Cancer Chemother Pharmacol (2009) 63:1103–1110
DOI 10.1007/s00280-008-0838-z

ORIGINAL ARTICLE

Role of BCRP as a biomarker for predicting resistance to 5-fluorouracil in breast cancer

Jianhui Yuan · Hui Lv · Bo Peng · Chengkun Wang · Yanhui Yu · Zhimin He

Received: 16 June 2008 / Accepted: 12 September 2008 / Published online: 27 September 2008

Abstract
Purpose Chemotherapy is not only important but also necessary for the patient of breast cancer. Breast cancer resistance protein (BCRP), an atypical drug efflux pump, mediates multidrug resistance in breast cancer. The aim of this study is to search new substrate of BCRP. The result will guide the drug selection of chemotherapy in BCRP-positive breast cancer.
Methods PA317/Tet-on/TRE-BCRP cell induced with doxycycline was used to screen the possible substrates of BCRP by MTT assay. The suspicious substrate [5-Fluorouracil (5-Fu)] was further confirmed in PA317 and breast cancer cell MCF-7 by HLCP, apoptosis assay (staining and FACS) and RNAi technique.
Results Mitoxantrone, 5-Fu, adriamycin, Methotrexate, Pirarubicin, and Etoposide were identified as substrates of BCRP. However, Paclitaxel, Vincristine, Vindesine, Mitomycin C, and cisplatin were not mediated by BCRP. 5-Fu was identified as substrate of BCRP for the first time. The further study showed that the intracellular retention dose of 5-Fu and the 5-Fu induced cellular apoptosis all decreased when BCRP highly expressed. Furthermore, 5-Fu accumulation and 5-Fu induced DNA damage increased when BCRP was silenced by RNAi in breast cancer cells.
Conclusions 5-Fluorouracil may be a specific substrate which can be bound by BCRP. BCRP can predict the sensitivity of breast cancer to 5-Fu. And BCRP-targeted therapy will reverse the resistance of breast cancer to 5-Fu.

Jianhui Yuan and Hui Lv were contributed equally to this work.

J. Yuan · H. Lv · B. Peng · C. Wang · Y. Yu · Z. He (✉)
Cancer Research Institute, Xiangya School of Medicine, Central South University, Xiangya Road #110, 410078 Changsha, Hunan, People's Republic of China
e-mail: hezhimin2005@yahoo.com

Keywords BCRP · 5-Fluorouracil · Multidrug resistance · RNAi

Abbreviations

ABC	ATP-binding cassette
BCRP	Breast cancer resistance protein
5-Fu	5-Fluorouracil
MDR	Multidrug-resistant
MX	Mitoxantrone

Introduction

The overexpression of the ATP-binding cassette (ABC) transport protein protects cells from cytotoxic drugs due to drug efflux, and is a major mechanism responsible for multidrug resistance (MDR). Breast cancer resistance protein (BCRP), also called ABCG2, is a ABC half-transporter which was previously isolated from adriamycin-resistant MCF-7 breast cancer cells [1]. BCRP overexpression has been observed in several drug-resistant cell lines and tumors, which indicates its importance in the multidrug-resistant phenotype of cancer cells [2–5]. The ABCG2 drug efflux pump can bind and transport a variety of chemotherapeutic agents including Mitoxantrone, topotecan, SN-38 (the active metabolite of irinotecan), and flavopiridol [4–8]. We also proofed in previous study that BCRP has taken an important role in the MDR in breast cancer cells [9]. As we all know, chemotherapy is not only important but also necessary for the patient with breast cancer. When BCRP has been detected, to distinguish the effective anti-cancer drugs from chemotherapeutic agents which are usually used in treatment of breast cancer is very important for reasonable chemotherapy. The present study was designed to examine whether BCRP expression was correlated with the resistance to these

chemotherapeutic agents by using PA317/Tet-on/TRE-BCRP cell induced with doxycycline, which was established by our lab [10]. We observed that there was an effect-dose relation between resistant degree of cell to 5-fluorouracil (5-Fu) and expression level of BCRP in the cells. Furthermore, the results were confirmed in BCRP-overexpressive breast cancer cell (MCF-7/BCRP). And the resistance to 5-Fu could be reversed by knocking down BCRP through RNAi.

