal transition, and tumor angiogenesis [26–28].

In this study, we observed that miR-188-5p is upregulated in doxorubicin-resistant human breast cancer cells. MiR-188-5p regulates the cellular response to doxorubicin at least partially by targeting FBXW7/c-Myc signaling. Furthermore, we identified honokiol as an effective inhibitor of miR-188-5p. Downregulation of miR-188-5p by honokiol significantly enhances doxorubicin sensitivity. Our study finds an important role of miR-188-5p in the development of doxorubicin resistance in breast cancer, and enriches our understanding of the mechanism of action of honokiol in cancer therapy.

Materials and methods

Cell lines and reagents

Both breast cancer cell lines MCF-7 and MDA-MB-231 were from the China Center for Type Culture Collection (CCTCC). These two cell lines were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 50 units/ml penicillin and 50 μg/ml streptomycin at 37 °C under 5% CO_2. Doxorubicin-sensitive parental cell lines MCF-7 and MDA-MB-231 were gradually cultured with increasing concentrations of doxorubicin (from 0.01 to 5 μM, Sigma-Aldrich, St. Louis, MO, USA) to make them resistant to doxorubicin to obtain two doxorubicin-resistant breast cancer cell lines MCF-7/ADR and MDA-MB-231/ADR. When cells were able to survive at any given concentration of the drug, they were passaged at concentrations 1.5- to twofold higher. MCF-7/ADR and MDA-MB-231/ADR cells were maintained in medium containing 5 μM doxorubicin and passaged for 2–4 weeks in medium lacking doxorubicin prior to use. Honokiol was also purchased from Sigma-Aldrich and dissolved in dimethyl sulfoxide (DMSO). For honokiol treatment, an equivalent concentration of DMSO was used as a vehicle control.

Transfection

To study the regulation of miR-188-5p in the response of breast cancer cells to doxorubicin, miR-188-5p mimics were transfected into doxorubicin-sensitive MCF-7 and MDA-MB-231 cells, and miR-188-5p inhibitors were transfected into doxorubicin-resistant MCF-7/ADR and MDA-MB-231/ADR cells. We also transfected miR-188-5p mimics, siFBXW7, and c-Myc plasmids into MCF-7/ADR and MDA-MB-231/ADR cells to evaluate whether miR-188-5p and FBXW7/c-Myc signaling are involved in the chemosensitization effect of honokiol. MiR-188-5p mimics and inhibitors were purchased from GenePharma (Shanghai, China). SiFBXW7 and c-Myc plasmids were also designed and synthesized by GenePharma. Transfection was performed

using the Lipofectamine 2000 transfection kit (Invitrogen, CarIsbad, CA, USA).

QPCR

QPCR was used to measure the expression of miR-188-5p and FBXW7 at the mRNA level. The total RNA was isolated from cultured cells using Trizol reagent (Invitrogen), and the cDNA was synthesized using M-MLV (Invitrogen). For testing the level of miR-188-5p, the miRNAs from cultured cells were isolated using the mirVana miRNA isolation kit (Applied Biosystems, South San Francisco, CA, USA) and reverse-transcribed using the TaqMan microRNA reverse transcription kit (Applied Biosystems). Amplification was performed on an ABI Prism 7900HT platform (Applied Biosystems). MiR-188-5p was detected using the TaqMan microRNA assay kit (Applied Biosystems), and FBXW7 mRNA was detected using the SYBR Green qPCR kit (Roche Molecular Biochemicals, Mannheim, Germany). The expressions of miR-188-5p and FBXW7 mRNA were normalized to those of U6 and GAPDH, respectively, using the 2-ΔΔCt method. The primer sequences were as follows: miR-188-5p forward 5′-CAUCCCUUGCAUGGUGGAGGG-3′, miR-188-5p reverse 5′-CUCCACCAUGCAAGGGAUGUU-3′; FBXW7 forward 5′-CCACTGGGCTTGTACCATGTT-3′, FBXW7 reverse 5′-CAGATGTAATTCGGCGTCGTT-3′; U6 forward 5′-CTCGCTTCGGCAGCACA-3′, U6 reverse 5′-AACGCTTCACGAATTTGCGT-3′; GAPDH forward 5′-AGACAGCCGCATCTTCTTGT-3′, GAPDH reverse 5′-ATCCGTTCACACCGACCTTC-3’.

Western blot

The protein expression levels of FBXW7 and c-Myc were analyzed by Western blot. The following antibodies were used: anti-FBXW7 antibody (1:1000; Abcam, Cambridge, MA, USA) and anti-c-Myc antibody (1: 1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Their corresponding secondary antibodies were goat anti-rabbit (1:3000; Santa Cruz Biotechnology) and goat anti-mouse (1:3000; Santa Cruz Biotechnology) antibodies. GAPDH served as an internal control. Blots were visualized with a chemiluminescent detection system (ECL, Amersham Life Science, Buckinghamshire, England) and images were detected on a Fujifilm LAS-4000 scanner (Fujifilm, Tokyo, Japan).

