# Curcumin reverses doxorubicin resistance via inhibition the efflux function of ABCB4 in doxorubicin-resistant breast cancer cells

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**Abstract.** Doxorubicin is one of the most widely used chemotherapy agents for the treatment of breast cancer. However, the development of doxorubicin resistance limits the long-term treatment benefits in patients with breast cancer. Curcumin, a well-known dietary polyphenol derived from the rhizomes of turmeric (*Curcuma longa*), enhances the sensitivity of breast cancer cells to chemotherapeutic agents; however, the mechanisms underlying this phenomenon remain unclear. The aim of the present study was to evaluate the effect of curcumin on chemoresistance in doxorubicin-resistant breast cancerMCF-7/DOX and MDA-MB-231/DOX cell lines. Cell Counting Kit-8, monolayer transport, western blot and ATPase activity assays were performed during the study. The results revealed that curcumin significantly enhanced the effect of doxorubicin in doxorubicin-resistant breast cancer cells. The intracellular accumulation of doxorubicin was substantially increased following curcumin treatment in doxorubicin-resistant breast cancer cells, in a manner that was inversely dependent on the activity of ATP binding cassette subfamily B member 4 (ABCB4). Treatment with a combination of curcumin and doxorubicin decreases the efflux of doxorubicin in ABCB4-overexpressing cells. Furthermore, curcumin inhibited the ATPase activity of ABCB4 without altering its protein expression. In conclusion, curcumin reversed doxorubicin resistance in human breast cancer MCF-7/DOX and MDA-MB-231/DOX cells by inhibiting the ATPase activity of ABCB4. The study highlights the promising use of curcumin as a chemosensitizer in the treatment of breast cancer.

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## Introduction

Breast cancer is the most frequent malignancy among females worldwide, and results in ~0.5 million mortalities per year (1). Chemotherapy is the standard treatment method for breast cancer. The anthracycline antibiotic Adriamycin® (doxorubicin), isolated from *Streptomyces peucetius*, is a commonly used chemotherapeutic agent for the treatment of breast carcinoma (2,3). However, development of DOX resistance is one of the main reasons for low chemotherapeutic efficiency in patients with breast cancer (4). Therefore, improved treatment strategies for doxorubicin-resistant breast cancer may improve its clinical application.

Drug resistance may be mediated by different mechanisms. One of the most important mechanisms underlying chemoresistance involves the overexpression of ATP-binding cassette (ABC) transporters. The ABC transporters area large group of proteins involved in material transportation, cellular homeostasis and chemoresistance (5). The majority of eukaryotic ABC transporters are efflux transporters that facilitate the removal of a variety of structurally distinct chemotherapy drugs from the cytoplasm into the extracellular space (5). Elevated expression of ABC transporters has been reported to be associated with drug resistance *in vivo* and *in vitro* (6,7). ABC sub-family B member 4 (ABCB4) exhibits high sequence homology (~80%) with the classic drug efflux transporter ABCB1 (8). ABCB4 functions as an efflux pump to limit the intracellular accumulation of drugs, including taxanes, anthracyclines and vinca alkaloids (9). Increasing evidence suggests that ABCB4 is overexpressed in cancer cells following exposure to anticancer drugs including doxorubicin, and may serve an important role in drug resistance (10-12). A previous study revealed that ABCB4 is overexpressed in doxorubicin-resistant breast cancer cells, reduces chemotherapy drug sensitivity and may cause drug resistance (13). Thus, inhibiting the activity of ABCB4 may be an important target for the development of therapeutic approaches to counteract doxorubicin resistance.

Previous studies explored the use of combining traditional chemotherapeutic agents with natural compounds *in vitro*, with encouraging results (14,15). Curcumin, a yellow pigment derived from *Curcuma longa*, has been used as a traditional medicine for centuries (16). It has been reported

to exhibit anti-inflammatory, antioxidant, antimicrobial and anticancer properties, and has been investigated for the treatment of postoperative inflammation, osteoarthritis, diabetes, cardiovascular disease and cancer (17-24). It suppressed the initiation and progression of breast cancer in *in vitro* and *in vivo* and models (25,26). Additionally, curcumin may increase the therapeutic efficacy of chemotherapeutic agents and overcome multiple drug resistance in cancer through inhibition of ABC transporters activity, including ABCB1, ABCG2 and ABCCs (27-31). However, studies investigating the effect of curcumin on drug resistance in breast cancer remain limited and further research may identify novel transporters involved in the chemotherapy resistance reversal of curcumin.