Materials and methods

Cell culture

PA317/Tet-on/TRE-BCRP cells had been established and stored by ourselves, in which the expression of BCRP could be regulated by doxycycline (DOTC). PA317/Tet-on/TRE-BCRP cells were induced by 0, 0.1, 1, 3, 6 mg L^{-1} DOTC for 24 h before other experiments were taken. BCRP-overexpressive breast cancer cells (MCF-7/BCRP) were obtained by transfecting BCRP to MCF-7 cells, and MCF-7/BCRP/Bi cells were established by repressing BCRP expression through RNA interfering. JAR cells (endogenous expression of BCRP highly) were obtained from Institute of Biochemistry and cell biology, SIBS, CAS. All cells were cultured in DMEM medium (Gibco BRL) supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine at 37°C in a humidified atmosphere containing 5% CO_2.

MTT assay

Anticancer drugs employed in research were Paclitaxel (Taxol), 5-Fu, Mitomycin C (MMC), Etoposide (VP-16), Vincristine (VCR), adriamycin (ADM), Methotrexate (MTX), Pirarubicin (THP), Vindesine (VDS), Mitoxantrone (MX) and cisplatin (DDP). They were purchased from Sigma Chemical Co. or Shanghai Pharmacy Co., etc. MTT assays were performed as previously [9]. Cells were counted and then cultured in 96-wells plate, and treated with those anticancer drugs of different concentration for 72 h, respectively. The anti-proliferative effects of the drugs were evaluated by survival rate. Fifty percent cell growth inhibitory concentrations (IC_{50}) was calculated from linear regression analysis of the linear portion of the growth curves. Resistance index (RI) of cells to anticancer agents was calculated as (IC_{50} of drug-resistant cells)/(IC_{50} of parental cell). Experiment was duplicated for three times.

Detection of accumulation of 5-Fu by HPLC

Cells were treated with 600 mg L^{-1} 5-Fu for 2 h when cultured cell had arrived 70% degrees of fusion. After phosphate buffered solution (PBS) washing, the culture medium was changed to fresh medium for 1 h incubation. After trypsinization and PBS washing, cells were collected by 3,000×*g* centrifuge for 5 min at 4°C, then added 200 μL sterile water and ultrasounding for 20 s, supernatant was monitored using high-performance liquid chromatography (HPLC), as described previously [11].

Apoptosis assays by staining and flow cytometry

Apoptosis was monitored by using Hochest 33258 staining. As described by Kugawa et al. [12]. Cells treated with 60 or 600 mg L^{-1} 5-Fu for 24 h. After PBS washing, cell fixation fluid was added for 10 min at 4°C. Then 5 mg L^{-1} Hochest 33258 was added for staining 10 min at room temperature. The apoptotic cells were observed and counted through fluorescence microscope. Apoptosis was also monitored by flow cytometer. The treated cells were sent to Ding-guo Company (Beijing, China) and analyzed using a FACScan flow cytometer (Becton–Dickinson). Each experiment was performed in triplicate.

Single cell gel electrophoresis assay (comet assay)

5-Fluorouracil induced DNA damage of cell was measured with comet assay. The alkaline (pH > 13.0) single cell gel electrophoresis assay was performed by a modified method of Singh [13]. After treatment with 5-Fu, the cells were then washed in PBS and suspended in low melting agarose LMA at 37°C, and 80 μl of 0.75% LMA cell suspension (1×10^4/ml) was piped onto frosted glass microscope slide pre-coated with a 100 μl layer of 0.75% normal melting agarose NMA. The coverslips were placed gently to allow even spreading of gel. The slides were kept on ice for 5 min to allow the gel to solidify. The coverslips were removed and the third layer of 80 μl of 0.75% LMA was finally added and allowed to solidify on ice for 10 min. Then the coverslips were removed and the slides were immersed in freshly prepared ice-cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, 1% Triton X-100 and 10% DMSO, pH 10.0) to lyse the cell proteins and to allow DNA unfolding. After incubating at least 1 h at 4°C in the dark, the slides were covered with fresh buffer (1 mM Na_2EDTA and 300 mM NaOH, pH > 13.0) in a horizontal electrophoresis unit. The slides were allowed to rinse in this buffer for 20 min for DNA unwinding. Then, the DNA was electrophoresed at 20 V and 300 mA for 30 min at 4°C. After electrophoresis, the slides were washed gently with fresh prepared 0.4 M Tris–HCl, pH 7.5 for three times and then stained with 50 μl ethidium bromide (20 μg/ml) for 20 min. The pictures of 50 cells per treatment sample (25 cells/slide) were taken individually under a fluorescence microscope (Olympus, BX51) with digital camera (Olympus, DP50) at 200× magnification and the test was carried out three times. The percentage of tail DNA

(DNA in the comet tail) and Olive tail moment [tail DNA% × (tail meanX − head meanX) were analyzed using Comet Assay Software Project (CASP)].