Luciferase assay

Luciferase assay was performed to study the direct binding of miR-188-5p to the putative binding site in the 3′UTR of the FBXW7 mRNA. FBXW7 3′UTR was inserted to construct a pGL3-FBXW7 3′UTR wild-type plasmid, which was co-transfected with miR-188-5p mimics into MCF-7 and MDA-MB-231 cells. A pGL3-FBXW7 3′UTR mutant plasmid (containing a mutation of the complementary sites of miR-188-5p located within FBXW7 3′UTR) was also constructed as a control. The pGL3-FBXW7 3′UTR wild-type and pGL3-FBXW7 3′UTR mutant plasmids were constructed and purchased from Genecopoeia (Guangzhou, China). 24–36 h after transfection, the luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

WST-1 assay

Cell viability was detected by WST-1 assay. The cancer cells were incubated with different concentrations of doxorubicin in the absence or presence of honokiol in a 96-well plate for 24 h; then, the WST-1 reagent (25 μg/well, Roche) was added, and the plate was incubated for an additional 4 h. Finally, the OD value of each well was read using a microplate reader (BioTek Instruments, Winooski, VT, USA).

Flow cytometry

Flow cytometry was conducted to detect cell apoptosis. The cancer cells were incubated with doxorubicin in the absence or presence of honokiol for 24 h, and then apoptotic cells were stained with FITC-Annexin V and propidium iodide (BD Pharmingen, San Diego, CA, USA). Finally, the stained cells were detected on a FACScan flow cytometer (BD Biosciences, Mountain View, CA, USA).

Statistical analysis

Data were analyzed using the SPSS 22.0 statistical software. Each experiment was repeated at least three times and the data of each experiment were presented as means ± SEM. The statistical significance of between-group differences was determined using the *t*-test, and differences with a P value < 0.05 were considered statistically significant.

Results

Honokiol induces apoptosis in doxorubicin-resistant breast cancer cells

To investigate the effect of honokiol in doxorubicin-resistant human breast cancer cells, we treated doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines with doxorubicin in the absence or presence of honokiol. As shown in Fig. 1, doxorubicin induced apoptosis in MCF-7 and MDA-MB-231 cells, but not in MCF-7/ADR and MDA-MB-231/ADR cells. However,

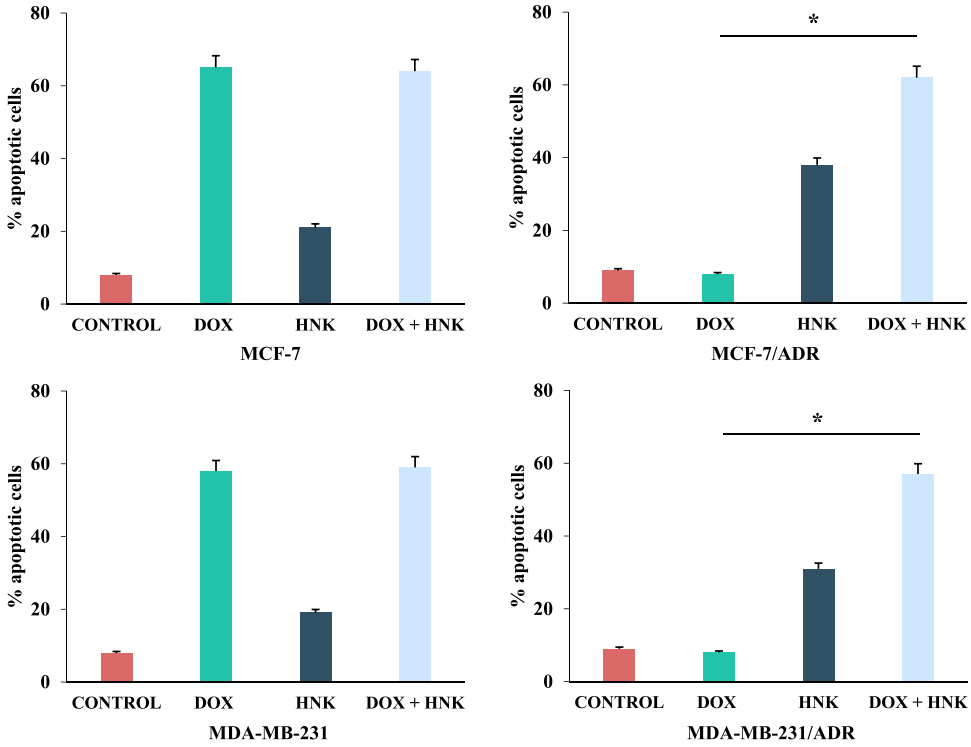

Fig. 1 Honokiol sensitizes doxorubicin-resistant breast cancer cells to doxorubicin-induced apoptosis. Doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines were treated with doxorubicin (5 μM) in the absence or presence of honokiol (20 μM) for 24 h. Cell apoptosis was determined by flow cytometry. *$P < 0.05$

the presence of honokiol significantly induced apoptosis in MCF-7/ADR and MDA-MB-231/ADR cells. The addition of honokiol restored the sensitivity of MCF7/ADR and MDA-MB-231/ADR cells to the apoptotic effect of doxorubicin. In addition, we noticed that honokiol alone showed a certain apoptotic effect in both doxorubicin-sensitive and doxorubicin-resistant cells. However, the effect was weaker than doxorubicin or honokiol plus doxorubicin combination treatment.