The present study used doxorubicin-resistant MCF-7/DOX and MDA-MB-231/DOX breast cancer cell lines to investigate the doxorubicin resistance reversal effect of curcumin and to explore its possible mechanism of action *in vitro*. The results revealed that curcumin inhibited the doxorubicin efflux function of ABCB4 without altering its protein expression, and may be one of the molecular mechanisms underlying the reversal of doxorubicin resistance. Curcumin appears to be a promising chemosensitizer and chemotherapeutic drug for breast cancer treatment.

## Materials and methods

*Cell culture*. The human breast cancer cell lines MCF-7 and MDA-MB-231, and Madin-Darby Canine Kidney (MDCKII) cells were purchased from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). MDCKII cells are an epithelial cell line of canine kidney origin that are frequently used to investigate drug transporters *in vitro*, as they exhibit low expression levels of transporter proteins and low metabolic activity (32). MCF-7 and MDCKII cells were cultured in complete DMEM (Thermo Fisher Scientific Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.). MDA-MB-231 cells were maintained in RPMI-1640 (Thermo Fisher Scientific Inc.) based culture medium supplemented with 10% FBS. The LZRS-IRES-eGFP plasmid expressing human ABCB4 was kindly donated by Professor Alfred Schinkel (Division of Molecular Oncology, The Netherlands Cancer Institute). For knockdown studies, the ABCB4 shRNA (5'-CUCAAUACGCGG CUAACAG-3'; Shanghai GenePharma Co., Ltd.) was cloned into the LZRS-IRES-eGFP vector. Doxorubicin-resistant breast cancer MCF-7/DOX and MDA-MB-231/DOX cells, MDCKII-patent (transfected with LZRS-IRES-eGFP-empty vector), MDCKII-ABCB4 (transfected with human LZRS-IRES-eGFP-ABCB4 expression vector), MCF-7/DOX and MDA-MB-231/DOX cells with ABCB4 knockdown (transfected with LZRS-IRES-eGFP-shABCB4) or overexpression (transfected with LZRS-IRES-eGFP-ABCB4 expression vector) were established in our laboratory as previously described (13). MCF-7/DOX and MDA-MB-231/DOX without transfection or treatment were used controls for ABCB4 knockdown/overexpression in the respective cell types. All the cell lines were maintained in an atmosphere containing 5% $CO_2$ at 37˚C as previously reported (13).

*Cell cytotoxicity assay*. The cytotoxicity of curcumin and the combination of curcumin (Sigma-Aldrich; Merck KGaA) and doxorubicin (Sigma-Aldrich; Merck KGaA) was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies) assay. Cells were seeded in a 96-well plate at a density of $5x10^3$ cells/well. Cells were incubated with various concentrations of curcumin (0, 2.5, 5, 10, 20, 50 and 100 $\mu$M) for 72 h. For the doxorubicin chemosensitivity assay, cells were incubated with a combination of 10 $\mu$M curcumin and varying concentrations of doxorubicin (0, 2.5, 5, 10, 20, 50 and 100 $\mu$M) for 72 h. DMSO was used as the control. Following incubation, 10 $\mu$l CCK-8 solution was added to each well, and cells were incubated for an additional 4 h. The absorbance was measured at a wave length of 570 nm using a fluorescence spectrofluorometer (cat no. F-7000; Hitachi Ltd.). $IC_{50}$ values were calculated using the cell survival data curve.

