

## Original Article

# 3-bromopyruvate enhanced daunorubicin-induced cytotoxicity involved in monocarboxylate transporter 1 in breast cancer cells

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**Abstract:** Increasing evidence demonstrates that the hexokinase inhibitor 3-bromopyruvate (3-BrPA) induces the cell apoptotic death by inhibiting ATP generation in human cancer cells. Interestingly, some tumor cell lines are less sensitive to 3-BrPA-induced apoptosis than others. Moreover, the molecular mechanism of 3-BrPA-triggered apoptosis is unclear. In the present study, we examined the effects of 3-BrPA on the viability of the breast cancer cell lines MDA-MB-231 and MCF-7. We further investigated the potential roles of monocarboxylate transporter 1 (MCT1) in drug accumulation and efflux of breast cancer cells. Finally, we explored whether 3-BrPA enhanced daunorubicin (DNR)-induced cytotoxicity through regulation of MCT1 in breast cancer cells. MTT and colony formation assays were used to measure cell viability. Western blot analysis, flow cytometric analysis and fluorescent microscopy were used to determine the molecular mechanism of actions of MCT1 in different breast cancer cell lines. Whole-body bioluminescence imaging was used to investigate the effect of 3-BrPA *in vivo*. We found that 3-BrPA significantly inhibited cell growth and induced apoptosis in MCF-7 cell line, but not in MDA-MB-231 cells. Moreover, we observed that 3-BrPA efficiently enhanced DNR-induced cytotoxicity in MCF-7 cells by inhibiting the activity of ATP-dependent efflux pumps. We also found that MCT1 overexpression increased the efficacy of 3-BrPA in MDA-MB-231 cells. 3-BrPA markedly suppressed subcutaneous tumor growth in combination with DNR in nude mice implanted with MCF-7 cells. Lastly, our whole-body bioluminescence imaging data indicated that 3-BrPA promoted DNR accumulation in tumors. These findings collectively suggest that 3-BrPA enhanced DNR antitumor activity in breast cancer cells involved MCT-1, suggesting that inhibition of glycolysis could be an effective therapeutic approach for breast cancer treatment.

**Keywords:** Breast cancer, DNR, 3-BrPA, MCT1

## Introduction

Cancer cells have different metabolic requirements compared to most normal differentiated cells. Unlike normal cells, cancer cells primarily produce energy via glycolysis even in the presence of oxygen [1, 2]. This phenomenon, called the Warburg effect, has received increasing attention in recent years [3]. Regardless of the underlying mechanism such as mitochondrial dysfunction, hypoxia, tumor cell signaling, or metabolic enzyme expression, its final result is

the same: glycolysis-dependent production of ATP by tumor cells [4]. Because glycolysis produces less ATP than oxidative phosphorylation, tumor cells must metabolize more glucose to maintain their energy supply than normal cells [5]. Therefore, high glycolytic activity is essential for the survival and growth of tumor cells, and inhibition of glycolysis may selectively kill them via ATP deprivation.

Multidrug resistance (MDR) is a unique broad-spectrum resistance phenomenon [6]. Despite

continual introduction of new chemotherapeutic drugs and improvements in clinical chemotherapy, overcoming MDR is still one of the greatest challenges in cancer chemotherapy [7, 8]. MDR is produced by mutation (e.g., lactamase in bacteria) or overexpression (e.g., the ABC transporters in tumor cells) of proteins that antagonize drug activity [9, 10]. The ABC transporters including P-glycoprotein, are located in the cell membrane, and are highly dependent on ATP for activity [11, 12]. Inhibition of glycolysis and consequent inactivation of the ABC transporters promote intracellular retention of anti-cancer agents, thus accentuating their cytotoxic effects on malignant cells [13, 14].

3-bromopyruvate (3-BrPA) and 2-deoxyglucose (2-DG) are analogs of pyruvate and glucose [15], respectively, that competitively inhibit the activity of hexokinase (HK), the enzyme that converts glucose to glucose-6-phosphate in the glycolytic pathway [16]. 3-BrPA is currently believed to have more anti-cancer activity than 2-DG: it inhibits glycolysis at a significantly lower  $K_i$  than 2-DG and also inhibits oxidative phosphorylation, leading to metabolic oxidative stress [17, 18]. Moreover, 3-BrPA is a strong alkylating agent with similar chemical properties and structure as lactic acid, the final product of glycolysis [19, 20]. It uses lactic acid carriers to enter cells, and upon entry, is immediately pharmacologically active [21]. Additionally, 3-BrPA may be cytotoxic for tumor cells with different aerobic glycolytic phenotypes and has extensive application prospects in combination therapy [22]. However, sensitivity to 3-BrPA differs in different tumor cell lines, and drug combination problems are also worthy of attention.

In the current study, we explored the effects of 3-BrPA on the viability of the breast cancer cell lines. We also determined whether 3-BrPA enhanced daunorubicin (DNR)-induced cytotoxicity. Furthermore, we investigated the potential roles of monocarboxylate transporter 1 (MCT1) in drug accumulation and efflux of breast cancer cells. Finally, we dissect the molecular mechanism of 3-BrPA-induced anti-tumor activity in breast cancer cells.

### Material and methods

#### *Reagents and antibodies*

3-BrPA, daunorubicin (DNR), and propidium iodide were purchased from Sigma Chemical

Co. (St. Louis, MO, USA). Anti-MCT1 antibody and rabbit anti- $\beta$ -actin were obtained from Millipore (Temecula, CA, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively.

#### *Cell lines and cell culture*

Human breast cancer cell lines MDA-MB-231 and MCF-7 were obtained from American Type Culture Collection (Rockville, MD, USA). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (10 U/ml), streptomycin (100 U/ml), and HEPES (25 mM). Cells were maintained at 37°C in a 5% CO<sub>2</sub> humidified atmosphere.

#### *Cell viability assay*

Cells in 96-well plates received different concentrations of 3-BrPA or DNR 24 h after seeding at  $1 \times 10^4$  cells/well. They were refed with phosphate-buffered saline (PBS) containing 5 mg/ml MTT 24 h after addition of compounds. After 4-h incubation at 37°C, the MTT solution was removed and replaced with 150  $\mu$ l of dimethylsulfoxide/well. Absorbance was measured in a plate reader at 490 nm.