Honokiol sensitizes doxorubicin-resistant breast cancer cells to doxorubicin treatment

We then investigated whether honokiol could sensitize doxorubicin-resistant breast cancer cells to doxorubicin treatment. Doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines were treated with different concentrations of doxorubicin in the absence or presence of honokiol followed by WST-1 assay for cell viability. In MCF-7 and MDA-MB-231 cells, cell viability decreased distinctly along with increasing concentrations of doxorubicin, regardless of the absence or presence of honokiol. In MCF-7/ADR and MDA-MB-231/ADR cells, cell viability did not show an obvious decrease with increasing concentrations of doxorubicin. However, the presence of honokiol significantly inhibited the growth of MCF-7/ADR and MDA-MB-231/ADR cells (Fig. 2).

Upregulation of miR-188-5p is associated with doxorubicin resistance

Recent studies have demonstrated that some miRNAs are related to chemoresistance. As shown in Fig. 3a, the upregulation of miR-188-5p was observed in doxorubicin-resistant breast cancer cells. The expression level of miR-188-5p in MCF-7/ADR and MDA-MB-231/ADR cells was significantly higher than that in the parental MCF-7 and MDA-MB-231 cells, respectively.

We further evaluated whether the upregulation of miR-188-5p would reduce the sensitivity of breast cancer cells to doxorubicin. MiR-188-5p mimics were transfected into doxorubicin-sensitive MCF-7 and MDA-MB-231 cells. Results of the WST-1 assay showed that miR-188-5p mimic-transfected MCF-7 and MDA-MB-231 cells were significantly less sensitive to doxorubicin-induced cell death than miR-NC mimic-transfected cells (Fig. 3b). Therefore, the upregulation of miR-188-5p induces the resistance of breast cancer cells to doxorubicin.

On the other hand, we evaluated whether the downregulation of miR-188-5p could reverse the resistance of doxorubicin-resistant breast cancer cells to doxorubicin. MiR-188-5p inhibitors were transfected into doxorubicin-resistant MCF-7/ADR and MDA-MB-231/ADR cells. The WST-1 assay showed that miR-188-5p inhibitor-transfected MCF-7/ADR and MDA-MB-231/ADR cells were more sensitive to doxorubicin-induced cell death than

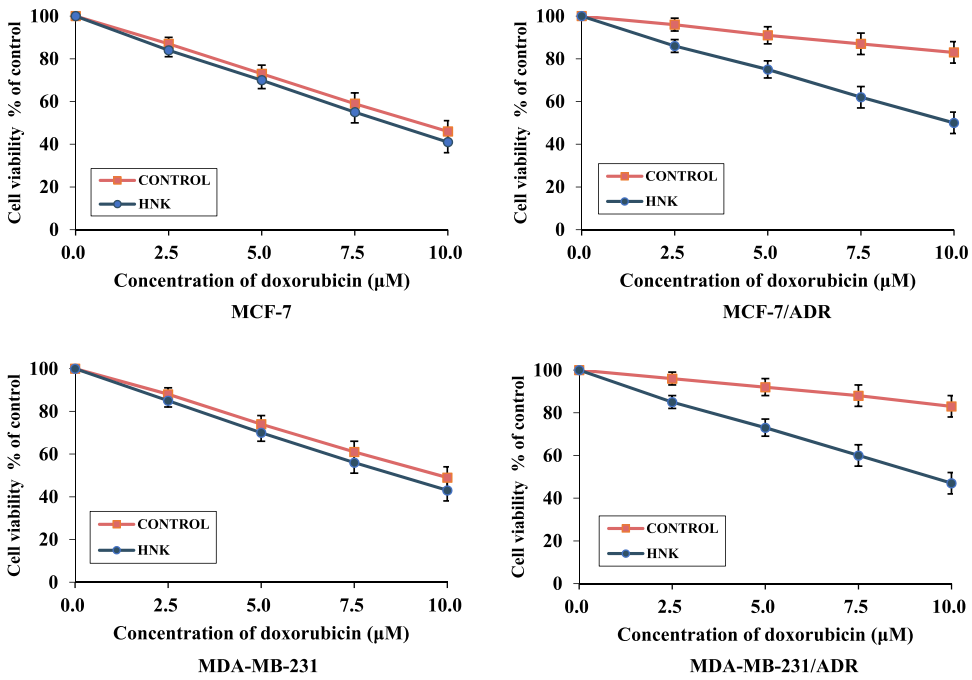

Fig. 2 Honokiol sensitizes doxorubicin-resistant breast cancer cells to doxorubicin treatment. Doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines were treated with different concentrations of doxorubicin in the absence or presence of honokiol (20 μM) for 24 h followed by WST-1 assay for cell viability