Statistical analysis

Statistic analyses were carried out using the SPSS 10.0 program for Windows. Student's *t* test was used to evaluate the statistical significance. A P value <0.05 was set as the criteria for statistical significance.

Results

The sensitivity of PA317/Tet-on/TRE-BCRP cells to various anti-cancer drugs

PA317/Tet-on/TRE-BCRP cell with 0, 0.1, 1, 3, 6 mg L^{-1} DOTC induction for 24 h, respectively, were treated with anti-cancer drugs mentioned above at various concentrations for 72 h. The IC_{50} were calculated by MTT assay. Then RI of cells to anticancer drugs was determined. Table 1 listed the results. RI was showed in the brackets. RI of PA317/Tet-on/TRE-BCRP to some drugs heightened along with up-expression of BCRP, such as MX, 5-Fu, MTX, ADM, THP, and VP-16. The max RI to these drugs was 82.8, 10.58, 8.6, 17.3, 15.8 and 17.6, respectively compared with control group. The 5-Fu was found to be the substrate of BCRP for the first time. The resistance of 5-Fu was closely correlated with BCRP levels, similar with MX and ADM which are the classic substrates of BCRP. However, the sensitivity of cell to Taxol, DDP, VDS, MMC, and VCR did not change regardless of the BCRP expression. These results were indicated that 5-Fu could be new substrates of BCRP.

Effect of BCRP expression on intracellular accumulation of 5-Fu

PA317/Tet-on/TRE-BCRP cells were inducted by 0, 0.1, 1, 3, 6 mg L^{-1} DOTC for 24 h, respectively. And in another

Table 1 IC_{50} values of different anticancer drugs in PA317/Tet-on/TRE-BCRP cells and RI of the cells

Drugs	PA317/vector	PA317/Tet-on/TRE-BCRP					JAR
	0	0	0.1	1	3	6 (μg DCL/ml)	0
5-Fu	136 ± 2.0	447 ± 3.0	900 ± 1.0*	1,233 ± 8.1**	1,353 ± 7.4**	1,440 ± 6.2**	1,430 ± 6.1
	(1)	(3.28)	(6.62)	(9.06)	(9.96)	(10.58)	
MTX	131 ± 1.0	238 ± 4.0	426 ± 2.0	773 ± 5.4*	973 ± 4.3*	1,127 ± 7.1**	1,140 ± 7.2
	(1)	(1.82)	(3.25)	(5.9)	(7.43)	(8.6)	
ADM	1.70 ± 0.14	2.20 ± 0.21	10.5 ± 0.71*	17.2 ± 0.53**	21.1 ± 0.42**	29.4 ± 0.64**	29.1 ± 0.61
	(1)	(1.29)	(6.18)	(10.1)	(12.4)	(17.3)	
THP	0.8 ± 0.06	1.50 ± 0.18	5.60 ± 0.32*	7.09 ± 0.33**	8.00 ± 0.52**	12.6 ± 0.47**	13.1 ± 0.68
	(1)	(1.61)	(7)	(8.86)	(10)	(15.8)	
VP-16	1.40 ± 0.08	16.1 ± 0.15**	18.3 ± 0.23**	21.3 ± 0.18*	23.0 ± 0.09**	24.7 ± 0.27**	24.2 ± 0.18
	(1)	(11.5)	(13.1)	(15.2)	(16.4)	(17.6)	
MX	0.05 ± 0.01	0.15 ± 0.06	0.72 ± 0.03**	2.23 ± 0.08**	3.24 ± 0.12**	4.14 ± 0.06**	3.78 ± 0.08
	(1)	(3)	(14.4)	(44.6)	(64.8)	(82.8)	
Taxol	65.0 ± 0.6	53.2 ± 0.5	54.0 ± 0.7	62.0 ± 0.7	58.2 ± 0.5	70.0 ± 0.8	80.0 ± 0.2
	(1)	(0.8)	(0.83)	(0.95)	(0.89)	(1.07)	
DDP	18.0 ± 0.5	18.2 ± 0.4	27.6 ± 0.4	24.7 ± 0.7	24.2 ± 0.5	24.1 ± 0.8	20.0 ± 0.2
	(1)	(1)	(1.5)	(1.4)	(1.3)	(1.3)	
VDS	3.60 ± 0.08	3.68 ± 0.08	3.74 ± 0.05	3.78 ± 0.06	4.03 ± 0.07	4.04 ± 0.07	5.90 ± 0.05
	(1)	(1)	(1.03)	(1.05)	(1.12)	(1.12)	
MMC	100 ± 1.0	159 ± 4.0	158 ± 5.1	188 ± 9.6	189 ± 8.2	172 ± 7.3	120 ± 5.4
	(1)	(1.6)	(1.58)	(1.88)	(1.89)	(1.72)	
VCR	3.70 ± 0.09	7.5 ± 0.07	10.7 ± 0.17	12.8 ± 0.15	14.5 ± 0.12	14.9 ± 0.11	16.1 ± 0.21
	(1)	(2)	(2.89)	(3.46)	(3.92)	(4.03)	