#### *Colony formation assays*

Cells in 12-well plates received different concentrations of 3-BrPA or DNR 24 h after seeding at  $5 \times 10^3$  cells/well. Approximately 1 week later, colonies were visualized under a microscope, and then stained with crystal violet and counted the cell numbers.

*Propidium iodide staining* cells in 6-well plates ( $2 \times 10^6$ /well) were cultured for 24 h to reach exponential growth. Cells received different concentrations of 3-BrPA, DNR, or both for 24 h and were stained with 600  $\mu$ l propidium iodide/well for 2 h and evaluated via flow cytometry.

#### *Intracellular ATP measurement*

Cells were seeded at  $2 \times 10^5$  cells/well in a 6-well plate for 24 h. Cellular ATP levels were determined using the Cell Titer-Glo Luminescent Cell Viability Assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Luminescence levels were measured using a microplate reader.

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### *Drug accumulation and efflux assay*

Cells were cultured in the presence or absence of 3-BrPA for 1 h followed by treatment with 6  $\mu\text{mol/L}$  DNR for 1 h. After washing, intracellular fluorescence levels were determined via flow cytometry [accumulation phase (AP)]. For determining drug efflux levels, the cells were further incubated in the presence or absence of 3-BrPA for 1 h before determination of intracellular fluorescence levels [efflux phase (EP)]. The difference in mean fluorescence intensity (MFI) between the AP and background represented the intracellular drug concentration during the AP. The difference in MFI between the EP and background represented intracellular drug concentration during EP.

### *Western blot analysis*

Cells were rinsed with ice-cold PBS and lysed in radioimmune precipitation buffer for 30 min on ice. Cell lysates were centrifuged at  $12,000 \times g$  for 30 min at  $4^{\circ}\text{C}$ . Proteins in the supernatant were separated on 15% SDS-polyacrylamide gels and subsequently transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes were incubated with primary antibody overnight at  $4^{\circ}\text{C}$  and subsequently with the corresponding secondary antibody.  $\beta$ -actin was used as the loading control.

### *Plasmid transfection*

The pCMV-HA-MCT1 plasmid and control plasmid were obtained from GenePharma. Plasmids were transfected into MDA-MB-231 cells using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were harvested 24 h after transfection for western blot analysis.

### *Mitochondrial membrane potential*

Cells in 12-well plates ( $2 \times 10^5$ ) cells/well were cultured for 24 h to reach exponential growth before treatment. Changes in mitochondrial membrane potential were evaluated using a mitochondrial membrane potential assay kit (Keygen Biotech, Nanjing, China) according to the manufacturers' instructions. Stained cells were visualized using a microscope.

### *Live cell imaging system*

Cells in 6-well plates ( $2 \times 10^5$  cells/well) were cultured for 24 h to reach exponential growth.

Cells received 3-BrPA for 1 h, followed by 6  $\mu\text{mol/L}$  DNR for 1 hour. Drug fluorescence in cells was visualized using a live cell imaging system.

### *In vivo experiments*

Five-week-old male nude mice were subcutaneously inoculated  $1 \times 10^7$  MCF-7 cells. We allowed 2 weeks for palpable subcutaneous tumors to form before administering 3-BrPA and DNR. When tumors were  $> 100 \text{ mm}^3$  in size, mice were intraperitoneally injected every day with 0.2 ml of PBS, DNR ( $0.5 \text{ mg kg}^{-1}$ ), 3-BrPA ( $8 \text{ mg kg}^{-1}$ ), or DNR plus 3-BrPA. Tumor volume and mouse body weight were measured before each injection. Tumor volume was calculated as  $\text{length} \times \text{width} \times \text{height}/2$ . All animal studies and procedures were approved by the BBMC Institutional Animal Care and Use Committee.

### *Whole-body bioluminescence imaging*

Whole-body bioluminescence imaging was performed in mouse studies to monitor the distribution of chemotherapy drugs in breast tumors. After tumors were  $> 500 \text{ mm}^3$  in size, the mice were injected with 0.2 ml of PBS, DNR ( $0.5 \text{ mg kg}^{-1}$ ) or DNR plus 3-BrPA. Mice were anesthetized with 2% isoflurane and imaged using the Caliper IVIS Lumina II system (Caliper, Hopkinton, MA, USA) 30 minutes after drug injection.

### *Statistical analysis*

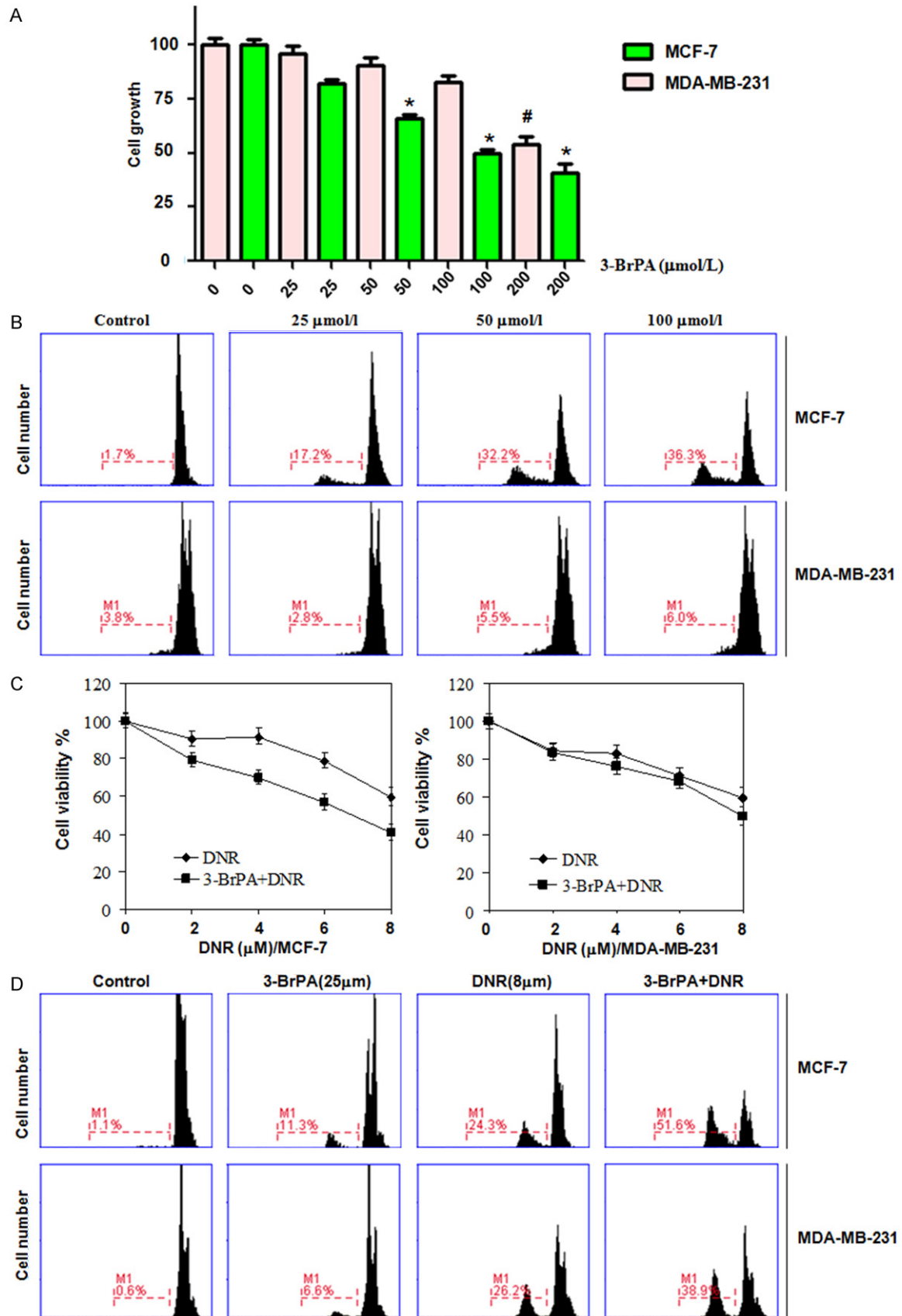
Statistical analyses were carried out using by Student's test. The symbol \*\* indicates that the values are significantly different from those of the control (\* $P < 0.05$ ).