*Measurement of intracellular doxorubicin accumulation*. The intracellular accumulation of doxorubicin in cells was measured using a flow cytometer. The cells were cultured in 6-well plates at a density of $1x10^6$ per well, and were cultured to 80% confluence with or without 10 $\mu$M curcumin treatment for 72 h. The cells were subsequently treated with 30 $\mu$M doxorubicin for 4 h, and washed three times with chilled PBS. The cell-associated mean fluorescence intensity of doxorubicin was detected using FACSCalibur (Beckman Coulter, Inc.) with excitation/emission wavelengths of 485/580 nm. The results were analyzed though CyExpert 2.0 (Beckman Coulter, Inc.) software.

*Reverse transcription-quantitative PCR (RT-qPCR)*. Total RNA was extracted from the cells with TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The extracted RNA was reverse transcribed into cDNA using the Revert Aid™ H Minus First Strand cDNA Synthesis kit (Takara Bio, Inc.) according to the manufacturer's protocols. qPCR was performed using the SYBR® Green PCR Master mix (Takara Bio, Inc.). The following primer pairs were used for the qPCR: *ABCB4*, forward 5'-TGGCCCTGGTTGGAAGTAGTG-3' and reverse 5'-AGAAGGATCTTGGGGTTGCGAA-3'; and β-actin, forward 5'-GGATGCAGGAGATCACTG-3' and reverse 5'-CGATCCACACGGAGTACTT-3'. qPCR was conducted as follows: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. Relative quantification of *ABCB4* mRNA was analyzed using the $2^{-\Delta\Delta Cq}$ method relative to the β-actin expression level in each sample (33).

*Protein extraction and western blot analysis*. Total protein was extracted using radioimmunoprecipitation protein lysis buffer (Beyotime Institute of Biotechnology) and quantified using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Protein extracts (50 $\mu$g/lane) were separated by via SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Inc.). The membranes were blocked for 2 h at room temperature with 5% non-fat milk. Subsequently, the membranes were incubated with primary antibodies against ABCB4 (1:1,000; cat. no. ab24108, Abcam) or β-actin (1:5,000; cat. no. A5441, Sigma-Aldrich; Merck KGaA) at 4˚C overnight. The membranes were subsequently incubated with horseradish peroxidase-conjugated secondary antibodies

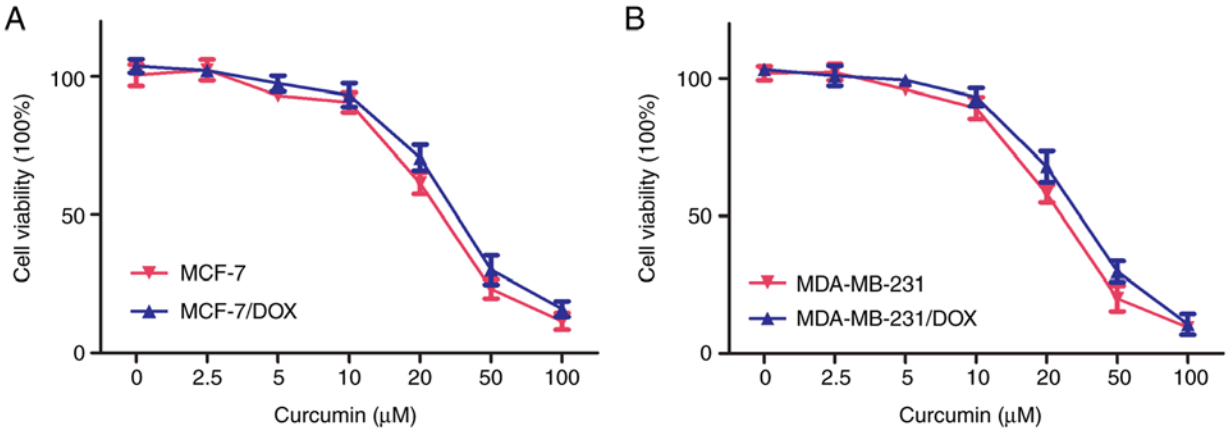


Figure 1. Curcumin inhibits proliferation of (A) MCF-7, MCF-7/DOX, and (B) MDA-MB-231 and MDA-MB-231/DOX cells. Cells were treated with varying concentrations of curcumin and cell proliferation was determined using the Cell Counting Kit-8 assay at 72 h. Each value represents the mean ± SEM from at least three independent experiments. DOX, doxorubicin-resistant.

