

The PI3K/Akt inhibitor LY294002 reverses BCRP-mediated drug resistance without affecting BCRP translocation

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Abstract. Cellular responses toward cytotoxic drugs are influenced by crosstalk between oncogenic signals and resistance mechanisms. Inhibition of the PI3K/Akt pathway is effective in sensitizing cancer cells of various organs, although the mechanisms largely remain to be elucidated. Breast cancer resistance protein (BCRP)/ABCG2, a drug efflux pump, confers resistance to multiple anticancer agents such as SN-38 and topotecan. Previous studies reported that inhibition of the PI3K/Akt pathway, by gene knockout or PI3K inhibitors, modulated BCRP-mediated drug transport via BCRP translocation in hematopoietic stem cells, renal polarized cells and glioma stem-like cells of mammals. In this study, we assessed the effects of PI3K inhibitors, LY294002 and wortmannin, on BCRP-mediated anticancer drug resistance of human cancer MCF-7 and A431 cells. LY294002, but not wortmannin, reversed the BCRP-mediated SN-38 and topotecan resistance. LY294002 treatment did not affect total or cell surface BCRP levels as determined by western blotting and flow cytometry but blocked BCRP-mediated topotecan efflux in a dose-dependent manner. Immunohistochemical analyses also demonstrated unchanged cellular BCRP distribution. BCRP overexpression in MCF-7 and A431 cells did not confer LY294002 resistance, suggesting that LY294002 is not a transported substrate of BCRP. LY294002 is a derivative of quercetin, a member of flavonoids. Taken together, these results suggest that LY294002 inhibits BCRP-mediated drug transport not by BCRP translocation through the PI3K/Akt signal but putatively as a competitive inhibitor in a major subset of cancer cells. Due to its dual effects, LY294002 could be a lead compound for developing more effective and tolerable reagents for cancer treatment.

Introduction

The outcome of cancer chemotherapy is affected by multifactorial resistance mechanisms. One of the well-studied effectors of drug resistance are the membrane ATP-binding cassette (ABC) transporters, particularly P-glycoprotein/MDR1, the multidrug resistance related proteins MRP1, and breast cancer resistance protein (BCRP)/ABCG2 (1). Other mechanisms of drug resistance involve alteration in the apoptotic response, activation of DNA repair or stimulation of detoxifying systems, such as the exocytotic pathway for reducing intracellular drug concentration (2,3). On the other hand, cellular responses toward cytotoxic drugs are also controlled by crosstalk between oncogenic signaling pathways and resistance mechanisms. For example, enhanced sensitivity to drug treatment was observed in the presence of signaling inhibitors. Phosphatidylinositol 3-kinase (PI3K) inhibition was particularly effective in sensitizing pancreatic adenocarcinoma cells (4), ovarian cancer cells (5,6), and breast cancer cells (7,8). However, the mechanisms of how PI3K/Akt signaling pathways control drug-mediated apoptosis as well as resistance to drug-induced cell death are poorly understood.

BCRP, a half-size ABC transporter (9-11), localizes in the apical membrane of cells, across which its substrates are excreted out of cells. BCRP transports certain chlorophyll metabolites, steroid metabolites and xenobiotics and is presumed to play a protective role against toxic substances in the maternal-placental barrier, the digestive tract and the blood-testis barrier (12-15). BCRP is a molecular determinant of side-population (SP) cells, which are enriched in various stem cells (16). It also mediates the concurrent resistance to chemotherapeutic agents, such as mitoxantrone, SN-38 (an active metabolite of irinotecan), topotecan and gefitinib, by pumping them out of the cell (9-11,17-19).

Previous studies reported that inhibition of the PI3K/Akt pathway, by gene knockout or PI3K inhibitors, modulated BCRP-mediated drug transport via BCRP translocation in SP cells in the bone marrow, renal polarized cells, and glioma stem-like cells of mammals (20-22). In the present study, we examined the effects of two representative PI3K inhibitors, LY294002 and wortmannin, on the BCRP-mediated drug resistance. We show that LY294002 reversed BCRP-mediated drug resistance in human cancer cells but wortmannin did not.

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LY294002 treatment affected neither BCRP expression levels nor subcellular localization. LY294002 inhibited BCRP-mediated drug efflux in a dose-dependent manner, but BCRP overexpression in cancer cells did not result in LY294002 resistance. Our results hence suggest that LY294002 reverses BCRP-mediated drug resistance without BCRP translocation and may function as a competitive inhibitor of BCRP-mediated transport in a major subset of cancer cells.

Materials and methods

Reagents. LY294002, wortmannin and estrone were purchased from Wako (Osaka, Japan). Topotecan was purchased from Bosche Scientific (New Brunswick, NJ, USA) and gefitinib was obtained from Toronto Research Chemicals (North York, Ontario, Canada). SN-38 was generously provided by Yakult Honsha (Tokyo, Japan).