## **Results**

### *Differential 3-BrPA-mediated cytotoxicity in breast cancer cells*

To investigate the effects of 3-BrPA on cytotoxicity, MCF-7 and MDA-MB-231 cells were treated with different concentrations of 3-BrPA for 24 h. As determined by MTT assays, 50  $\mu\text{M}$  and 100  $\mu\text{M}$  3-BrPA inhibited cell proliferation and induced apoptosis in MCF-7 cells, but not in MDA-MB-231 cells (**Figure 1A** and **1B**). Specifically,  $\text{IC}_{50}$  for 3-BrPA treatment in MCF-7 and MDA-MB-231 cells are about 100 and 240  $\mu\text{M}$ , respectively. Moreover, 200  $\mu\text{M}$  3-BrPA

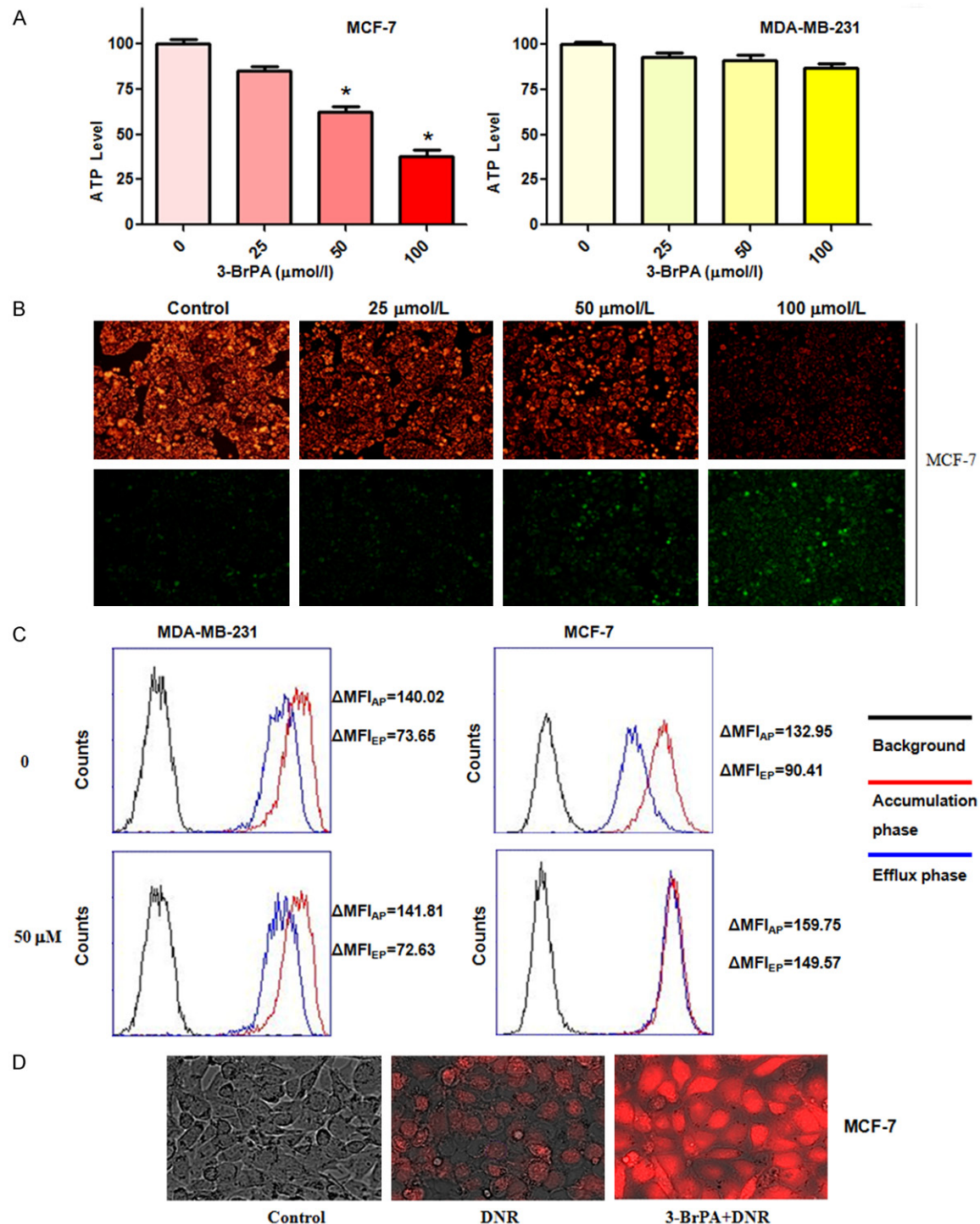
# DNR enhances 3-BrPA antitumor activity