(1:2,000; cat. no. ab97023, Abcam) for 2 h at room temperature. Protein bands were visualized using the ECL™ Prime (GE Healthcare) and a LAS-3000 imager (Fujifilm).

*MDCKII monolayer transport assay*. Cellular transport assay was performed as described previously (13). Briefly, MDCKII-parent (transfected with LZRS-IRES-eGFP-empty vector) and MDCKII-ABCB4 (transfected with LZRS-IRES-eGFP-ABCB4 expression vector) cells ($1x10^6$/insert) were seeded onto microporous polycarbonate membrane inserts (pore size, 3 $\mu$m; diameter, 24 mm; Costar; Corning, Inc.). The plates were incubated at 37˚C with 5% $CO_2$ for 3 days and subsequently used for assays. Prior to experimentation, cells were cultured in Opti-MEM (Invitrogen; Thermo Fisher Scientific, Inc.) with or without 100 $\mu$M verapamil (Sigma-Aldrich; Merck KGaA) or 10 $\mu$M curcumin for 2 h. Opti-MEM was subsequently replaced with fresh DMEM supplemented with 10% fetal calf serum (Gibco; Thermo Fisher Scientific, Inc.) and 100 nM ($^3$H) doxorubicin with or without 100 $\mu$M verapamil or 10 $\mu$M curcumin. The radioactivity of transported ($^3$H) doxorubicin was determined using a liquid scintillation counter (cat no. LS6500; Beckman Coulter, Inc.). The effect of curcumin on ABCB4-mediated doxorubicin transport was calculated as the fraction of doxorubicin recovered in the acceptor compartment vs. the fraction added in the donor compartment at the beginning of the experiment.

*ATPase activity assay*. ATPase activity was assayed to determine the functionality of the ABC transporter. MDCKII-ABCB4 cells ($1x10^6$) were incubated at 37˚C for 30 min in the presence of sodium orthovanadate (0.3 mM) in ATPase assay buffer (50 mM KCl, 5 mM sodium azide, 2 mM EDTA, 10 mM $MgCl_2$ and 1 mM dithiothreitol; pH 6.8) to measure the amount of inorganic phosphate that was released. Membrane proteins were isolated from cells using a Membrane and Cytosol Protein Extraction kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. A total of 30 $\mu$g membrane extracts were incubated with increasing concentration of curcumin (0, 5, 10, 15 and 20 $\mu$M) in the presence and absence of 5 $\mu$M verapamil. The reaction was initiated by the addition of 5 mM ATP and incubated for 20 min at 37˚C. The reaction was terminated by the addition of 0.1 ml 5% SDS solution, and the inorganic phosphate released was quantified with a colorimetric reaction at 340 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

*Statistical analysis*. Statistical analysis was performed using SPSS software (version 20; IBM Corp.). All values are presented as the mean ± SEM. One-way ANOVA and Tukey's post hoc test were performed to analyze the differences between the different groups. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*ABCB4 has a role in the effect of curcumin on doxorubicin resistance*. Prior to testing the reversal effects of curcumin, its cytotoxicity was evaluated using the CCK-8 assay. Curcumin (20 $\mu$M) exhibited moderate cytotoxic effects on parental and resistant cells following 72 h of treatment (Fig. 1). The majority (>90%) of parental and doxorubicin-resistant cells survived at a concentration of 10 $\mu$M curcumin, suggesting that curcumin is safe to be used up to a concentration of 10 $\mu$M. Thus, 10 $\mu$M curcumin was used for the subsequent assays using MCF-7/DOX and MDA-MB-231/DOX cells.