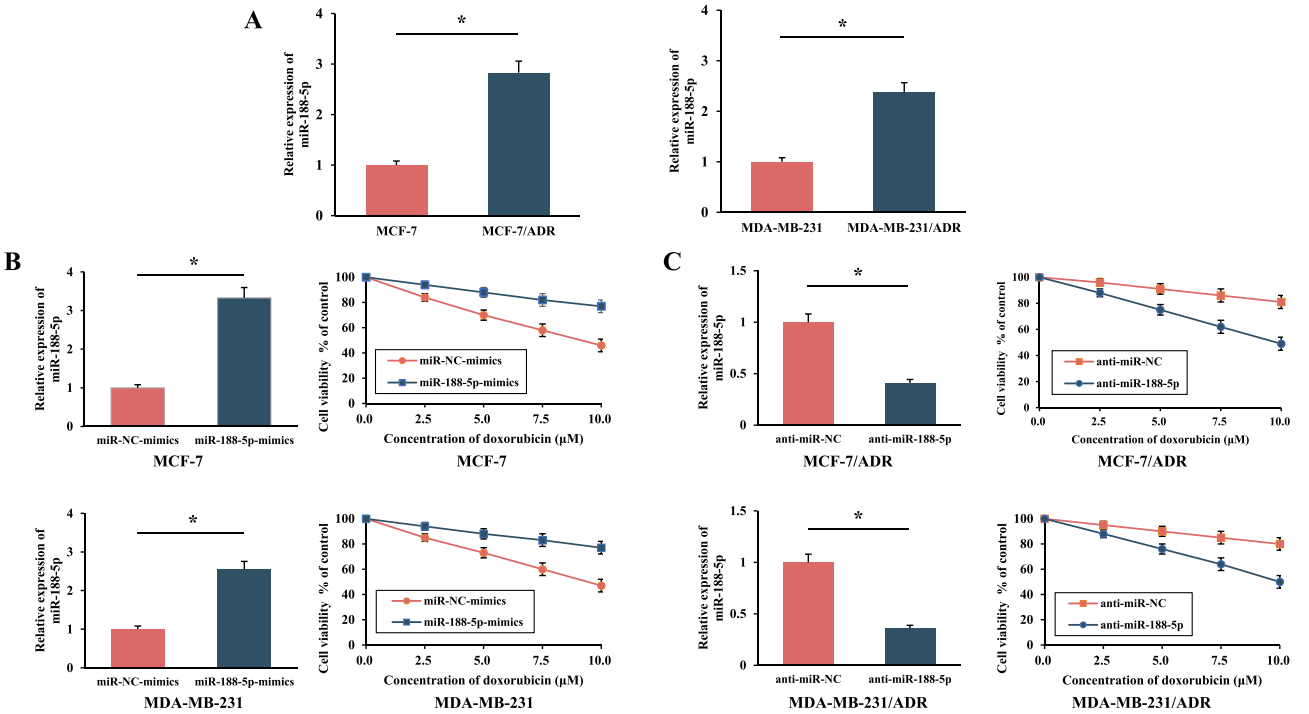

Fig. 3 Upregulation of miR-188-5p is associated with doxorubicin resistance. **a** QPCR for the expression levels of miR-188-5p in doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines. **b** MCF-7 and MDA-MB-231 cells were transfected with miR-188-5p mimics followed by qPCR for miR-188-5p expression, and then the tumor cells were treated with different concentrations of doxorubicin for 24 h followed by WST-1 assay for cell viability. **c** MCF-7/ADR and MDA-MB-231/ADR cells were transfected with miR-188-5p inhibitors followed by qPCR for miR-188-5p expression, and then the tumor cells were treated with different concentrations doxorubicin for 24 h followed by WST-1 assay for cell viability. $^{*}P < 0.05$

anti-miR-NC-transfected cells (Fig. 3c), suggesting that the downregulation of miR-188-5p reversed the resistance of doxorubicin-resistant breast cancer cells to doxorubicin.