PA317 cells, PA317/Tet-on/vector cells, PA317/Tet-on/TRE-BCRP cells with 0, 0.1, 1, 3, 6 mg L^{-1} DOTC induction for 24 h and JAR cells were detected. Each group of cells was pre-cultured for 24 h in 96-well multiplates, and then incubated with anticancer drugs at various concentrations for 72 h. The IC_{50} values represent the mean values ± SD, $n = 3$

* $P < 0.05$; ** $P < 0.01$. The RI was showed in brackets

* $P < 0.05$; ** $P < 0.01$ vs. PA317/vector (RI)

6 mg L^{-1} DOTC induction group, 10 μmol L^{-1} Ko143 was added for 15 min. All these groups were treated with 600 mg L^{-1}5-Fu for 2 h. PA317/Tet-on/vector cell was used as control. The accumulation of 5-Fu in cells was determined by HPLC assay. The concentration of 5-Fu in PA317/Tet-on/TRE-BCRP cells is 3.41 ± 0.17, 2.39 ± 0.23, 1.70 ± 0.08, 0.90 ± 0.05, 0.42 ± 0.02, 1.75 ± 0.1 mg L^{-1}, respectively ($P < 0.05$). As shown in Fig. 1, there was shown negative correlation between BCRP expression and cellular accumulation of 5-Fu. And cellular accumulation of 5-Fu would be raised when Ko143 (specific inhibitor of BCRP) was added. There results proved that resistance of 5-Fu could be mediated by BCRP.

Effect of BCRP expression on 5-Fu-induced apoptosis

Different cell group were treated with 60 or 600 mg L^{-1} 5-Fu, respectively. Apoptotic rate of each group were determined by Hochest 33258 staining and flow cytometry. As shown in Fig. 2. The rank order of apoptotic rate was PA317/Tet-on/TRE-BCRP cell which treated with 6 mg L^{-1} DOTC group < PA317/Tet-on/TRE-BCRP cell which treated with 1 mg L^{-1} DOTC group < PA317/Tet-on/TRE-BCRP cell which treated with 0 mg L^{-1} DOTC group. Furthermore, BCRP inhibitor Ko143 could reverse the 5-Fu-induced apoptosis. These results were indicated that 5-Fu-induced apoptosis could be reduced by BCRP expression.