Preliminary results revealed that ABCB4 was overexpressed in MCF-7/DOX and MDA-MB-231/DOX cells, and that it results in drug resistance (12). The present study investigated the role of ABCB4 in the effects of curcumin on doxorubicin resistance. The effect of curcumin on doxorubicin cytotoxicity in control the group, ABCB4 knockdown and ABCB4 overexpression in MCF-7/DOX and MDA-MB-231/DOX cells was investigated. As presented in Table I, compared to the DMSO-treated cells, curcumin reduced the $IC_{50}$ value of doxorubicin in all the analyzed cells. The most significant reduction was observed in ABCB4 overexpressing cells, while the least significant reduction was found in ABCB4 knockdown cells. The intracellular accumulation of doxorubicin in these cells was subsequently detected in the presence or absence of curcumin. As expected, compared with DMSO-treated group, co-incubation of doxorubicin and curcumin resulted in a significant increase in intracellular concentration of

Table I. Effect of curcumin on doxorubicin sensitivity in MCF-7/DOX and MDA-MB-231/DOX cells with different ABCB4 expression levels.

| | Half maximal inhibitory concentration ($\mu$M) | |
|---|---|---|
| Treatment group | MCF-7/DOX | MDA-MB-231/DOX |
| Control+DMSO | 19.5±2.1 | 25.8±3.2 |
| Control+curcumin | 12.8±1.4[a] | 15.3±1.3[a] |
| ABCB4-knockdown+DMSO | 11.8±1.3 | 18.1±2.0 |
| ABCB4-knockdown+curcumin | 8.6±0.9[b] | 12.9±1.1[b] |
| ABCB4-overexpression+DMSO | 26.6±3.2 | 34.6±4.2 |
| ABCB4-overexpression+curcumin | 10.9±1.4[c] | 14.1±1.6[c] |

Data are presented as the mean ± SEM of at least three independent experiments. [a]P<0.05 vs. control + DMSO group; [b]P<0.05 vs. ABCB4-knoc kdown + DMSO group, [c]P<0.01 vs. ABCB4-overexpression+DMSO group. DOX, doxorubicin-resistant; ABCB4, ATP binding cassette subfamily B member 4; DMSO, dimethyl sulfoxide.

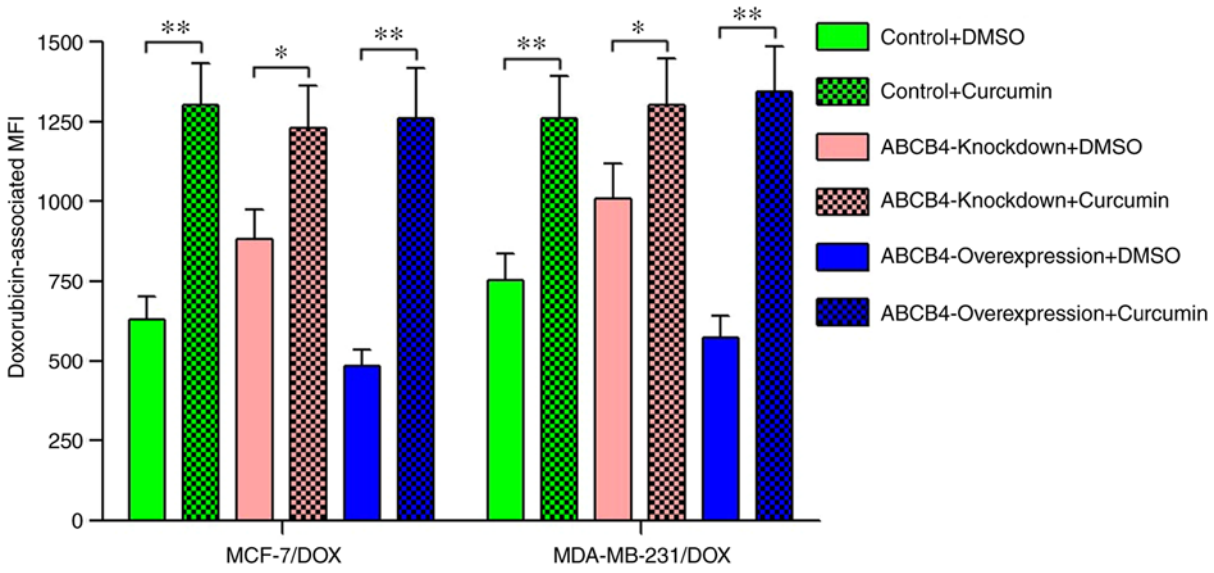


Figure 2. Effect of curcumin on the intracellular accumulation of doxorubicin in doxorubicin-resistant breast cancer cells with different ABCB4 expression levels. Cells were treated with 30 $\mu$M doxorubicin for 4 h. *P<0.05, **P<0.01. Values are presented as the mean ± SEM from at least three independent experiments. MFI, mean fluorescence intensity; ATP binding cassette subfamily B member 4; DOX, doxorubicin-resistant.

doxorubicin, and the rate of increase was proportional to the level of ABCB4 expression (Fig. 2).

