Silencing the expression and function of breast cancer resistance protein in MCF-7/MX100 cells by shRNA expressing lentivirus

Caihong Yu[1a], Siyun Xu[1a], Aiming Yu[2a], Yanqing Liu[1], Haihong Hu[1], Liping Li[1], Lushan Yu[1], Hui Zhou[1], Huidi Jiang[1], Su Zeng[1*]

1. Laboratory of Pharmaceutical Analysis and Drug Metabolism, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

2. Department of Biochemistry & Molecular Medicine, UC-Davis Medical Center, Sacramento, CA 95817, USA

Abstract: Overexpression of breast cancer resistance protein (ABCG2/BCRP) in cancer cells may cause tumor resistance to chemotherapeutic drugs. RNA interference (RNAi) can selectively silence the expression of a target gene of interest. In the present study, we aimed to modulate the BCRP expression and examine the functional consequence using RNAi approach. Three siRNAs (si-BCRP1, si-BCRP2 and si-BCRP3) targeting BCRP were evaluated in drug-resistant MCF-7/MX100 cells overexpressing BCRP. The BCRP expression at the mRNA and protein levels was inhibited by si-BCRP2 and si-BCRP3 over 90% and 70%, respectively. As a result, the intracellular mitoxantrone accumulation was sharply increased in MCF-7/MX100 cells after the transfection. Furthermore, shRNA sequences bearing si-BCRP2 and siBCRP3 were cloned into lentiviral expression plasmid (pTRIPZ) to package lentivirus, and MCF-7/MX100 cells stably expressing siRNA targeted to human ABCG2/BCRP were established by lentivector-mediated gene transfer system. The stable cells exhibited an increased miotxantrone accumulation, among which the BCRP expression at the mRNA level was reduced by Lenti-BCRP2 and Lenti-BCRP3 around 72% and 56%, respectively. Moreover, the BCRP expression at the protein level was reduced by 70% and 53%, respectively. Furthermore, the cell lines were used to screen active ingredients in traditional herbal medicines in order to evaluate BCRP substrates or inhibitors. Our data suggested that the BCRP knockdown cell lines could serve as good cell models for preclinical studies.

Keywords: BCRP, RNA interference, Lentiviral expression system, Drug resistance reversal, Mitoxantrone, TCM

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1. Introduction

Multi-drug resistance (MDR) often exists in malignant tumor chemotherapy[1], leading to a low therapeutic effect. Some MDR mechanisms have been clarified, including increased activity of drug efflux pump (such as ABC efflux transporter and other relevant transporters), regulation of cell apoptosis signaling pathways, change and repair of drug target sites, as well as some unconventional mechanisms[2]. Above all, the abnormal expression of ABC transporter unavoidably plays an important role in MDR[3], such as *P*-glycoprotein (*P*-gp), breast cancer resistance protein (BCRP), MDR associated protein (MRP) and lung resistance protein (LRP). Particularly, the role of BCRP in tumor drug resistance has attracted further attention in recent years[4–6]. Studies showed that a variety of refractory tumors, such as breast cancer[7], lung cancer[8], acute myelogenous leukemia[9], are related to a high BCRP expression, resulting in atypical MDR.

How to reverse the MDR by BCRP becomes one of the hot topics. BCRP inhibitors can be divided into three categories[10]. One is specific inhibitors, such as FTC, Ko134, Ko143 and SN443. The second is broad-spectrum inhibitors, such as GF120918 and estrone, and the third group includes flavonoid compounds and their derivatives. However, the inhibitors can also produce serious toxicity; therefore, they are limited in clinical use. Besides, antisense nucleic acids and hammerhead ribozymes[11,12], which cause mRNA degradation, can reverse MDR. However, it is hard to obtain an efficient and stable transfection technically and functionally.

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[a]These authors equally contribute to this work.
[*]Corresponding author. Tel./Fax: 86-571-88208407, E-mail: zengsu@zju.edu.cn

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Currently, RNA interference has become more popular, which causes mRNA degradation or inhibits protein translation through the RNA silencing complex (RISC) combined with corresponding complementary mRNA[13,14]. Since secondary structure of mRNA is affected to a smaller extent, RNA interference is more stable and easily to be performed compared with antisense nucleic acids and hammerhead ribozymes. However, it still has disadvantages, such as transient suppression and low stability[15,16]. Therefore, in the present study, we aimed to establish stable BCRP knockdown cell lines by lentivector-mediated gene transfer system. This system might be used to reverse tumor drug resistance and screen BCRP substrates and inhibitors.