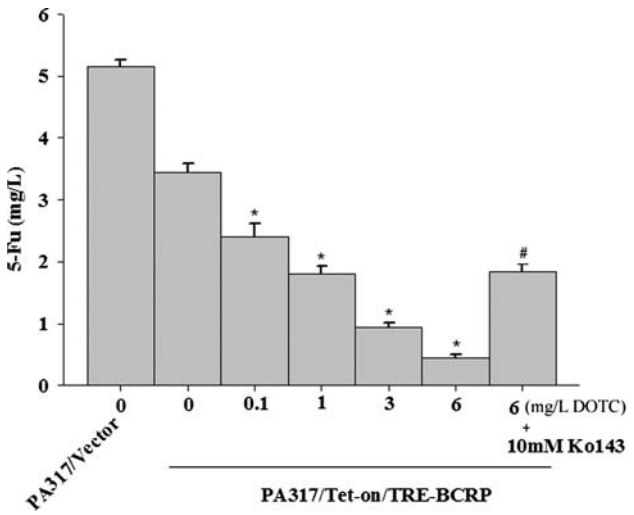

Fig. 1 Effect of different expression levels of BCRP on the accumulation of 5-Fu in PA317/Tet-on/TRE-BCRP cells PA317/Tet-on/vector cells, PA317/Tet-on/TRE-BCRP cells with 0, 0.1, 1, 3, 6 mg L^{-1} DOTC induction for 24 h were treated with 600 mg L^{-1}5-Fu for 2 h. Then the accumulation of 5-Fu in cells was determined by HPLC assay. The cellular accumulation of 5-Fu was decreased when BCRP highly expressed. There was negative correlation between BCRP expression and cellular accumulation of 5-Fu. And this effect could be reversed by Ko143. These data are representative of three independent experiments (* significantly different from at PA317/Tet-on/TRE-BCRP cells with 0 mg L^{-1} DOTC induction at $P < 0.05$. # significantly different from PA317/Tet-on/TRE-BCRP cells with 6 mg L^{-1} DOTC induction at $P < 0.05$)

Effect of BCRP expression on accumulation of 5-Fu in breast cancer cells

MCF-7/vector cells, MCF-7/BCRP cells and MCF-7/BCRP/Bi cells were treated with 600 mg L^{-1} 5-Fu for 2 h. The accumulation of 5-Fu in these cells were detected by HPLC. As shown in Fig. 3, the concentration of 5-Fu was decreased in the MCF-7/BCRP cells compared with MCF-7/vector cells. However, the concentration of 5-Fu was almost increased to the level of MCF-7/vector cells when BCRP was knock-down by RNAi. Also, the same result was gotten when Ko143 was used ($P < 0.05$). These results indicated that the 5-Fu was pumped out by BCRP. 5-Fu was the substrate of BCRP in breast cancer cells.

The resistance of breast cancer cells to 5-Fu was reversed by shut-down BCRP expression

The shRNA-mediated reversal of the multidrug-resistant phenotype was assessed by comparison of the cell survival assay in shRNA-treated cells and control. MCF-7/BCRP/Bi showed a slightly enhanced chemosensitizing activity to 5-Fu compared to MCF-7/BCRP/vector cells ($P < 0.05$) (Fig. 4). That means the sensitivity to 5-Fu was regained when BCRP expression was silenced. This also proofed that the resistance of 5-Fu was mediated by BCRP and the 5-Fu was the substrate of BCRP in breast cancer cells.

The 5-Fu induced DNA damage was reversed by RNAi in breast cancer cells

MCF-7/BCRP/vector cells and MCF-7/BCRP/Bi cells were treated with 600 mg L^{-1} 5-Fu for 48 h then the DNA damage was measured with comet assay. As shown in Fig. 5, the cell with damaged DNA showed a head and a tail just like a comet. The head DNA swelled and the tail DNA shortened in the MCF-7/BCRP/vector cells (Fig. 5a) compared with MCF-7/BCRP/Bi cells (Fig. 5b). The tail DNA percentage (Fig. 5c) and Olive tail moment (Fig. 5d) of the MCF-7/BCRP/Bi cells were significantly increased compared with MCF-7/BCRP/vector cells, $P < 0.01$. This suggested the DNA damage was strengthened. These results confirmed that the resistance of 5-Fu in breast cancer cells was mediated by BCRP. And this effect could be reversed by RNAi.

Discussion

As drug exporters of the ABC family, P-glycoprotein (P-gp/ABCB1) [14], multidrug resistance-associated protein