Establishment of BCRP-transfected cells. Breast cancer MCF-7 cells and epidermoid carcinoma A431 cells were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 7% fetal bovine serum (FBS) at 37°C in a humidified incubator with 5% CO₂. Establishment of MCF-7/BCRP cells has been previously described (23). A BCRP cDNA fragment was inserted into the pCAGIPuro plasmid (pCAGIPuro-BCRP) (24). A431 cells that stably expressed exogenous BCRP (A431/BCRP) were established by lipofection with pCAGIPuro-BCRP. The transfected cells were selected with 0.5 µg/ml puromycin for 7 days, and >100 clones of resistant cells were mixed and pooled for subsequent experiments. Expression levels of exogenous BCRP were stable for at least six months.

Cell growth inhibition assay. Effects of PI3K inhibitors on cell growth were evaluated by measuring cell growth after incubation at 37°C for 4 days in the absence or presence of various concentrations of each PI3K inhibitor. Cells were seeded in 12-well plates in the absence or presence of each PI3K inhibitor for 4 days. Cell numbers were determined by using a Coulter counter (Beckman Coulter, Fullerton, CA, USA) and presented as percentages relative to those of control cells.

Next, effects of the PI3K inhibitors on anticancer drug resistance were evaluated by measuring cell growth after incubation at 37°C for 4 days in the absence or presence of various concentrations of anticancer drugs in combination with each PI3K inhibitor. Cell numbers were determined and presented as percentages relative to those of control cells cultured in the absence of anticancer drugs.

Western blot analysis. Cells (1 × 10⁶) were incubated in DMEM with or without various concentrations of LY294002 for 24 h. Western blot analysis was performed under the reducing conditions as previously described (23). The blots were incubated with 5 µg/ml of the mouse anti-BCRP monoclonal antibody, BXP-21 (Chemicon, Temecula, CA, USA). GAPDH expression was also analyzed as a loading control.

Fluorescence-activated cell sorting (FACS) analyses. Effects of specific compounds on the cellular accumulation of topotecan were determined by flow cytometry. Trypsinized cells

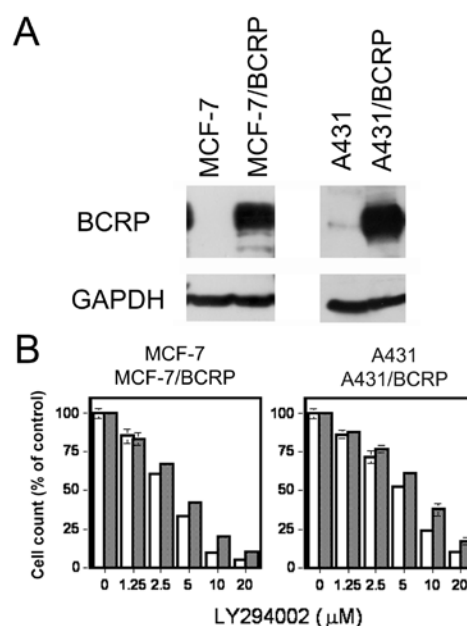


Figure 1. (A) BCRP expression in the BCRP-transfected cells. Protein sample (20 µg of MCF-7 and MCF-7/BCRP and 10 µg of A431 and A431/BCRP) was loaded in each lane, and western blot analysis was performed under the reducing conditions. BCRP was detected using BXP-21. (B) LY294002 resistance of the BCRP-transfected cells. MCF-7/BCRP and MCF-7 cells (3 × 10⁴/well) as well as A431/BCRP and A431 cells (5 × 10³/well) were seeded into 12-well plates, and cultured in the absence or presence of various concentrations of LY294002 for 4 days. Cell numbers were determined, and presented as percentages relative to those of control cells cultured without LY294002. Left, MCF-7 and MCF-7/BCRP cells. Right, A431 and A431/BCRP cells. Open bars, parental cells. Dotted bars, BCRP-transfected cells. The data shown are the means ± SD of triplicate determinations, and are representative of two independent experiments. Where a vertical bar is not shown, the SD is within the bar.

(5 × 10⁵) were incubated in the presence of indicated concentrations of reagents for 60 min at 37°C. After addition of topotecan to the medium, the cells were incubated for an additional 30 min at 37°C, washed in ice-cold phosphate-buffered saline (PBS), and subjected to fluorescence analysis by using a FACSCalibur (Becton-Dickinson, San Jose, CA, USA).

Amounts of cell surface BCRP were measured by FACS. Cells were incubated in DMEM for 12 h without or with 2 µM (MCF-7/BCRP and MCF-7) or 1 µM (A431/BCRP or A431) LY294002. Trypsinized cells (5 × 10⁵–1 × 10⁶) were incubated with 10 µg/ml of biotinylated anti-human ABCG2, 5D3 (eBiosciences, San Diego, CA, USA) or mouse IgG-BIOT conjugate (Beckman Coulter) for 30 min on ice in the dark, washed and incubated with 10 µg/ml of R-phycoerythrin-conjugated streptavidin (Becton-Dickinson) for an additional 30 min on ice in the dark. After washing, the fluorescence staining level was analyzed with a FACSCalibur.