2. Materials and methods

2.1. Chemicals

Restriction endonucleases BamH I and Hind III were purchased from Fermentas (Hanover, MD, USA). T4 DNA ligase, DNA markers, IANprep Mini plasmid kit and TIANgel Midi purification kit were obtained from TIANGEN (Beijing, China). QIAprep Spin Miniprep Kit was provided by QIAGEN (Hilden, Germany). RNase inhibitor, peptone and yeast extract were supplied from BBI (Boston, USA). Bromophenol blue, Triton X100, Tween-20, NP-40, 2-mercaptoethanol and ethidium bromide (EB) were purchased from Bio-Rad (Hercules, USA). PCR primers, glycerol, sodium dodecyl sulfate (SDS), acrylamide–bis-acrylamide (37.5:1, v/v), PVDF membrane, HEPES, TEMED and EDTA (AR) were all obtained from Sangon Co. (Shanghai, China). Agarose was provided by Oxoid (Basingstoke, United Kingdom). RPMI-1640 culture medium and fetal calf serum (FCS) were supplied from Gibco (California, USA). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA). BCA kit was obtained from Beyotime (Beijing, China). SDS, Tris, glycine and 20% Tween-20 were bought from Amresco (Solon, USA). BXP-53 monoclonal antibody was obtained from MERCK (Darmstadt, German). Goat anti-mouse IgG (H+L) and goat anti-rat IgG were purchased from Affinity BioReagents (Golden, USA). Mitoxantrone, icariin, isorhamnetin and dihydromyricetin were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and formic acid (LC) were supplied from Shanghai Ludu Chemical Reagent Factory (Shanghai, China).

2.2. Cell Lines and culture

The parental cell line, MCF-7, was obtained from Professor Yang B (Zhejiang University). Cancer resistance cell line, MCF-7/MX100, was established in our laboratory as previously reported[17]. MCF-7 cells were maintained in RPMI-1640 medium containing 10% FCS, 100 units/mL penicillin and streptomycin. MCF-7/MX100 cells were maintained in RPMI-1640 medium containing 10% FCS and 100 nM mitoxantrone. Cells were incubated in a humidified atmosphere with 5% CO_2 supply.

2.3. Transfection of synthesized siRNA

Three BCRP siRNAs were synthesized by Shanghai GenePharma Co., Ltd. Table 1 lists their sequences. MCF-7/MX100 cells were plated at a density of 5×10^6 cells/well and transfected with 20 pmol siRNA using Lipofectamine™ 2000. The BCRP expression and function were determined by quantitative polymerase chain reaction (qPCR), flow cytometry and western blotting analyses.

2.4. Preparation of anti-ABCG2 shRNA expressing lentivirus

RNA interference sequences (si-BCRP2 and si-BCRP3) were inserted into lentiviral expression plasmid pTRIPZ to package lentivirus. Briefly, the shRNA templates were designed, and Table 2 lists their sequences. Then the shRNA sequences were amplified by PCR using

Table 1. Sequences of siRNAs

siRNA groups	Sense (5′–3′)	Antisense (5′–3′)
NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
si-BCRP1	GGAGGCAAAUCUUCGUUAUTT	AUAACGAAGAUUUGCCUCCTT
si-BCRP2	GGCCUUGGGAUACUUUGAATT	UUCAAAGUAUCCCAAGGCCTT
si-BCRP3	GCCUACCUGAAAUUGUUAUTT	AUAACAAUUUCAGGUAGGCTT
siRNA to GAPDH	GUAUGACAACAGCCUCAAGTT	CUUGAGGCUGUUGUCAUACTT

Table 2. Sequences of shRNA templates

shRNA groups	Sequence
NC	Purchased from Thermo Fisher
BCRP2	TGCTGTTGACAGTGAGCGCA**GGCCTTGGGATACTTTGAA**ATAGTGAAGCCACAGATGTAT-**TTCAAAGTATCCCAAGGCC**TATGCCTACTGCCTCGGA
BCRP3	TGCTGTTGACAGTGAGCGCA**GCCTACCTGAAATTGTTAT**ATAGTGAAGCCACAGATGTAT-**ATAACAATTTCAGGTAGGC**TATGCCTACTGCCTCGGA

the primers as follows: 5′-CAGAAGGCTCGAGAA-GGTATATTGCTGTTGACAGTGAGCG-3′ (forward) and 5′-CTAAAGTAGCCCCTTGAATTCCGAGGCA-GTAGGCA-3′ (reverse). The shRNA was inserted into lentiviral expression plasmid pTRIPZ, which was digested by EcoRI and XhoI. Lenti293t cells were plated at a density of 2–3 $\times 10^5$ cells/10-mm dish, which were used to package lentivirus once a 90% confluence was obtained. Fresh medium was changed after 10 h to avoid toxicity.

2.5. Transduction of shRNA/BCRP lentivirus into MCF-7/MX100 cells

After 48 h of packaging, the supernatant (Leti-BCRP2 and Leti-BCRP3) was filtered by a 0.45-μm filter and added into MCF-7/MX100 cells with 8 μg/mL poly-brene. Then the interference effects were identified by qPCR, flow cytometry and western blotting after puromycin screening (1 μg/mL) and doxycycline induction (1 μg/mL).