Honokiol enhances doxorubicin sensitivity by downregulating miR-188-5p

Natural products can exert anti-cancer effects through regulating microRNAs. Thus, we speculated whether honokiol enhances doxorubicin sensitivity by modulating miR-188-5p. As shown in Fig. 4a, miR-188-5p expression was downregulated in MCF-7/ADR and MDA-MB-231/ADR cells after honokiol treatment. Further, MCF-7/ADR and MDA-MB-231/ADR cells were transfected with miR-188-5p mimics and treated with doxorubicin in the presence of honokiol followed by apoptosis assay. The presence of honokiol induced apoptosis in MCF-7/ADR and MDA-MB-231/ADR cells. However, miR-188-5p mimic transfection resulted in resistance to the honokiol-induced apoptotic effect (Fig. 4b). These results indicate that the upregulation of miR-188-5p is associated with doxorubicin resistance in human breast cancer cells and that honokiol enhances doxorubicin sensitivity by downregulating miR-188-5p.

FBXW7 is a direct target of miR-188-5p

Given that honokiol enhances doxorubicin sensitivity by downregulating miR-188-5p, we next investigated the molecular mechanism by which miR-188-5p regulates the response of breast cancer cells to doxorubicin. We studied its target genes and focused on the genes associated with breast cancer progression and the development of chemoresistance. Using the open miRNA database miRBase, *FBXW7* was identified as a candidate target gene of miR-188-5p, because miR-188-5p and FBXW7 3′UTR have complementary sites (Fig. 5a).

Western blot analysis was performed and the downregulation of FBXW7 was detected in MCF-7/ADR and MDA-MB-231/ADR cells, suggesting that it may be involved in doxorubicin resistance of human breast cancer cells. FBXW7 is a well-known E3 ubiquitin ligase of c-Myc. Accordingly, the expression levels of c-Myc protein were markedly increased in MCF-7/ADR and MDA-MB-231/ADR cells (Fig. 5b).

We then studied the correlation between miR-188-5p and FBXW7. As shown in Fig. 5c, the expression of FBXW7 mRNA was significantly decreased in miR-188-5p mimic-transfected MCF-7 and MDA-MB-231 cells, and significantly increased in miR-188-5p inhibitor-transfected MCF-7/ADR and MDA-MB-231/ADR cells.

To further confirm the possibility that miR-188-5p targets *FBXW7*, we used a dual luciferase reporter system to study the direct interaction between miR-188-5p and *FBXW7*. Upon transfection of the miR-188-5p mimic, the luciferase activity of FBXW7 was significantly decreased in MCF-7 and MDA-MB-231 cells. In contrast, miR-188-5p mimic transfection did not change the luciferase activity of mutant FBXW7 (containing mutations in the complementary sites of miR-188-5p located within FBXW7 3′UTR) (Fig. 5d), suggesting that miR-188-5p directly interacts with FBXW7 3′UTR and inhibits FBXW7 expression.

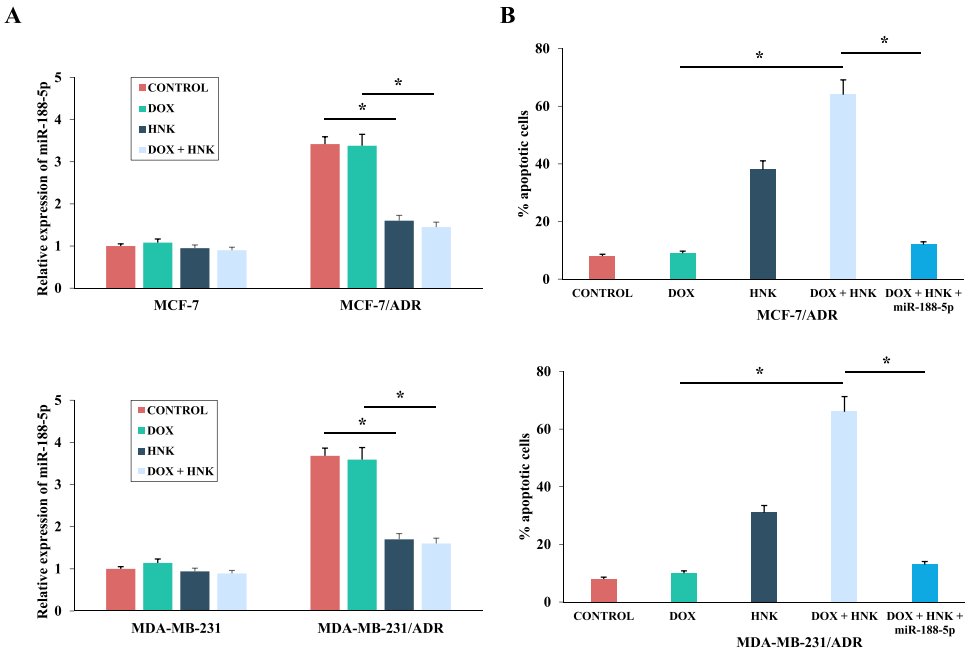

Fig. 4 Honokiol enhances doxorubicin sensitivity by downregulating miR-188-5p. **a** Doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines were treated with doxorubicin (5 μM) in the absence or presence of honokiol (20 μM) for 24 h followed by qPCR for miR-188-5p expression. **b** MCF-7/ADR and MDA-MB-231/ADR cells were transfected with miR-188-5p mimics and then treated with doxorubicin (5 μM) in the absence or presence of honokiol (20 μM) for 24 h followed by apoptosis assay. $^*P < 0.05$