## DNR enhances 3-BrPA antitumor activity

**Figure 1.** Differential 3-BrPA-mediated cytotoxicity in breast cancer cells. A. MCF-7 and MDA-MB-231 cells were treated with 25, 50, 100, or 200  $\mu\text{mol/L}$  3-BrPA for 24 h. Then, cell viability was analyzed by the MTT assay. Data represent mean  $\pm$  SEM of three independent experiments. B. MCF-7 and MDA-MB-231 cells were treated with various concentrations 3-BrPA (25, 50, or 100  $\mu\text{mol/L}$ ) for 24 h before being analyzed using flow cytometry. C. Cells were treated with DNR (2, 4, 6, or 8  $\mu\text{mol/L}$ ) or co-treated cells with DNR (2, 4, 6, or 8  $\mu\text{mol/L}$ ) and 3-BrPA (25  $\mu\text{mol/L}$ ) for 24 h. Cell viability was analyzed by the MTT assay. Data represent mean  $\pm$  SEM of three independent experiments. D. MCF-7 and MDA-MB-231 cells were treated with 3-BrPA (25  $\mu\text{mol/L}$ ), DNR (8  $\mu\text{mol/L}$ ), or 3-BrPA+DNR for 24 h before being analyzed using flow cytometry.



## DNR enhances 3-BrPA antitumor activity

**Figure 2.** 3-BrPA damages mitochondrial membrane potential and inhibits ATP generation in MCF-7 cells. A. Intracellular ATP levels were measured in MCF-7 and MDA-MB-231 cells treated with 25, 50, or 100  $\mu\text{mol/L}$  3-BrPA for 5 h. Data represent mean  $\pm$  SEM of three independent experiments. \* $P < 0.05$  vs. control; \*\* $P < 0.01$  vs. control. B. MCF-7 cells were treated with indicated concentrations of 3-BrPA (25, 50, or 100  $\mu\text{mol/L}$ ) for 24 h, and the mitochondrial membrane potential were assessed by JC-1 staining and fluorescence microscopy. C. MCF-7 and MDA-MB-231 cells were cultured in the presence or absence of 3-BrPA (50  $\mu\text{mol/L}$ ), and 30 minutes later, 8  $\mu\text{mol/L}$  DNR was added and intracellular fluorescence levels were analyzed by flow cytometry after incubating for 30 minutes. Then cells were further incubated in the presence or absence of 3-BrPA (50  $\mu\text{mol/L}$ ) for 1 h, and intracellular fluorescence levels were analyzed by flow cytometry. Data represent mean  $\pm$  SEM of three independent experiments. AP (accumulation phase); EP (efflux phase); MFI (mean fluorescence intensity). D. MCF-7 cells were treated with 3-BrPA (50  $\mu\text{mol/L}$ ) or 3-BrPA+DNR for 24 h, and cell fluorescence was detected by live cell imaging system.

exhibited anti-tumor activity to a greater extent in MCF-7 cells compared with MDA-MB-231 cells (**Figure 1A** and **1B**). These results show that 3-BrPA is more cytotoxic for MCF-7 cells than MDA-MB-231 cells.

### *3-BrPA sensitized MCF-7 cells to DNR-induced cell growth inhibition and apoptosis*

To determine whether 3-BrPA sensitized breast cancer cells to chemotherapeutic agents, we co-treated cells with DNR and 3-BrPA. Co-treated MCF-7 cells were less viable than cells receiving only DNR (**Figure 1C**).  $\text{IC}_{50}$  for DNR treatment was reduced from about 13.8  $\mu\text{M}$  in MCF-7 cells to 7  $\mu\text{M}$  in MCF-7 cells treated with 3-BrPA (**Figure 1C**). Co-treatment, however, did not decrease the viability of MDA-MB-231 cells (**Figure 1C**).  $\text{IC}_{50}$  of DNR treatment was about 8  $\mu\text{M}$  in MDA-MB-231 cells to 9.5  $\mu\text{M}$  in 3-BrPA treated MDA-MB-231 cells (**Figure 1C**). Furthermore, propidium iodide staining showed that 3-BrPA in combination with DNR caused to a great induction on apoptosis in MCF-7 cells compared with MDA-MB-231 cells (**Figure 1D**). These results suggest that 3-BrPA sensitized MCF-7 cells but not MDA-MB-231 cells to DNR-induced cell growth inhibition and significant apoptosis.

### *3-BrPA damaged mitochondrial membrane potential and inhibited ATP generation in MCF-7 cells*

The loss of mitochondrial membrane potential and consequent production of reactive oxygen species (ROS) are the common landmark events in early apoptosis [23]. ROS closely contact mitochondrial antioxidants and induce apoptosis by releasing cytochrome c from mitochondria. ROS also inhibit the production of ATP, which in turn further increases ROS generation and apoptosis. Thus, a vicious cycle ensues. Therefore, we detected whether 3-BrPA inhibited ATP levels in breast cancer cells. We found

that 3-BrPA decreased ATP levels in MCF-7 cells, but not in MDA-MB-231 cells (**Figure 2A**). To further assess changes in mitochondrial membrane potential, we used JC-1 as a fluorescent marker. When mitochondrial membrane potential is intact, cells fluoresce red. In contrast, when it is destroyed, cells fluoresce green. Consistently, MCF-7 cells treated with 3-BrPA showed clear damage to the mitochondrial membrane potential, appearing green after JC-1 staining (**Figure 2B**). Taken together, 3-BrPA damaged mitochondrial membrane potential and decreased ATP generation in MCF-7 cells.