2.6. qPCR

Total RNA was extracted from MCF-7/MX100 cells and reversely transcribed to cDNA. BCRP expression at the mRNA level was determined by real-time qPCR (Mastercycler® ep Realplex, Eppendorf Company) using specific primers as follows, 5′-TCTGGCATTTGTTT-CCTC-3′ (forward) and 5′-CTCCTGGCCCTCTACT-CT-3′ (reverse). GAPDH served as a housekeeping gene using primers as follows: 5′-ATCACCATCTT-CCAGGAGCGA-3′ (forward) and 5′-GCTTCACCA-CCTTCTT GATGT-3′ (reverse). The relative level of BCRP expression was determined using the ΔΔCT method, and then the differences among groups or treatments were compared accordingly.

2.7. Western blotting

Whole-cell proteins (30 μg) were separated on 8% SDS-poly-acrylamide gels and electrotransfered onto nitrocellulose membranes. Membranes were then incubated in fat-free dry milk at room temperature for 2 h. Blots were incubated overnight with the BXP-53 monoclonal antibody at 4 °C, followed by a 2-h incubation using horseradish peroxidase (HRP) conjugated goat anti-rat IgG. Finally, blots were visualized with an enhanced chemiluminescence detection system.

2.8. Flow cytometry

Briefly, 48 h after the transfection, MCF-7/MX100 cells were incubated in 1 μM mitoxantrone at 37 °C for 60 min. Cells incubated in the absence of drug were used as negative controls. Cells were then washed twice with ice-cold PBS buffer, resuspended and subjected to flow cytometry analysis (λex = 635 nm, λem = 670 nm).

2.9. Identification of substrates or inhibitors using the BCRP stably knockdown cells

The MCF-7/MX100 cell line stably expressing ABCG2/BCRP shRNAs was used to screen active ingredients in traditional herbal medicines, such as dihydromyricetin (30 μM), icariin (10 μM) and isorhamnetin (25 μM). For accumulation study, the incubation time was set up at 45 min. For interaction study, mitoxantrone was incubated for 30 min after a pre-incubation with other compounds for 15 min. The samples were analyzed by UPLC-MS/MS[17]. Then the concentration of ingredients was calculated, and the accumulation rate (per minute) was compared based on the same protein contents.

2.10. Statistical analysis

Student's *t*-test was used to evaluate statistical significance. A P value<0.05 was considered as statistically significant.

3. Results and discussion

3.1. Suppression of BCRP expression and function by siRNAs

In the present study, we evaluated three siRNAs (si-BCRP1, si-BCRP2 and si-BCRP3) using MCF7/MX100 cells. The results from qPCR and western blotting showed that the BCRP expression was significantly decreased in MCF-7/MX100 cells after transfection with si-BCRP2 and si-BCRP3. In particular, si-BCRP2 and si-BCRP3 inhibited the BCRP expression at the mRNA level by 97% and 95%, respectively (Fig. 1A). At the protein level, the BCRP expression was reduced by 76% and 71%, respectively (Fig. 1B). The intracellular accumulation of mitoxantrone in MCF-7/MX100 cells was enhanced after transfection with si-BCRP2 and si-BCRP3 (P<0.05) (Fig. 1C). These results indicated that si-BCRP2 and si-BCRP3 were effective in suppressing the BCRP expression and function.

3.2. Development of BCRP shRNA expressing lentivirus to suppress BCRP expression and function

Our results also showed that the inhibitory effect of si-BCRP2 and si-BCRP3 diminished over time (data not shown). Therefore, to achieve a stable knockdown of BCRP for long-term investigation, two BCRP shRNAs were designed according to the sequences of si-BCRP2 and si-BCRP3, and cloned into the lentiviral vector pTRIPZ. The pTRIPZ plasmid harbors puromycin resistance genes, which could be used for stable transfection. Moreover, it is an inducible expression plasmid, and the inserted gene and its own red fluorescence protein (RFP) gene can be expressed only after the doxycycline induction.

Subsequently, MCF-7/MX100 cells were infected by the sh-BCRP2 or sh-BCRP3 expressing lentivirus to establish stable BCRP knock-down cells. The interference effect was identified after puromycin screening (1 μg/mL) and doxycycline induction (1 μg/mL). The RFP fluorescence from the lentivirus plasmid was observed at 570 nm→595 nm after the doxycycline induction, and the infection rate was satisfactory (Fig. 2A). RT-qPCR indicated that the BCRP expression at the mRNA level was suppressed around 72% and 56% by Lenti-BCRP2 and Lenti-BCRP3, respectively (Fig. 2B). Western blotting showed that the BCRP expression at the protein level was reduced around 70% and 53%, respectively (Fig. 2C). Meanwhile, the intracellular mitoxantrone accumulation was significantly increased by transfection of Lenti-BCRP2 or Lenti-BCRP3 (P<0.05, Fig. 2D). The successful suppression of BCRP expression and activity in stable BCRP knock-down cells suggested that these cells were an appropriate new model system for evaluation of BCRP-mediated drug disposition mechanisms.