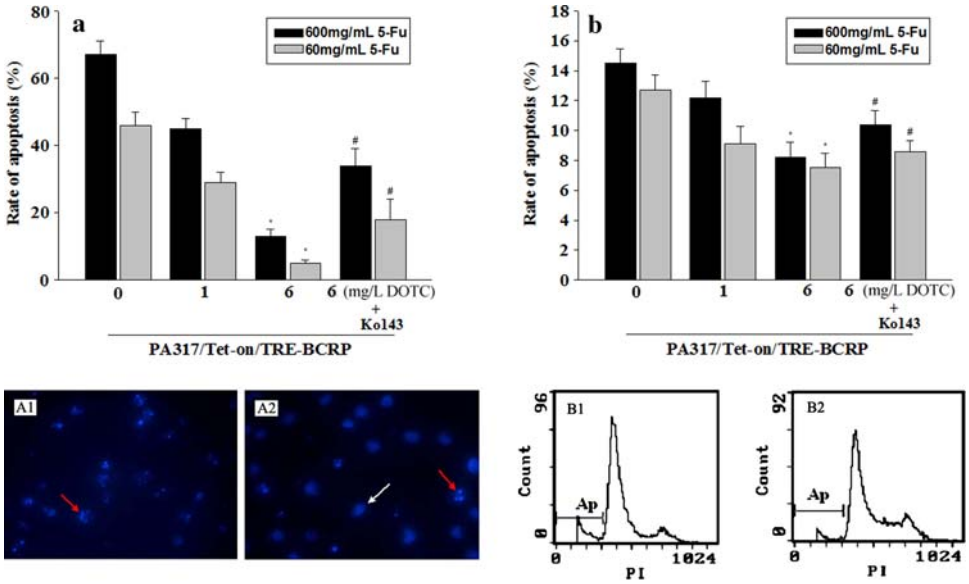

Fig. 2 Effect of BCRP expression on 5-Fu-induced cellular apoptosis. Apoptosis detected by Hochest 33258 staining (**a**), and flow cytometry analysis (**b**), a, b, c, d were treated with 60 or 600 mg L^{-1} 5-Fu for 24 h, respectively. The rank order of apoptotic rate was c < b < a, d > c. These data are representative of three independent experiments (* significantly different from a group at $P < 0.05$, # significantly different from c group at $P < 0.05$). This indicated expression of BCRP could reduce the 5-Fu-induced cellular apoptosis. This effect could be reversed by inhibiting the role of BCRP. a: PA317/Tet-on/TRE-BCRP cells inducted with 0 g L^{-1} DOTC; b: PA317/Tet-on/TRE-BCRP cells inducted with 1 mg L^{-1} DOTC; c: PA317/Tet-on/TRE-BCRP cells inducted with 6 mg L^{-1} DOTC; d: PA317/Tet-on/TRE-BCRP cells inducted with 6 mg L^{-1} DOTC + Ko143 (10 μM). *Red arrow* apoptotic cell, *White arrow* normal cell, *Ap* apoptosis peak. Apoptotic cell would be stained into brightly blue because of their chromatin condensation, while normal cell was stained into even slightly blue

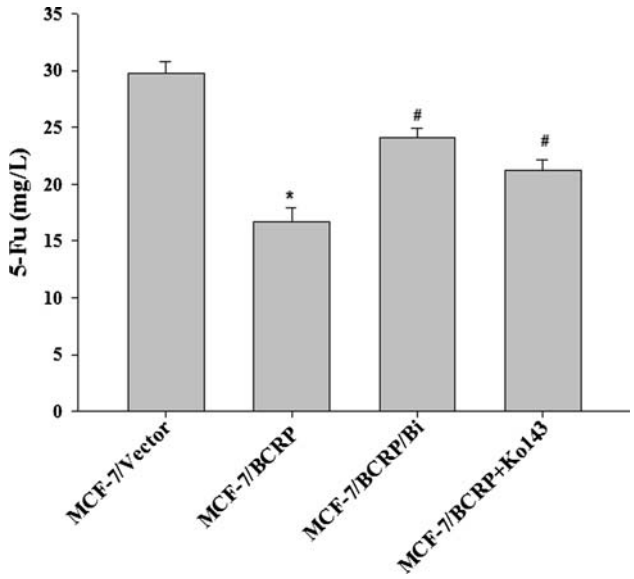

Fig. 3 Effect of BCRP expression on the accumulation of 5-Fu in breast cancer cells MCF-7/vector cell, MCF-7/BCRP cell, MCF-7/BCRP/Bi cell were treated with 600 mg L^{-1}5-Fu for 2 h, then the concentration of 5-Fu in cells was determined by HPLC assay. The cellular concentration of 5-Fu was decreased in MCF-7/BCRP cell compared with MCF-7/vector cell. This effect can be reversed by Ko143 or RNAi (* significantly different from MCF-7/vector cell at $P < 0.05$, # significantly different from MCF-7/BCRP cell at $P < 0.05$)