*Curcumin did not alter the expression level of ABCB4*. The reversal of ABCB4-mediated doxorubicin resistance may occur by ABCB4 down regulation or inhibition of the function of ABCB4. Therefore, the effect of curcumin on the expression level of ABCB4 following treatment of the doxorubicin-resistant cell lines MCF-7/DOX and MDA-MB-231/DOX with 10 $\mu$M curcumin for 0, 24, 48 and 72 h was investigated. The results revealed that following treatment with curcumin, the mRNA and protein levels of ABCB4 in the two cell lines were not significantly altered (Fig. 3). Therefore, curcumin may inhibit the transport function of ABCB4 to reduce doxorubicin resistance.

*Curcumin inhibits ABCB4-mediated doxorubicin transport*. In order to clarify whether the reversal of doxorubicin resistance by curcumin occurs due to inhibition of the efflux function of ABCB4, an efflux assay was performed. The amount of ($^3$H)-doxorubicin that was pumped into the extracellular medium was increased in MDCKII-ABCB4 cells compared with MDCKII-parent cells (Fig. 4). Incubation with curcumin significantly reduced the efflux of ($^3$H)-doxorubicin from MDCKII-ABCB4 cells. Verapamil is an inhibitor of ABCB4 function and was used as control. Verapamil decreased the efflux of ($^3$H)-doxorubicin in MDCKII-ABCB4 cells. Curcumin and verapamil did not alter the rate of efflux of ($^3$H)-doxorubicin in the MDCKII-parent cells.

*Curcumin inhibits ABCB4 ATPase activity*. To assess whether curcumin interacts directly with ABCB4, the effect of curcumin on ABCB4 ATPase activity was investigated in crude membranes expressing ABCB4. The results revealed that curcumin slightly stimulated basal ATP hydrolysis

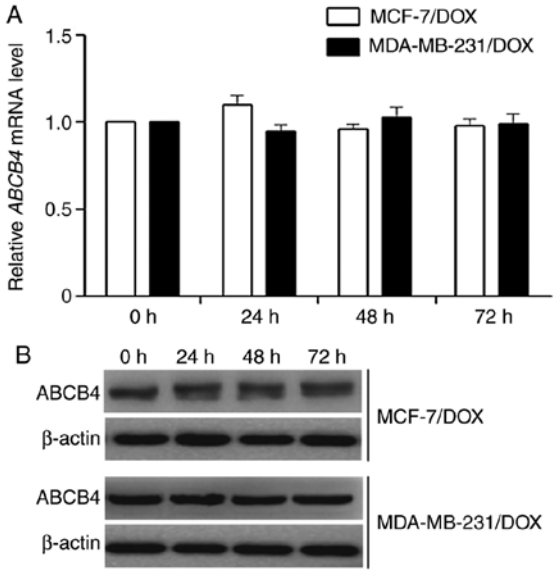


Figure 3. Effect of curcumin on the expression of ABCB4 in doxorubicin-resistant breast cancer cells. (A) mRNA and (B) protein levels of ABCB4 were detected by reverse transcription polymerase chain reaction and western blotting, respectively. Values presented are the mean ± SEM from at least three independent experiments. DOX, doxorubicin-resistant; ABCB4, ATP binding cassette subfamily B member 4.