3.3. Application of stable BCRP knockdown cells for the screening of BCRP substrates and inhibitors

Based on the above-mentioned findings, we assessed BCRP substrates and inhibitors using the BCRP knockdown cells (Lenti-BCRP2) and the negative control cells (Lenti-NC). The Lenti-BCRP2 and Lenti-NC cells were treated with doxycycline (Lenti-BCRP2$^+$ and Lenti-NC$^+$) to induce BCRP2 and control shRNA expression. In contrast, the cells treated with drug vehicle (Lenti-NC$^-$ and Lenti-BCRP2$^-$) were used as controls. The four groups of cells were then subjected to accumulation study to determine whether the phytochemicals, dihydromyricetin, isorhamnetin and icariin were substrates of BCRP. Mitoxantrone, a known substrate of BCRP, was used as a positive control. Drug concentrations were determined by UPLC-MS/MS assays. The data showed that the accumulation of mitoxantrone and isorhamnetin was significantly increased about 100% and 50%, respectively, after BCRP was knocked down in the cells (Fig. 3). The results indicated that isorhamnetin might be a BCRP substrate as well. Estradiol was utilized to test if the BCRP inhibition enhanced the isorhamenetin transport. When co-incubated with 100 μM of estradiol, the intracellular accumulation of isorhamnetin was significantly increased except that the accumulation was associated with a minimal expression of BCRP (Fig. 3C) in BCRP knockdown cells (Lenti-BCRP2$^+$, Fig. 4). The results indicated that BCRP might play an important role in the isorhamnetin transport, and isorhamnetin could be a potential substrate of BCRP.

Furthermore, we tested whether the stable BCRP knockdown cells were suitable for studying BCRP inhibitors. BCRP substrate mitoxantrone was used as a model drug. Therefore, Lenti-NC$^-$, Lenti-NC$^+$, Lenti-BCRP2$^-$ and

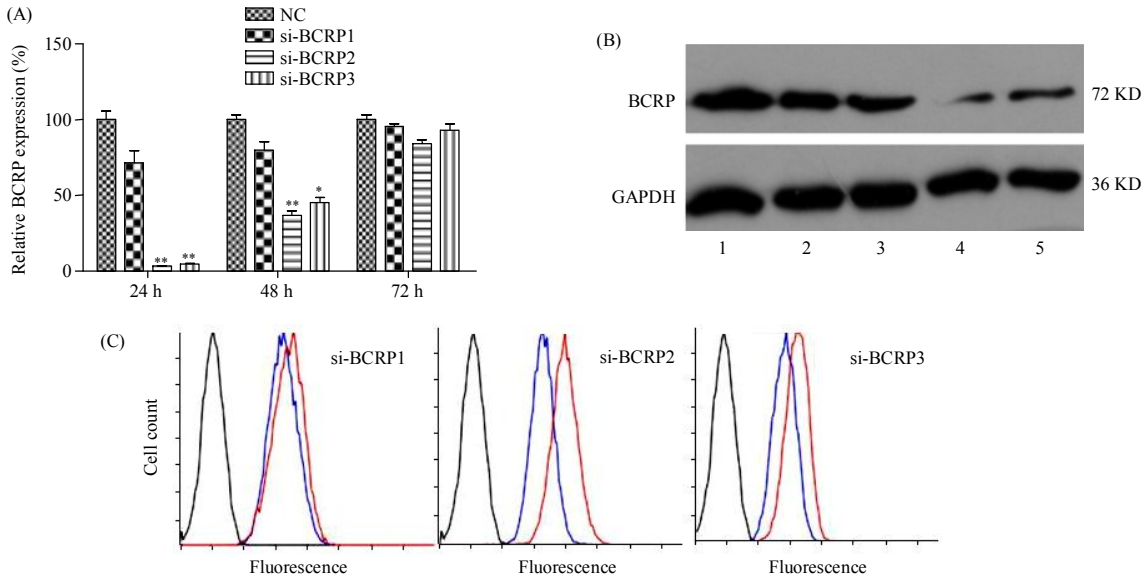

Figure 1. Inhibition of BCRP expression and function by siRNAs. (A) RT-qPCR assay indicated that si-BCRP2 and si-BCRP3 sharply reduced the BCRP expression at the mRNA level in MCF-7/MX100 cells (P<0.05, n = 3 in each group). (B) Immunoblot analysis showed that the BCRP expression at the protein level was suppressed by si-BCRP2 and si-BCRP3. Lane 1: MOCK cells (MCF-7/MX100 cells), Lane 2: NC (MCF-7/MX100 cells transfected with negative control siRNA), Lane 3: si-BCRP1, Lane 4: si-BCRP2, Lane 5: si-BCRP3. (C) Flow cytometry analyses revealed that the intracellular mitoxantrone accumulation was significantly increased in MCF-7/MX100 cells after transfection with si-BCRP2 and si-BCRP3 (black: blank, blue: negative control, red: siRNA).