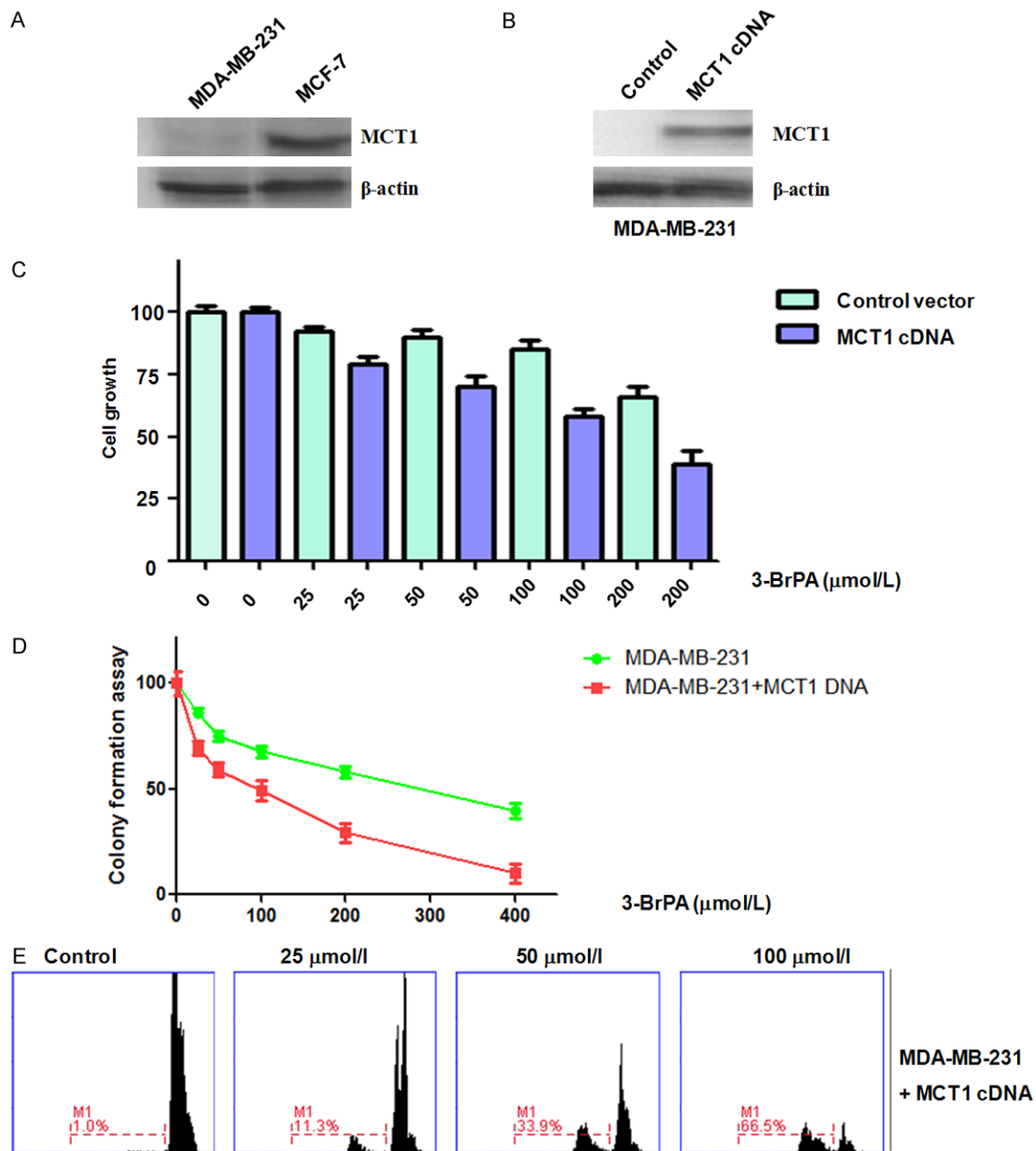
### *3-BrPA enhances DNR accumulation and efflux in MCF-7 breast cancer cells*

The anticancer activity of 3-BrPA requires depletion of the cellular ATP pool, as does ablation of MDR because of the ATP dependence of the ABC transporters [24]. To assess whether 3-BrPA could enhance DNR accumulation due to reduced ATP production, the effects of glycolysis inhibition on DNR accumulation and efflux were conducted in breast cancer cells. As determined by monitoring MFI via flow cytometry, 3-BrPA markedly increased DNR levels in MCF-7 cells (from 90.41 to 149.57), but not in MDA-MB-231 cells (**Figure 2C**). A similar result was obtained using a live imaging system. 3-BrPA increased DNR levels in MCF-7 cells (**Figure 2D**), but not in MDA-MB-231 cells (**Figure 4B**). This pattern is consistent with that seen in the viability (**Figure 1C**). Our results suggest that 3-BrPA enhanced DNR accumulation and efflux partly through reducing ATP levels in MCF-7 cells.

### *Overexpression of MCT1 enhanced 3-BrPA-induced cell growth inhibition and apoptosis*

It has been known that monocarboxylate transporter 1 (MCT1) is a potential marker for 3-BrPA