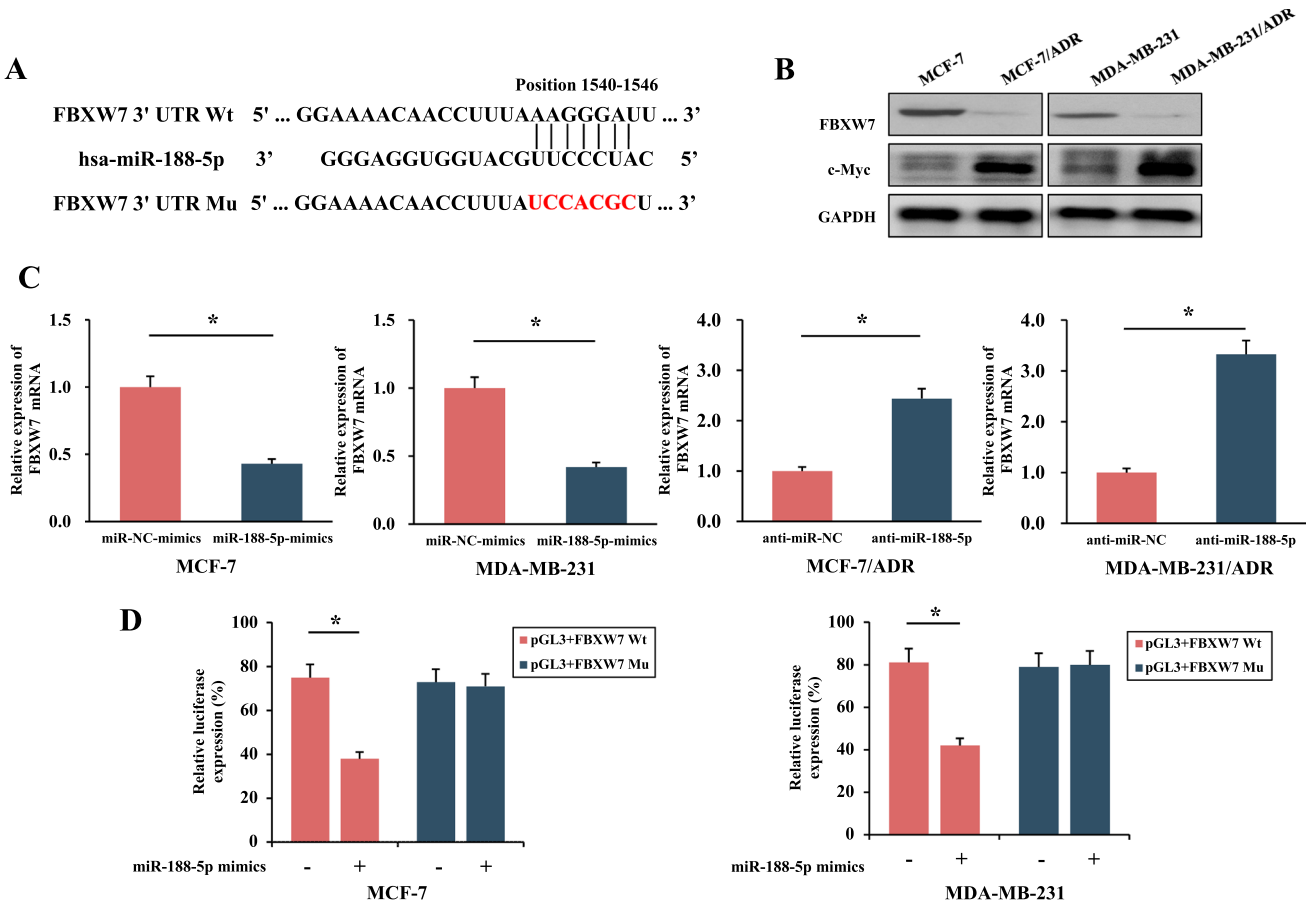

Fig. 5 MiR-188-5p directly targets FBXW7 3′UTR and inhibits FBXW7 expression. **a** The complementary sites of miR-188-5p located within FBXW7 3′UTR. **b** Western blot for FBXW7 and c-Myc protein expression in doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines. **c** QPCR for FBXW7 mRNA expression in MCF-7 and MDA-MB-231 cells transfected with miR-188-5p mimics, and MCF-7/ADR and MDA-MB-231/ADR cells transfected with miR-188-5p inhibitors. **d** Luciferase activity of wild-type but not mutant FBXW7 was significantly inhibited by miR-188-5p mimics. $^{*}P < 0.05$