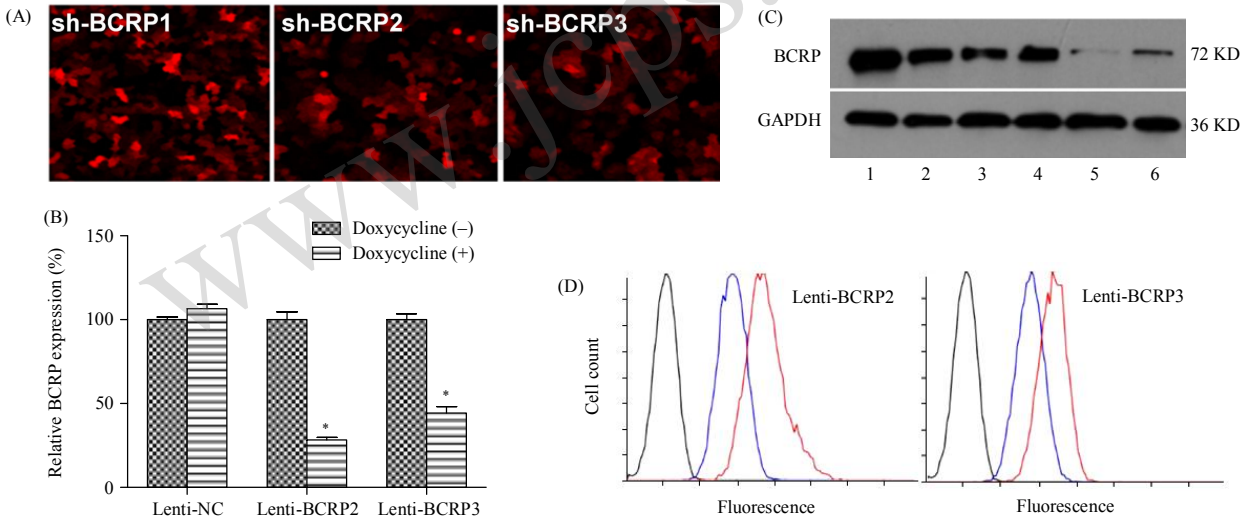

Figure 2. Inhibition of BCRP expression and function by shRNAs. (A) The RFP fluorescence from the lentivirus plasmid observed after 72 h showed that the transfection rate was about 80%. (B) RT-qPCR assay showed that Lenti-BCRP2 and Lenti-BCRP3 significantly reduced the BCRP expression at the mRNA level in MCF-7/MX100 cells (P<0.05, n = 3 in each group). (C) Immunoblot analysis demonstrated that the BCRP expression at the protein level was suppressed by Lenti-BCRP2 and Lenti-BCRP3. Lane 1: Lenti-NC$^-$ (Lenti-NC cells uninduced with doxycycline), Lane 2: Lenti-BCRP2$^-$ (Lenti-BCRP2 cells uninduced with doxycycline), Lane 3: Lenti-BCRP3$^-$ (Lenti-BCRP3 cells uninduced with doxycycline), Lane 4: Lenti-NC$^+$ (Lenti-NC cells induced with doxycycline), Lane 5: Lenti-BCRP2$^+$ (Lenti-BCRP2 cells induced with doxycycline), Lane 6: Lenti-BCRP3$^+$ (Lenti-BCRP3 cells induced with doxycycline). (D) Flow cytometry analyses indicated that the intracellular mitoxantrone accumulation was significantly increased in cells infected with Lenti-BCRP2 or Lenti-BCRP3 (black: blank, blue: negative control, red: lentivirus).