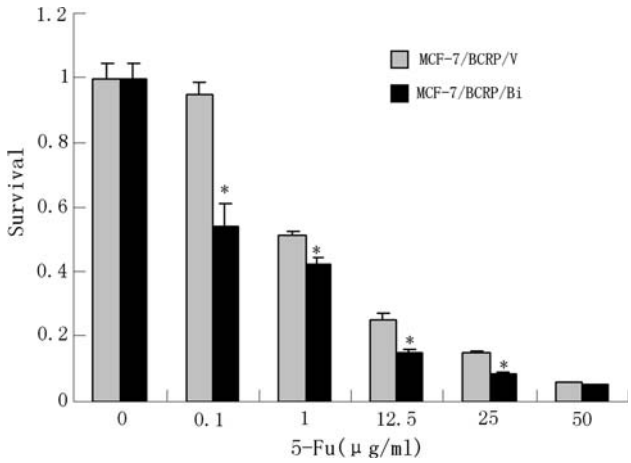

Fig. 4 The resistance to 5-Fu of breast cancer cells was reversed by shut-down BCRP expression MTT cytotoxicity assay was performed as described in "Materials and methods" MCF-7/BCRP/V cell, MCF-7/BCRP/Bi cell were treated with various concentration of 5-Fu. The survival of MCF-7/BCRP/Bi cell was decreased compared to MCF-7/BCRP/V cell. This indicated that the sensitivity of MCF-7/BCRP/Bi cells to 5-Fu is increased compared to MCF-7/BCRP/V. Each *bar* represent the mean ± SD ($n = 3$) (* significantly different from MCF-7/BCRP/V cell at $P < 0.05$)

(MRP/ABCC1) [15] and the breast cancer resistance protein (BCRP/ABCG2) [1] transporters confer MDR by actively exporting many anticancer drugs from cells. There is considerable, not complete, overlap in anti-cancer substrate specificity among them. Though BCRP, compared to P-gp and MRP, mediates an atypical MDR [6, 16]. It was very important to set up the exact substrate preference of BCRP because that the overlap may have clinical consequences for both MDR in tumors and anti-cancer drug

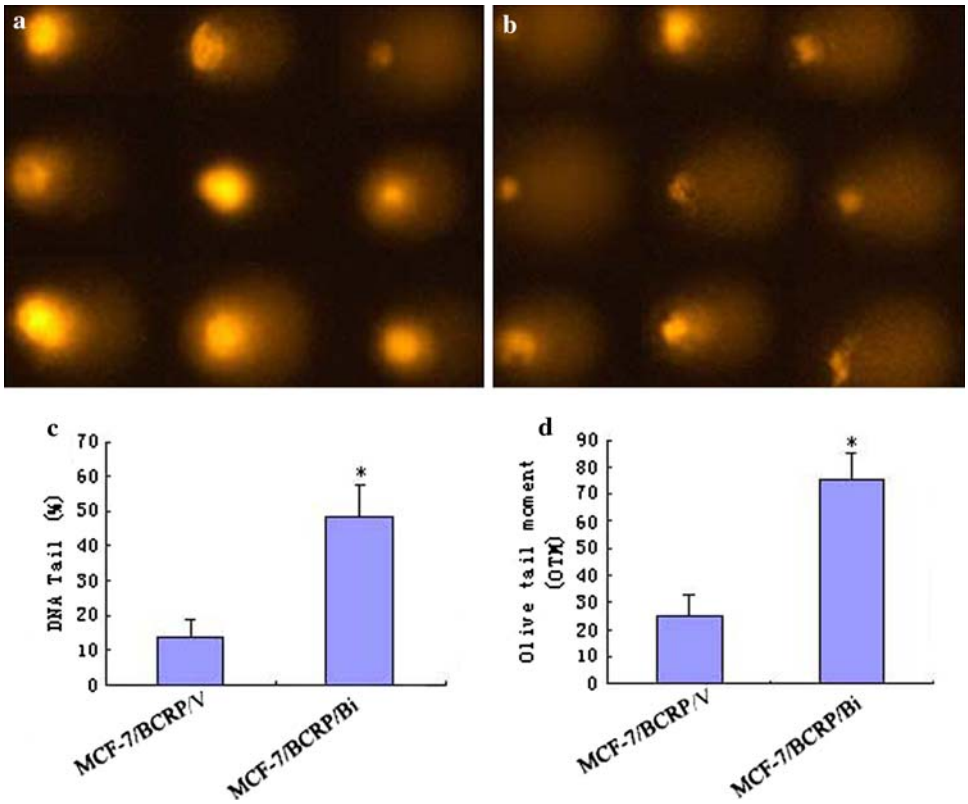

Fig. 5 The 5-Fu induced DNA damage was reversed by RNAi in breast cancer cells. Results of single cell gel electrophoresis analysis. The tail DNA percentage and Olive tail moment of the MCF-7/BCRP/Bi cells **b**, were significantly increased compared with the MCF-7/BCRP/V cells **a**. The *bars* represent the mean values ± SD of tail DNA% **c**, and Olive tail moment **d**, in three experiments. Thirty cells were evaluated in each test (* significantly different from MCF-7/BCRP/V cell at $P < 0.01$). These suggested that the DNA damage was weakened. The MCF-7/BCRP/Bi cells were become sensitivity to 5-Fu after the expression of BCRP was silenced