FBXW7/c-Myc signaling is involved in the chemosensitization effect of honokiol

Finally, we focused on FBXW7/c-Myc signaling to further explain the chemosensitization effect of honokiol. As showed in Fig. 6a, the expression level of FBXW7 was upregulated, and accordingly, the expression level of c-Myc was downregulated in MCF-7/ADR and MDA-MB-231/ADR cells by honokiol treatment. Moreover, the presence of honokiol induced apoptosis in MCF-7/ADR and MDA-MB-231/ADR cells; however, FBXW7 silencing or c-Myc transfection resulted in resistance to the honokiol-induced apoptotic effect (Fig. 6b). Therefore, FBXW7/c-Myc signaling is involved in the chemosensitization effect of honokiol. Downregulation of miR-188-5p by honokiol significantly enhances doxorubicin sensitivity through FBXW7/c-Myc signaling.

Discussion

The key finding of this study was the important role of miR-188-5p in the development of doxorubicin resistance in breast cancer cells. Upregulation of miR-188-5p induces the resistance of breast cancer cells to doxorubicin, while the downregulation of miR-188-5p reverses the resistance of doxorubicin-resistant breast cancer cells to doxorubicin. Therefore, miR-188-5p becomes a potential target for chemosensitization in breast cancer. In addition, *FBXW7* was proven to be a target gene of miR-188-5p. MiR-188-5p regulates the cellular response to doxorubicin at least partially by targeting FBXW7/c-Myc signaling.

As an oncogenic or tumor-suppressive miRNA, miR-188-5p has been reported to be either upregulated or downregulated in various types of cancer [21–25]. In vitro experiments show that miR-188-5p plays important roles in

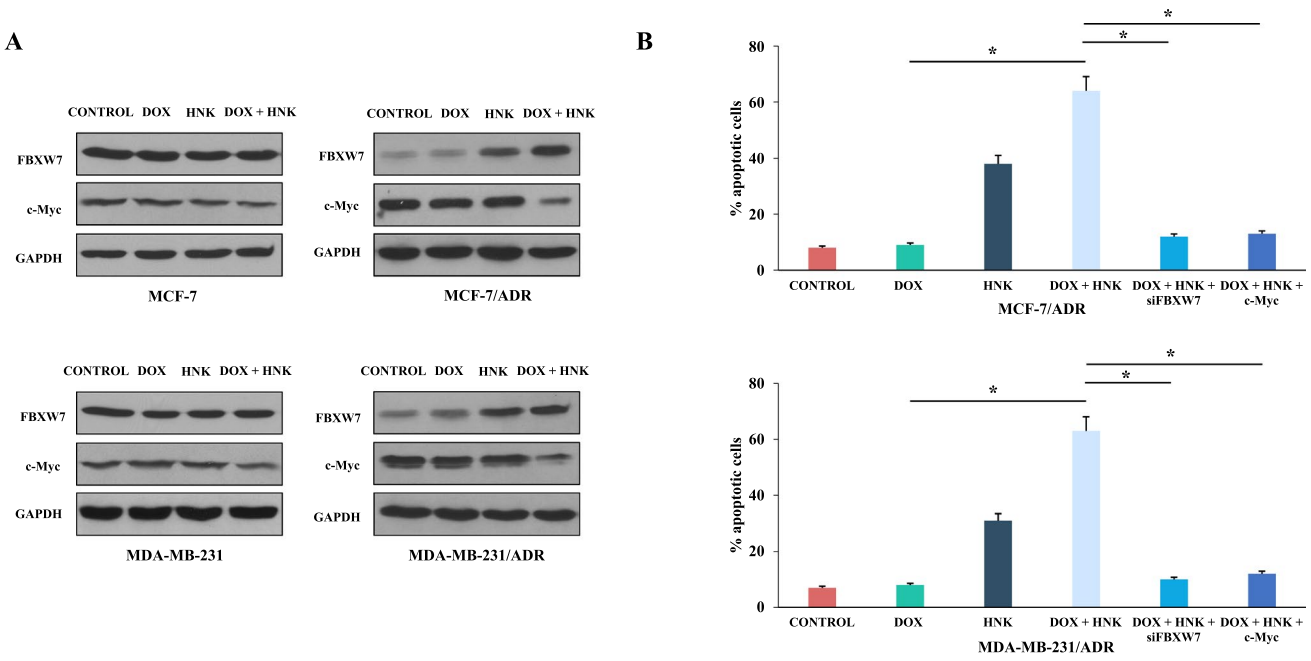

Fig. 6 FBXW7/c-Myc signaling is involved in the chemosensitization effect of honokiol. **a** Doxorubicin-sensitive (MCF-7 and MDA-MB-231) and doxorubicin-resistant (MCF-7/ADR and MDA-MB-231/ADR) breast cancer cell lines were treated with doxorubicin (5 μM) in the absence or presence of honokiol (20 μM) for 24 h followed by western blot for FBXW7 and c-Myc protein expression. **b** MCF-7/ADR and MDA-MB-231/ADR cells were transfected with siFBXW7 or c-Myc and then treated with doxorubicin (5 μM) in the absence or presence of honokiol (20 μM) for 24 h followed by apoptosis assay. $^*P < 0.05$