Lenti-BCRP2$^+$ cells were pre-incubated with dihydromyricetin (30 μM), isorhamnetin (25 μM) or icariin (10 μM) for 15 min, and then the cells were subjected to the assay of mitoxantrone (5 μM) accumulation. The data showed that, after the isorhamnetin pre-incubation, the intracellular mitoxantrone levels were significantly increased in Lenti-NC$^-$, Lenti-NC$^+$ and Lenti-BCRP2$^-$ cells (Fig. 5), which all had high levels of BCRP expression (Fig. 3). In contrast, isorhamnetin had no effect on mitoxantrone accumulation in Lenti-BCRP$^+$ cells. In

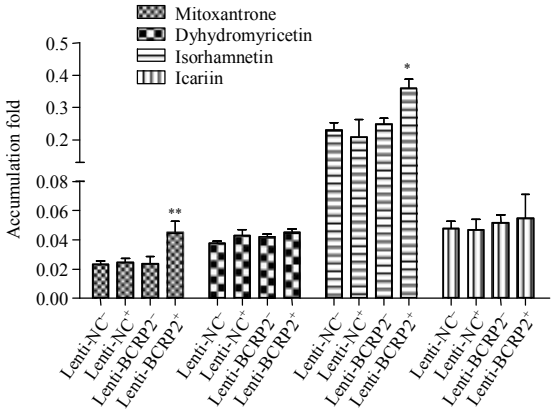

Figure 3. Accumulation of mitoxantrone, dihydromyricetin, isorhamnetin and icariin in MCF-7/MX100 cells following the manipulation of BCRP expression with shRNAs. The data suggested that isorhamnetin might be a substrate of BCRP.

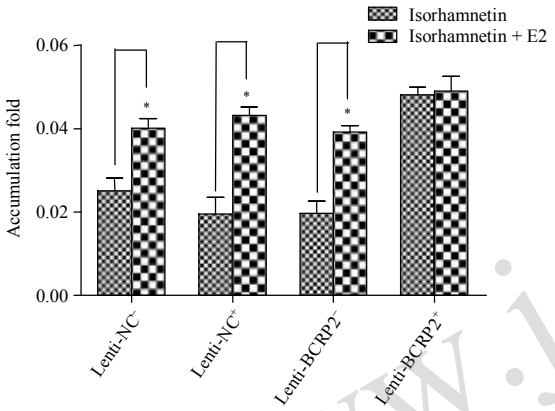

Figure 4. Inhibition of BCRP by estradiol (E2) increased the intracellular accumulation of isorhamnetin in MCF-7/MX00 cells (Lenti-NC^{-}, Lenti-NC^{+} and Lenti-BCRP^{-}) with high levels of BCRP expression. In contrast, E2 showed no effect on the isorhamnetin accumulation in BCRP knockdown cells (Lenti-BCRP^{+}).

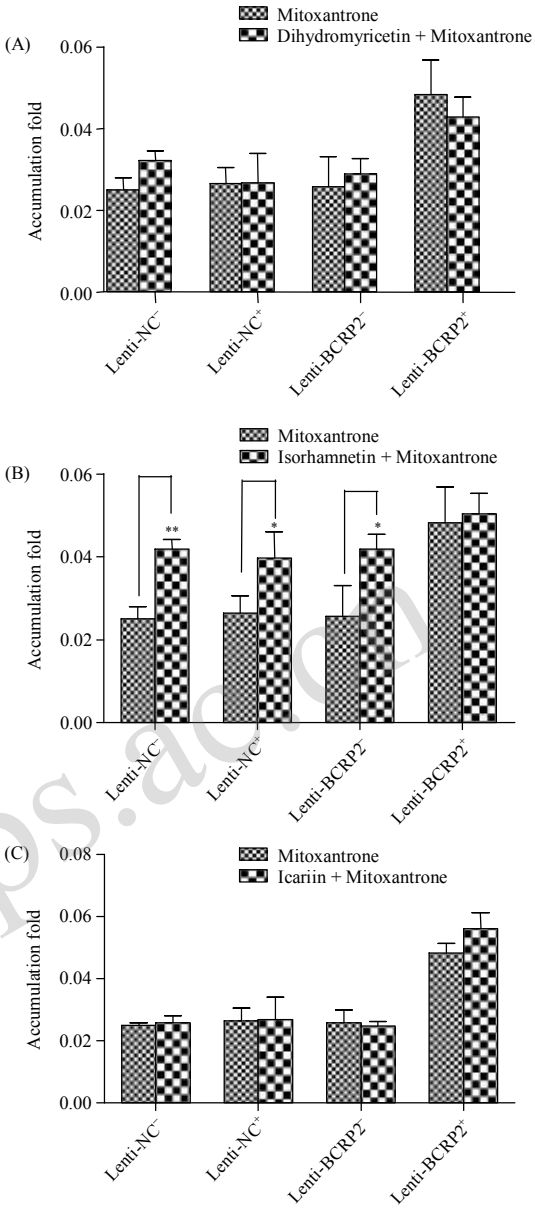

Figure 5. Effects of dihydromyricetin (A), isorhamnetin (B) and icariin (C) on intracellular accumulation of mitoxantrone. The data indicated that the intracellular mitoxantrone accumulation was significantly increased by isorhamnetin in MCF-7/MX100 cells with high levels of BCRP expression. This effect was minimal in BCRP knockdown cells (Lenti-BCRP^{+}).

addition, dihydromyricetin and icariin exhibited no effects on the mitoxantrone transport in all groups. The results indicated that isorhamnetin inhibited the BCRP-mediated cellular drug disposition.