pharmacology. Therefore, we established a inducible BCRP-expressive model (PA317/Tet-on/TRE-BCRP) with doxycycline (DOTC) inducement in previous experiment [10]. And there was a positive correlation between the level of BCRP expression and dose of DOTC inducement in this model. In this study, we utilized it to screen clinical anticancer drugs and found that MX, 5-Fu, MTX, ADM, THP, and VP-16 were the possible substrates of BCRP. The transport of Taxol, DDP, VDS, MMC, and VCR could not be mediated by BCRP. Although some of substrates have been reported [4, 6–8, 17], the results herein are, to our knowledge, the first to show a strict association between BCRP expression and a variety of chemotherapy drug sensitivity. Especially, the 5-Fu was first identified as a possible substrate of BCRP. This finding was very interesting and significant.

5-Fluorouracil is a pyrimidine analog that is an antineoplastic antimetabolite. It interferes with DNA synthesis by blocking the thymidylate synthetase conversion of deoxyuridylic acid to thymidylic acid. It is typical anti-cancer drug acting especially on tumor cell cycle. 5-Fu can result in cell apoptosis by cytotoxic effect [18] and is usually used in breast cancer, ovarian carcinoma, and other malignancies as first line chemotherapy drug [19–21]. There is little report that the resistance of 5-Fu is mediated by P-gp, MRP or BCRP although the resistance has been found. Our earlier research [22] had shown that 2.5% of primary breast cancer cells were displayed middle-degree resistance to 5-Fu and 20% for low-degree resistance. The mechanisms for resistance to 5-Fu are not fully defined. In this study, we found 5-Fu was the substrate of BCRP and further confirmed corresponding relation between BCRP-expressive level and intracellular concentration of 5-Fu by HPLC in PA317/Tet-on/TRE-BCRP and MCF7/BCRP cells (Figs. 1, 3). Moreover, BCRP could antagonize cell apoptosis induced by 5-Fu through staining and FACS (Fig. 2).These finding provided new clue to assess the potential mechanism of 5-Fu in chemoresistance.

Breast cancer resistance protein is expressed in a number of normal tissues. Such as the canalicular membrane of liver hepatocytes, the apical membrane of the epithelium in the small and large intestine, the ducts and lobule of the breast, the luminal surface of brain capillaries, and human placenta [23], etc. The localization of BCRP in the intestine and liver suggests that BCRP has the potential to strongly affect both the absorption and secretion of substrate drugs [24, 25]. BCRP is also overexpression in about 20–30% of breast cancer [5], 30–40% of acute leukemia [3], 30–35% of ovarian carcinoma [26], 50% of non-small cell lung cancer [27], etc. And BCRP mediate the MDR of these malignant tumors. Distributing property and physiological, pathological function of BCRP imply that it is a biomarker. In fact, we have found that BCRP-targeted RNAi therapy was tremendously effective in reversing BCRP-mediated MX resistance in previous job [9]. Here we discovered the target therapy also could reversed BCRP-mediated

resistance of MCF7/BCRP cells to 5-Fu (Fig. 4) and increased DNA damage of MCF7/BCRP cells induced by 5-Fu (Fig. 5). These results further ascertained the correlation between BCRP and 5-Fu.

In summary, BCRP can reduce the intracellular accumulation of the MX, 5-Fu, MTX, ADM, THP, and VP-16, but not effect the intracellular accumulation of the Taxol, DDP, VDS, MMC, and VCR. Furthermore, 5-Fu may be a specific substrate which can be bound by BCRP. It is likely that BCRP function will influence the resistance of 5-Fu in breast cancer cells. This is the new finding for us that 5-Fu is the substrate of BCRP. It also indicated that Taxol, DDP, VDS, MMC, and VCR could be used when BCRP was detected. However, MX, MTX, ADM, THP, and VP-16, 5-Fu could not. It is not only in breast cancer but also in other cancers. These observations may result in the development of strategies to prevent resistance in clinical when the BCRP is detected, and illustrate the importance of identifying the transporters which play a role in MDR.

Acknowledgments We thank Dr. Alfred H. Schinkel for kind gift of Ko143. Financial support: the Natural Science Foundation of Hunan Province, China (02JJY2052); the Basic Research Special Program of the Ministry of Science and Technology of China (2003CCC00700).

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