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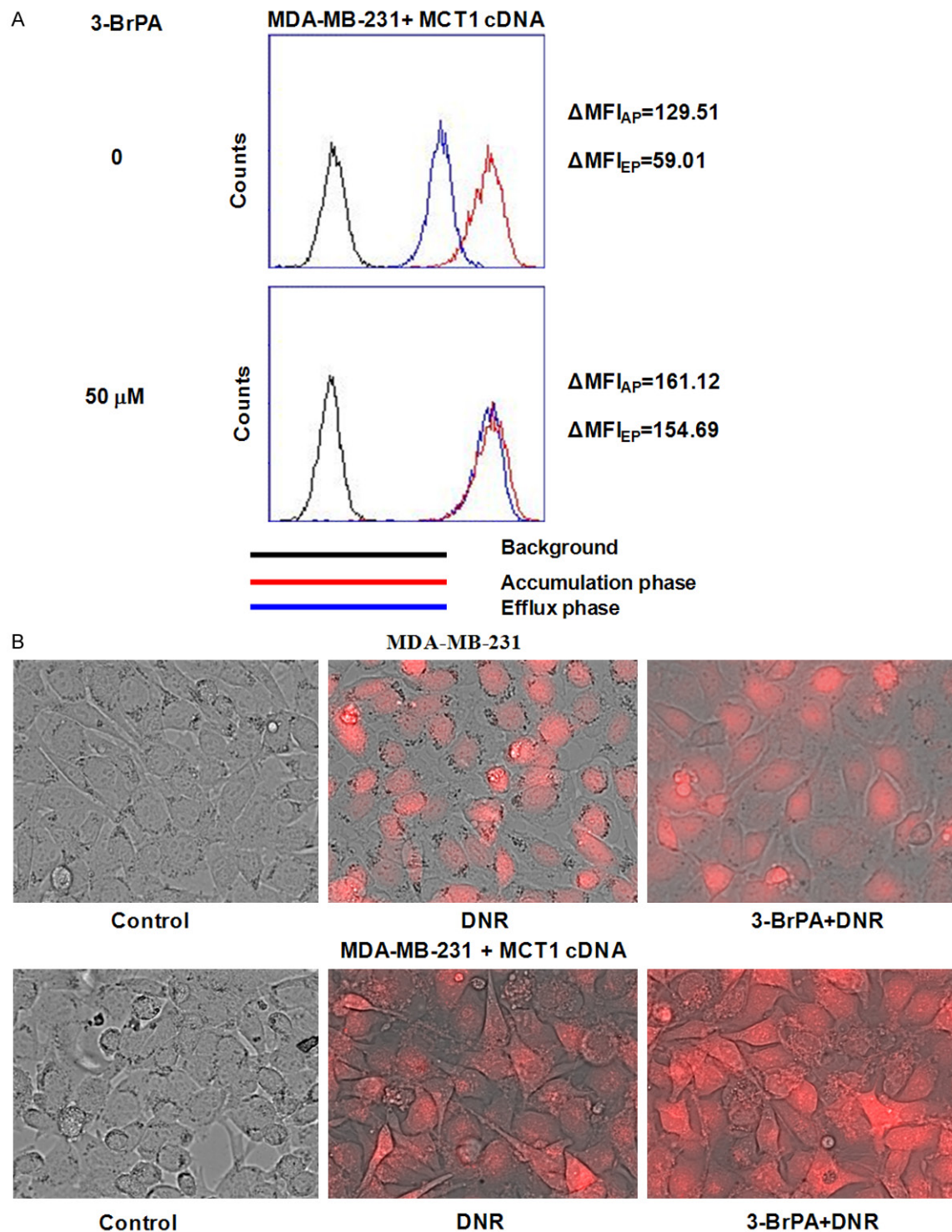


**Figure 3.** Overexpression of MCT1 enhances 3-BrPA-induced apoptosis in MDA-MB-231 cells. **A.** MDA-MB-231 and MCF-7 cells were exposed to 100 μmol/L 3-BrPA for 24 h. At the end of the treatment, the total cell lysates were prepared, and an equal amount of protein was separated by SDS-PAGE, followed by western blotting. The membranes were probed with MCT1. **B.** MDA-MB-231 cells were transfected with the control or MCT1 cDNA. After 24 h, the whole-cell lysates were subjected to western blot analysis. **C-E.** MDA-MB-231 cells were transfected with the control or MCT1 cDNA. After 24 h, the cells were cultured with 3-BrPA (25, 50, 100, 200 μmol/L) for 24 h. Then cell viability, colony formation and apoptosis were investigated. Data represent mean ± SEM of three independent experiments.

sensitivity in human cancer cells [25]. To identify whether MCT-1 is involved in 3-BrPA-induced cell growth inhibition in breast cancer cells, we examined the expression of MCT1 in the two breast cancer cell lines. We observed high expression of MCT1 in MCF-7 cells and

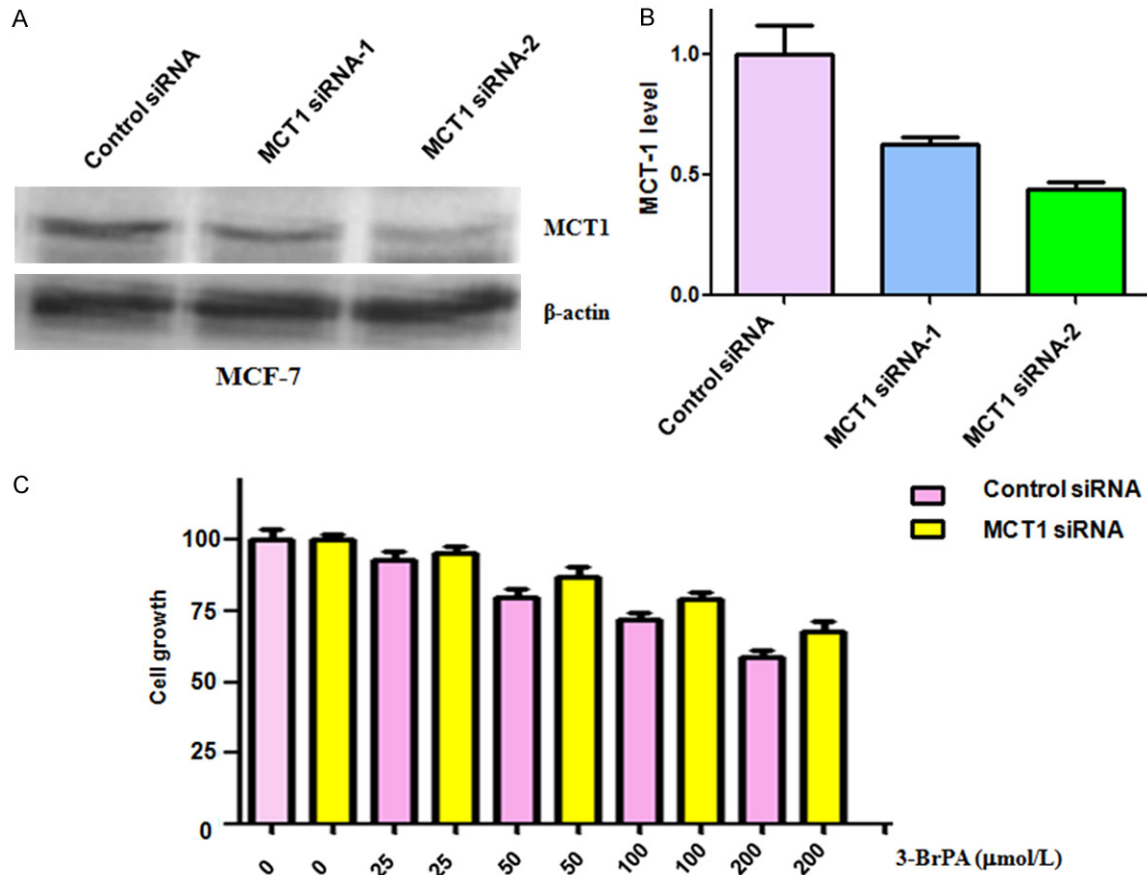
undetectable level of MCT1 in MDA-MB-231 cells (Figure 3A). To further explore the function of MCT1 in 3-BrPA sensitivity, MDA-MB-231 cells were transfected with MCT1 cDNA. Overexpression efficiency was confirmed by western blotting and we found higher expression of