3.4. Discussion

RNA interference technology has been widely reported in BCRP studies. miR-328[18] and miR-519[19] can reduce the BCRP expression in MCF-7/MX100 cells, leading to increased drug resistance, while the miRNA antagonist can partly reverse the drug resistance.

In the present study, we investigated the suppression function of three new siRNAs (si-BCRP1, si-BCRP2 and si-BCRP3) in MCF-7/MX100 cells. Among these siRNAs, si-BCRP2 and si-BCRP3 exhibited strong inhibitory effect on MCF-7/MX100 cells. The transfection efficiency was much improved in our study compared with previous investigations[20,21], and the delivery system might be applied to other siRNA investigations. However, these data also indicated that not all siRNAs were effective. Therefore, how to design effective siRNA has been a key question and many scientists have elaborated on it[22–24]. There are currently no reliable methods to identify the ideal sequence for a siRNA although a number of parameters. Nowadays, rational siRNA design schemes have been developed based on

an understanding of the RNAi mechanism and naturally occurring miRNA function. Besides, despite our transfection efficiency was dramatically improved and the inhibitory rate of si-BCRP2 and si-BCRP3 was much higher compared with Yang et al's study[21], it has been reported that the siRNA inhibitory effect is unstable and can just last for 48 h. The mainly reason could be that siRNAs are easy to suffer from degradation by intracellular nuclease[13]. Therefore, the development of higher efficiency and more stable gene-silencing RNAi-mediated system is desirable in order to broaden its application.

Lv et al.[20] used the shRNA expressing adenovirus vector to decrease the BCRP expression and increase the drug sensitivity by four times. However, the efficiency of transient transfection of pAVU6+27/siBCRP is only about 30%, which can not completely inhibit the BCRP expression. The results are satisfactory by stable transfection in their study. Yang et al.[21] found that although the inhibition ratio of siRNA is not significant enough by direct transfection, it can also partly reverse breast cancer drug resistance. However, it was obvious that the transient transfection of chemically synthesized siRNAs was restricted by low transfection efficiency and short-term cellular persistence of the siRNA molecules.

Therefore, to overcome the non-persistent inhibitory effect of siRNAs, the RNA interference sequences (si-BCRP2 and si-BCRP3) were inserted into lentiviral expression plasmid pTRIPZ to package lentivirus in our study. The pTRIPZ plasmid harbours puromycin resistance genes, which could be used for stable transfection. In addition, it is an inducible expression plasmid, therefore, the expression of the inserted gene and its own RFP gene should be induced by doxycycline. This expression system had many advantages that experiment could be performed in the same type of cells. What's more, uninduced cells were convenient for a follow-up study. In our investigation, we found that the BCRP expression was significantly reduced after infecting lentivirus into MCF-7/MX100 cell line. Meanwhile, the mitoxantrone accumulation was significantly improved in Lenti-BCRP3 (P<0.05).

4. Conclusions

Taken together, in the present study, we established a stable system of BCRP knockdown cell model by lentivector-mediated transduction, which might be used to reverse MDR and screen BCRP substrates and inhibitors. In addition, the knockdown cells might serve as a new model to evaluate the role of BCRP in drug discovery and development.

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References

[1] Liu, F.S. *Taiwan J. Obstet. Gynecol.* **2009**, *48*, 239–244.
[2] Lage, H. *Cell Mol. Life Sci.* **2008**, *65*, 3145–3167.
[3] Sharom, F.J. *Pharmacogenomics.* **2008**, *9*, 105–127.
[4] Lage, H.; Dietel, M. *Lancet. Oncol.* **2000**, *1*, 169–175.
[5] Ishikawa, T.; Nakagawa, H. *J. Exp. Ther. Oncol.* **2009**, *8*, 5–24.
[6] Wang, Y.Z.; Duan, X.L.; Li, Y.F.; Liang, X.J. *Prog. Biochem. Bioph.* **2009**, *36*, 1523–1529.
[7] Diah, S.K.; Smitherman, P.K.; Aldridge, J.; Volk, E.L.; Schneider, E.; Townsend, J.; Morrow, C.S. *Cancer Res.* **2001**, *61*, 5461–5467.
[8] Nagashima, S.; Soda, H.; Oka, M.; Kitazaki, T.; Shiozawa, K.; Nakamura, Y.; Takemura, M.; Yabuuchi, H.; Fukuda, M.; Tsukamoto, K.; Kohno, S. *Cancer Chemother. Pharmacol.* **2006**, *58*, 594–600.
[9] Yi, J.; Chen, J.; Sun, J.; Wei, H.L. *Zhonghua Yi Xue Za Zhi.* **2009**, *89*, 1741–1744.
[10] Ahmed-Belkacem, A.; Pozza, A.; Macalou, S.; Perez-Victoria, J.M.; Boumendjel, A.; Di Pietro, A. *Anticancer Drugs.* **2006**, *17*, 239–243.
[11] Kowalski, P.; Farley, K.M.; Lage, H.; Schneider, E. *Anticancer Res.* **2004**, *24*, 2231–2235.
[12] Kowalski, P.; Stein, U.; Scheffer, G.L.; Lage, H. *Cancer Gene. Ther.* **2002**, *9*, 579–586.
[13] Grimm, D. *Adv. Drug Deliv. Rev.* **2009**, *61*, 672–703.
[14] Chua, J.H.; Armugam, A.; Jeyaseelan, K. *Curr. Opin. Mol. Ther.* **2009**, *11*, 189–199.
[15] Yang, S.J.; Yang, L.J.; Jiang, J.; Wang, J.C.; Zhang, Q. *J. Chin. Pharm. Sci.* **2008**. *17*, 97–105.
[16] Yang, T.; Zhao, Z.X.; Xu, Z.Z.; Zhao, E.Y.; Liu, X.Y.; Chen, C.J.; Wang, J.C.; Zhang, Q. *J. Chin. Pharm. Sci.* **2011**, *20*, 590–596.