cancer progression and metastasis by regulating cancer cell proliferation and migration [21, 22, 24, 25]. However, limited clinical data are available on the relationship between miRNA expression patterns and breast cancer resistance, and the relationship between miR-188-5p expression and chemotherapy resistance has been rarely reported. In this study, we observed that miR-188-5p was upregulated in doxorubicin-resistant human breast cancer cells. The expression levels of miR-188-5p in MCF-7/ADR and MDA-MB-231/ADR cells were significantly higher than those in the parental MCF-7 and MDA-MB-231 cells, respectively. By regulating the expression level of miR-188-5p in cancer cells, we found that miR-188-5p regulated the response of breast cancer cells to doxorubicin in vitro. Upregulation of miR-188-5p induced the resistance of breast cancer cells to doxorubicin. However, downregulation of miR-188-5p reversed the resistance of doxorubicin-resistant breast cancer cells to doxorubicin.

Most miRNAs function through inhibiting mRNA translation of target genes [15]. One study reported that miR-188-5p targets the tumor-suppressor *phosphatase and tensin homolog (PTEN)* and further activates Wnt/β-catenin signaling [25]. Other reported targets of miR-188-5p also include *sal-like protein 4 (SALL4)*, *interleukin 6 signal transducer (IL6ST)*, *ras-related protein 2c (Rap2c)*, *zinc finger protein 91 (ZFP91)*, and *ubiquitin specific peptidase 47 (USP47)* [21, 22, 24, 29–32]. Here, we found that *FBXW7* is a novel target of miR-188-5p and is involved in the response of breast cancer cells to doxorubicin. FBXW7 is a well-known tumor suppressor. Loss of FBXW7 expression has been implicated in multiple human malignancies, including breast cancer [9]. It has been well established that the loss of FBXW7 expression increases the stability and activity of c-Myc [7, 8], thus protecting cancer cells from chemotherapy-induced apoptosis. In our miRNA database analysis, *FBXW7* was predicted as a target gene of miR-188-5p. A dual luciferase reporter system further confirmed that miR-188-5p directly interacts with FBXW7 3′UTR and inhibits FBXW7 expression. The luciferase activity of wild-type but not mutant FBXW7 (containing mutations in the complementary sites of miR-188-5p located within FBXW7 3′UTR) was significantly inhibited by miR-188-5p mimics.

Another important finding of this study was that honokiol enhances the sensitivity of breast cancer cells to doxorubicin by regulating miR-188-5p/FBXW7/c-Myc signaling. Honokiol sensitizes doxorubicin-resistant breast cancer cells to doxorubicin-induced apoptosis. Mechanically, honokiol enhances doxorubicin sensitivity by downregulating miR-188-5p. FBXW7/c-Myc signaling is also involved in the chemosensitization effect of honokiol. Honokiol induced apoptosis in MCF-7/ADR and MDA-MB-231/ADR cells; however, transfection of miR-188-5p mimics, FBXW7 silencing or c-Myc

transfection resulted in resistance to the honokiol-induced apoptotic effect. The anti-cancer activity of honokiol has been reported in a variety of cancers [26–28]. More recently, researchers have found that honokiol was able to potentiate the efficacy of other chemotherapeutic drugs [33, 34]. Various molecular targets and signaling pathways, such as the NF-κB, STAT3, EGFR, mTOR, and VEGF pathways, have been implicated in the biological effects of honokiol [26–28, 33, 34]. In addition, some studies have also reported on the regulatory effects of honokiol on miRNAs. For example, honokiol could alleviate sepsis-induced acute kidney injury in mice by modulating miR-218-5p [35]. Honokiol could suppress the cancer stem cell property, epithelial-mesenchymal transition, and metastasis of renal cancer cells by modulating miR-141 [36]. Here, we reported a new pathway, the miR-188-5p/FBXW7/c-Myc pathway, through which honokiol reverses chemoresistance. Cancer is basically a multi-factorial disease that requires the regulation of multiple targets and signaling pathways. Moreover, honokiol is pharmacologically safe [37, 38]. Therefore, honokiol is expected to be a promising anti-cancer drug.

In summary, downregulation of miR-188-5p by honokiol enhances doxorubicin sensitivity through FBXW7/c-Myc signaling in human breast cancer. Our study finds an important role of miR-188-5p in the development of doxorubicin resistance in breast cancer, and enriches our understanding of the mechanism of action of honokiol in cancer therapy. In this study, the in vitro cell experiments have achieved positive results. These results will encourage us to continue to conduct future in vivo experiments (the validation in animal models, etc.) to bring honokiol one step closer to clinical use.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest were disclosed.

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