**Figure 4.** Overexpression of MCT1 enhances DNR accumulation and efflux in MDA-MB-231 cells. **A.** MDA-MB-231 cells were transfected with the control or MCT1 cDNA. Cells were cultured in the presence or absence of 3-BrPA (50  $\mu\text{mol/L}$ ), and 30 minutes later, 8  $\mu\text{mol/L}$  DNR was added and intracellular fluorescence levels were analyzed by flow cytometry after incubating for 30 minutes. Then cells were further incubated in the presence or absence of 3-BrPA (50  $\mu\text{mol/L}$ ) for 1 h, and intracellular fluorescence levels were analyzed by flow cytometry. Data represent mean  $\pm$  SEM of three independent experiments. **B.** MDA-MB-231 cells were transfected with the control or MCT1 cDNA. After 24 h, cells were treated with 3-BrPA (50  $\mu\text{mol/L}$ ) or 3-BrPA+DNR for 24 h, and cell fluorescence was detected by live cell imaging system.





**Figure 5.** Depletion of MCT1 reduces 3-BrPA sensitivity to MCF-7 cells. A. MCF-7 cells were transfected with the control or MCT1 siRNA. After 24 h, the whole-cell lysates were subjected to western blot analysis. B. Quantitative results are illustrated for panel A. C. MCF-7 cells were transfected with the control or MCT1 cDNA. After 24 h, the cells were cultured with 3-BrPA (25, 50, 100, 200 μmol/L) for 24 h. Then cell viability was investigated by MTT assay. Data represent mean ± SEM of three independent experiments.

MCT1 in transfected cells (**Figure 2B**). Moreover, our MTT assay results showed that overexpression of MCT1 sensitized MDA-MB-231 cells to 3-BrPA, leading to cell growth inhibition (**Figure 3C**), suggesting that 3-BrPA inhibited cell growth partly involved in DNR level. In line with this, our colony formation assay revealed overexpression of MCT1 caused inhibition of cell growth (**Figure 3D**). Notably, MCT1 overexpressing cells were significantly more sensitive to 3-BrPA-induced apoptosis (**Figure 3E**), compared with control cells (**Figure 1B**).

#### *Overexpression of MCT1 enhanced DNR accumulation and efflux*

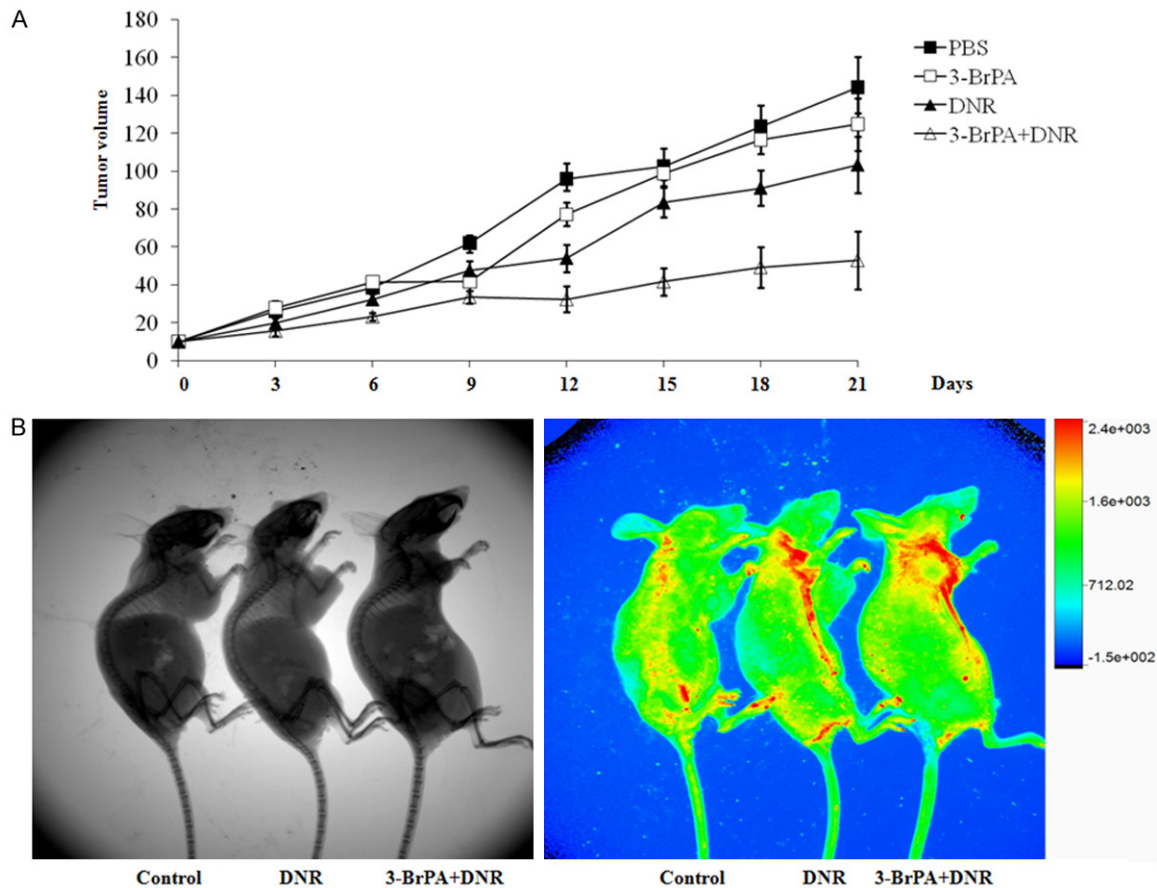
Next, we determined whether up-regulation of MCT1 affected DNR accumulation and efflux in MDA-MB-231 cells. Our flow cytometry result showed that overexpression of MCT1 signifi-

cantly increased DNR levels from MFI 59 to MFI 155 in MDA-MB-231 cells (**Figure 4A**), compared with control cells (**Figure 2C**). Consistently, over-expression of MCT1 led to DNR accumulation by a live imaging system (**Figure 4B**). More importantly, 3-BrPA increased DNR levels in MCT1 over-expressing MDA-MB-231 cells, but not in MDA-MB-231 cells (**Figure 4B**). Taken together, overexpression of MCT1 could increase DNR accumulation and efflux, leading to 3-BrPA-induced cell growth inhibition and apoptosis.