[17] Yu, C.H.; Xu, S.Y.; Yu, A.M.; Zeng, S. Proceedings of the 10th CSSX Conference and 3rd ISSX-CSSX Joint Workshop, Nanjing, China, **2012**, 35–36.
[18] Pan, Y.Z.; Morris, M.E.; Yu, A.M. *Mol. Pharmacol.* **2009**, *75*, 1374–1379.
[19] To, K.K.; Robey, R.W.; Knutsen, T.; Zhan, Z.R.; Ried, T.; Bates, S.E. *Mol. Cancer Ther.* **2009**, *8*, 2959–2968.
[20] Lv, H.; He, Z.; Liu, X.; Yuan, J.; Yu, Y.; Chen, Z. *J. Cell Biochem.* **2007**, *102*, 75–81.
[21] Yang, C.; Yin, G.; Gong, L.; Wang, J. *Immunol. J.* **2008**, *24*, 304–307.
[22] Elbashir, S.M.; Harborth, J.; Lendeckel, W.; Yalcin, A.; Weber, K.; Tuschl, T. *Nature.* **2001**, *411*, 494–498.
[23] Tuschl, T. *Mol. Interv.* **2002**, *2*, 158–167.
[24] Yue, C.; Wang, X.F.; Huang, Y.; Zhang, L.H.; Yang, Z.J. *J. Chin. Pharm. Sci.* **2012**, *21*, 5–20.

应用shRNA慢病毒技术抑制MCF7/MX100细胞中乳腺癌耐药蛋白的表达和功能

余彩虹[1a], 徐思云[1a], 喻爱明[2a], 刘彦卿[1], 胡海红[1], 李丽萍[1], 余露山[1], 周慧[1], 蒋惠娣[1], 曾苏[1*]

1. 浙江大学药学院 浙江省抗肿瘤药物临床前研究重点实验室 药物分析与药物代谢实验室, 浙江 杭州 310058
2. 纽约州立大学布法罗分校 药学院, 纽约 95817

摘要: 乳腺癌耐药蛋白(BCRP)在肿瘤细胞中的过表达可能导致肿瘤耐药性。RNA干扰是一种选择性抑制目的基因表达的实用技术。本研究旨在应用RNA干扰技术调控BCRP的表达和功能。首先在过表达BCRP的耐药细胞MCF-7/MX100中, 评价了三种靶向于BCRP的siRNAs (si-BCRP1, si-BCRP2和si-BCRP3)。研究发现si-BCRP2和si-BCRP3对BCRP的mRNA和蛋白的抑制率分别超过90%和70%, 进而导致MCF-7/MX100细胞中米托蒽醌的积聚显著上升。进一步的研究将生成si-BCRP2和si-BCRP3的shRNA序列克隆至慢病毒表达载体pTRIPZ中, 并采用慢病毒介导的转基因体系建立了稳定表达siRNA的MCF-7/MX100细胞。在该细胞系中, si-BCRP2和si-BCRP3对BCRP的mRNA的抑制率分别达到72%和56%, 对其蛋白的抑制率分别为70%和53%。在进一步的研究中, 该细胞系被用于中药活性成分的筛选, 以评价BCRP的底物和抑制剂。结果显示, BCRP低表达的MCF-7/MX100细胞可能作为良好的细胞模型被用于临床前研究。

关键词: 乳腺癌耐药蛋白, RNA干扰, 慢病毒表达系统, 耐药逆转, 米托蒽醌, 中药有效成分