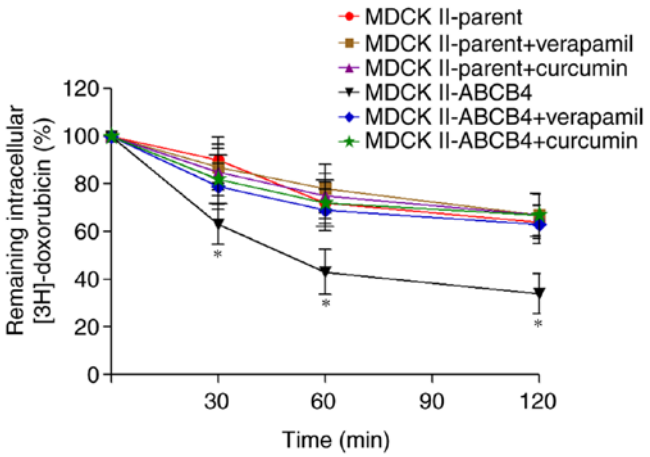


Figure 4. Inhibitory effect of curcumin on the ABCB4-mediated ($^3$H)-doxorubicin efflux. *P<0.05 vs. MDCKII-ABCB4 + curcumin. Values are mean ± SEM from at least three independent experiments. ABCB4, ATP binding cassette subfamily B member 4.

by ABCB4 at low concentrations (0.25-1 $\mu$M), but inhibited ATPase activity at higher concentrations. In addition, curcumin inhibited verapamil-stimulated ATPase activity in a concentration-dependent manner (Fig. 5).

## Discussion

Chemotherapy is the standard treatment for various types of cancer including breast cancer. However, the development of resistance to anticancer agents may complicate treatment. Overexpression of the ABC transporters has been directly implicated in resistance to a broad spectrum of chemotherapeutic drugs (34). Thus, ABC proteins may serve as a therapeutic target for overcoming drug resistance in cancer. The use of

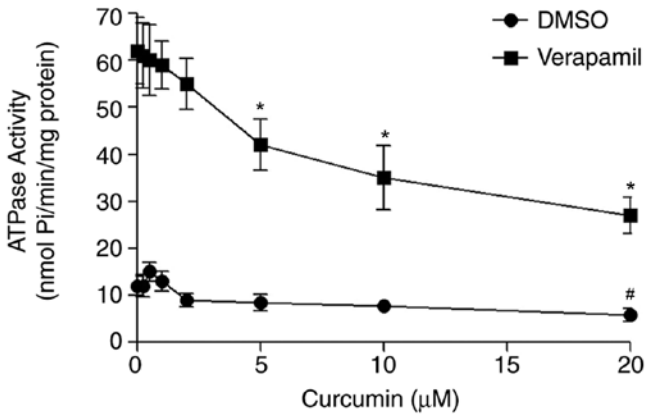


Figure 5. Effect of curcumin on basal and verapamil-stimulated ATPase activity of ATP binding cassette subfamily B member 4. Crude membranes were incubated with 5 $\mu$M verapamil or an equivalent volume of DMSO in the ATPase assay buffer. *P<0.05 vs. 0 $\mu$M curcumin (verapamil); #P<0.05 vs. 0 $\mu$M curcumin (DMSO). Values are presented as the mean ± SEM from at least three independent experiments. DMSO, dimethyl sulfoxide.

ABC transporter modulators for overcoming drug resistance has been previously investigated (35). There is an increasing interest in the use of combination therapy consisting of natural compounds and traditional chemotherapeutic agents, and this approach has produced promising results *in vitro* (14,15).