#### *Depletion of MCT1 inhibited 3-BrPA-induced cell growth*

To further define the role of MCT1 in 3-BrPA-induced cell proliferation, MCF-7 cells were transfected with MCT1 siRNA. Efficiency of MCT1 knockdown was validated by western

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**Figure 6.** 3-BrPA enhances the antitumor effects of DNR *in vivo*. **A.** MCF-7 cells were subcutaneously injected into the right flank of nude mice receiving vehicle and mice were treated with 3-BrPA (8 mg kg<sup>-1</sup>), DNR (0.5 mg kg<sup>-1</sup>) or DNR plus 3-BrPA every 2 days. Mice were sacrificed after 28 days. **B.** MCF-7 cells were subcutaneously injected into the right flank of nude mice. After tumors were > 500 mm<sup>3</sup> in size, the mice were injected with 0.2 ml of PBS, DNR (0.5 mg kg<sup>-1</sup>) or DNR plus 8 mg kg<sup>-1</sup> 3-BrPA. Mice were anesthetized with 2% isoflurane and imaged using the Caliper IVIS Lumina II system 30 minutes after drug injection.

blotting and we observed that MCT1 siRNA2 significantly inhibited the expression of MCT1 in MCF-7 cells (Figure 5A, 5B). We also detected the cell growth in MCT1 siRNA transfected MCF-7 cells treated with 3-BrPA. Our MTT assay result demonstrated that depletion of MCT1 rescued cell growth inhibition by 3-BrPA treatment in MCF-7 cells (Figure 5C), suggesting that 3-BrPA-mediated cell growth inhibition could be in part due to increased MCT levels.

### 3-BrPA enhances the antitumor effects of DNR *in vivo*

To assess the effects of 3-BrPA in combination of DNR *in vivo*, nude mice were injected subcutaneously with MCF-7 cells. Daily administration of both DNR and 3-BrPA clearly suppressed subcutaneous tumor growth compared with

DNR or 3-BrPA alone (Figure 6A). These results indicate that 3-BrPA sensitizes preformed tumors to DNR *in vivo*. We also investigated the distribution of DNR in nude mice receiving DNR alone or DNR plus 3-BrPA. The red fluorescence indicates the location of DNR in Figure 6B. It is clearly shown that DNR accumulated in tumor (Figure 6B). The results suggest that 3-BrPA could promote the accumulation of DNR in tumors.

### Discussion

Treatment of tumors is a major concern of the medical profession, and low sensitivity or resistance of tumor cells to chemotherapeutic drugs is an ongoing problem [26, 27]. Tumor cells proliferate rapidly and require the production of large amounts of proteins, nucleic acids, lipids,

and ATP [28]. Glycolysis rapidly supplies the necessary energy for these processes, as well as key precursors [29]. Cancer cells are more dependent on the glycolytic pathway for ATP generation than normal cells and eventually acquire drug resistance often because of aberrant expression of the drug-expelling ABC transporters [30]. Due to that transporters require ATP for activity [31, 32], inhibition of glycolysis may increase the concentration of chemotherapeutic agents in cancer cells [33, 34].

3-BrPA is an analog of the cellular metabolites pyruvic acid and lactic acid. As such, it effectively inhibits glycolysis and consequent ATP generation [35, 36]. Many new targets of 3-BrPA have been discovered in recent years, such as HK II, glyceraldehyde 3-phosphate dehydrogenase, and pyruvate dehydrogenase [37]. Tumor cells rely on glycolysis for ATP production and the ABC transporters require ATP for activity [38, 39]. Thus, 3-BrPA may effectively overcome MDR in tumors via glycolysis inhibition [40]. Consistently, our data show that 3-BrPA decreased ATP levels and increased DNR levels in MCF-7 cells. These observations suggest that 3-BrPA enhanced DNR accumulation partly via ATP deprivation, which reduced ABC transporter activity and consequent drug efflux from MCF-7 cells.

The present study shows that MCF-7 cells are more sensitive to 3-BrPA-induced anti-tumor activity than MDA-MB-231 cells, suggesting that 3-BrPA inhibits proliferation and induces apoptosis in some but not all tumor cell lines. Recently, a novel strategy for cancer treatment has been proposed [25]. It is based on data showing that deadly toxic substances can be transported into malignant cells via specific proteins highly expressed on the surface of cancer cells (e.g., MCT1). Consequently, cancer cells can absorb toxic substances that normal cells cannot, and this distinction can be exploited to selectively kill cancer cells. MCT1 is necessary and sufficient for 3-BrPA transport into cells, and tumor cell levels of MCT1 can be used to predict tumor cell sensitivity to 3-BrPA [25, 41]. In line with these, our results demonstrated that 3-BrPA enhanced the sensitivity of MCF-7 cells to DNR through MCT1. To support this concept, we found that MCT1 was more highly expressed in MCF-7 cells compared with MDA-MB-231 cells, in which expression was

barely detectable. Because cancer cells express more MCT1 than normal cells, tumor cells are more sensitive to 3-BrPA. Via identification of surface transporters, we should be able to determine how drugs enter cells and induce toxicity. Doing so would allow the selective treatment of tumor as compared with normal cells.

In conclusion, our results show that 3-BrPA induced cell growth inhibition and apoptosis, decreased ATP generation, and enhanced DNR accumulation in the MCF-7 breast cancer cell line. Our data further reveal that the different sensitivities of MCF-7 and MDA-MB-231 cells to 3-BrPA reflect differences in MCT1 expression levels. Therefore, 3-BrPA may be clinically useful for overcoming the resistance of tumors to chemotherapeutic agents and relevant to anti-cancer strategies attempting to target cancer metabolism. However, further investigation for molecular mechanism of 3-BrPA-triggered anti-tumor activities is warranted.

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#### Disclosure of conflict of interest

None.

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