There are an increasing number of studies highlighting the anticancer and chemosensitizing abilities of curcumin on various kinds of human cancer *in vivo* and *in vitro* (36,37). A number of studies have demonstrated the chemosensitizing ability of curcumin in doxorubicin-based chemotherapy (38,39). Curcumin reverses chemoresistance by inhibiting the expression and function of ABC transporters, including ABCB1, ABCG2 and ABCCs (27-31). One of the main mechanisms underlying doxorubicin resistance is the increased expression of the ABCB1 gene (40). High homology exists between ABCB1 and ABCB4, suggesting that ABCB4 may also serve a role in doxorubicin resistance (8). Doxorubicin-resistant variants of two commonly used breast cancer cell lines, ERα-positive MCF-7 cells and ERα-negative MDA-MB-231 cells, were previously established (13). Preliminary results revealed that ABCB4 was overexpressed in doxorubicin-resistant breast cancer MCF-7/DOX and MDA-MB-231/DOX cells and was regulated by promoter methylation. Furthermore, ABCB4 was upregulated in response to chemotherapy, and may result in drug resistance (13). Inhibition of the activity of ABCB4 may be one of the mechanisms underlying the effect of curcumin on doxorubicin resistance in breast cancer cells. In the present study, a combination of curcumin and doxorubicin markedly inhibited the proliferation of MCF-7/DOX and MDA-MB-231/DOX cells, and reversed doxorubicin resistance, consistent with previous observations (41). Furthermore, the intracellular accumulation of doxorubicin was substantially increased following curcumin treatment in doxorubicin-resistant breast cells, in a manner that was inversely dependent on the activity of ATP binding cassette subfamily B member 4 (ABCB4). Based on the results obtained in the current study, curcumin may reverse resistance by inhibiting ABCB4 activity. As the

cells were exposed to curcumin for 72 h in the present study, curcumin may exert its effects by either down regulating ABCB4 expression or inhibiting ABCB4 function. However, curcumin had no effect on the expression level of ABCB4 in the present study. Although curcumin down regulates a number of genes in cancer cells through epigenetic modification, it mainly induces specific and modest effects, rather than global and drastic changes (42). A previous study suggested that curcumin-induced epigenetic changes were interpreted as secondary to the effect of curcumin on gene expression, rather than direct effects onthe epigenetic status (43). This may partly explain the negative effects in this study. However, epigenetic modifications in the control of ABCB4 expression were reported in a previous study (13).

Curcumin may exert its effect by inhibiting the ABCB4-mediated doxorubicin efflux, thus reversing doxorubicin resistance. In order to investigate this potential mechanism, anABCB4-mediated ($^3$H)-doxorubicin efflux assay was performed in the current study. The results revealed that curcumin at 10 $\mu$M inhibited the efflux of doxorubicin in MDCKII-ABCB4 cells following incubation fordifferent durations. Thus, curcumin potentially reverses ABCB4-mediated doxorubicin resistance by inhibiting the activity of ABCB4, rather than downregulating ABCB4 expression. An ATPase assay demonstrated that curcumin slightly stimulated the basal ABCB4 ATPase activity at low concentrations (0.25-1 $\mu$M), but inhibited this activity at higher concentrations. Furthermore, curcumin had an inhibitory effect on the verapamil-stimulated ABCB4 ATPase activity at all the concentrations investigated. The results obtained suggested that curcumin may inhibit ABCB4 activity, and that it may compete for the binding site with substrates including verapamil and doxorubicin. Further research is required to elucidate the mechanism underlying any potential interaction between curcumin and ABCB4.

To date, the majority of studies investigating the effects of curcumin were performed *in vitro* (14,15,17,19,20). Despite the promising *in vitro* results obtained with even low concentrations of curcumin (10 $\mu$M) in the current study, curcumin has extremely low solubility in aqueous solution and poor bioavailability (44). Therefore, the clinical potential of curcumin is currently limited. A number of drug delivery systems have been developed to increase the solubility, stability and bioavailability of curcumin, including nanoparticles (45). Previous studies revealed that novel drug delivery strategies may enhance the delivery of curcumin to tumor tissues, and increase the bioavailability *in vivo* with a good safety profile. Curcumin did not induce hemolysis even at a high concentration of 120 $\mu$M (46-48). Therefore, the development of novel drug delivery technology may allow the clinical benefits of curcumin to emerge.

In conclusion, the results obtained in the present study may have important clinical implications for patients that develop doxorubicin-resistant breast cancer. Curcumin inhibits ATPase activity, reverses doxorubicin resistance and sensitizes human doxorubicin-resistant MCF-7/DOX and MDA-MB-231/DOX breast cancer cells, suggesting that curcumin may be a novel therapeutic approach to overcome breast cancer drug resistance.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

CW, LF and JH performed the experiments and analyzed the data. YD, BW and GX interpreted the data and drafted the manuscript. LW and HZ conducted the research design, supervised the work and wrote the final version of the manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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