Immunofluorescence analysis of BCRP. Cells were treated with 1 µM (A431/BCRP) or 2 µM (MCF-7/BCRP) of LY294002 for 12 h and collected with cell scrapers. The cells were routinely formalin-fixed, paraffin-embedded as a cell block and processed. Antigen-retrieval was performed by autoclaving (121°C) for 15 min. The slides were then incubated with 12.5 µg/ml of BXP-21 at 4°C overnight followed by incubation with a polyclonal anti-mouse immunoglobulin/FITC

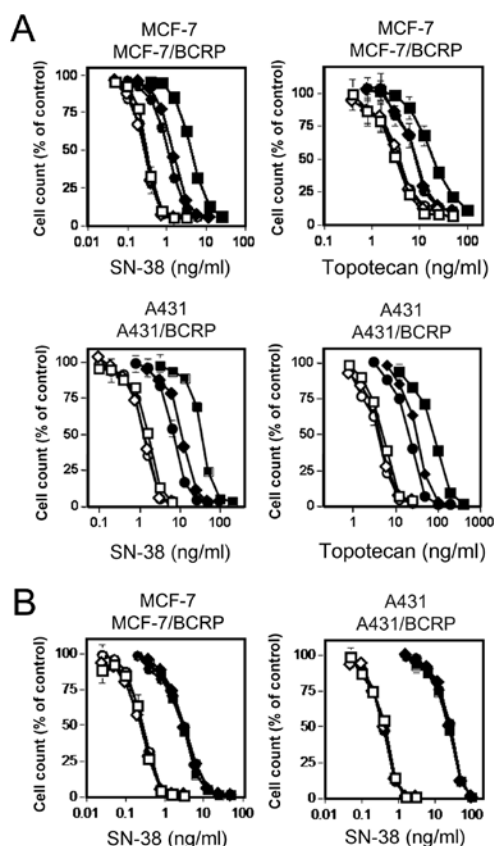


Figure 2. (A) Effects of LY294002 on anticancer drug resistances of BCRP-transfected cells. MCF-7/BCRP as well as MCF-7 cells (3×10^4 /well) and A431/BCRP as well as A431 cells (5×10^3 /well) were seeded into 12-well plates, and cultured in the absence or presence of various concentrations of anticancer drugs with or without LY294002 for 4 days. Cell numbers were determined, and presented as percentages relative to those of control cells cultured in the absence of anticancer agents. Open symbols, the parental cells. Closed symbols, the BCRP-transfected cells. The data shown are the means \pm SD of triplicate determinations, and are representative of three independent experiments. Where a vertical bar is not shown, the SD is within the symbol. Squares, without LY294002. Diamonds, 1 μ M (MCF-7) or 0.5 μ M (A431) of LY294002. Circles, 2 μ M (MCF-7) or 1 μ M (A431) of LY294002. (B) Effects of wortmannin on SN-38 resistance of BCRP-transfected cells. Experimental procedures and presentation are the same as described above. Open symbols, parental cells. Closed symbols, BCRP-transfected cells. Squares, without wortmannin. Diamonds, 1 μ M (MCF-7) or 0.5 μ M (A431) of wortmannin. Circle, 2 μ M (MCF-7) or 1 μ M (A431) of wortmannin.

secondary antibody (1:20 dilution) (Dako, Glostrup, Denmark) at 4°C for 2 h. BCRP was detected by fluorescence microscopy as green fluorescence and cell nuclei were indicated by purple fluorescence.

Results

Effects of LY294002 and wortmannin on drug resistances of BCRP-transfected cells. MCF-7/BCRP and A431/BCRP cells were found to overexpress BCRP (Fig. 1A) and they showed significantly higher resistances to SN-38 and topotecan than the corresponding parental cells. These transfected cells also acquired slightly more resistance to the PI3K inhibitors (Fig. 1B and data not shown), but the degrees of resistance did not seem to be significant considering the degrees of resistance against anticancer drugs. LY294002 at concentrations $<2 \mu$ M

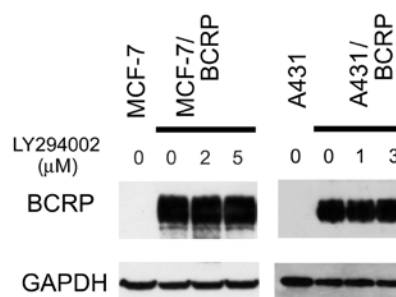


Figure 3. Effects of LY294002 on BCRP protein expression. Cells were cultured in the absence or presence of various concentrations of LY294002 for 24 h. After harvesting the cells, protein sample (20 μ g of MCF-7/BCRP and 5 μ g of A431/BCRP) was loaded in each lane and western blot analysis was performed. The data are representative of three independent experiments.

showed growth inhibitory effects $<30\%$ on MCF-7 and A431 cells after 4-day treatment. LY294002 significantly enhanced the cytotoxicities of SN-38 and topotecan on MCF-7/BCRP and A431/BCRP cells in a dose-dependent manner, while it only slightly affected the cytotoxicities on MCF-7 and A431 cells (Fig. 2A). Wortmannin at the concentrations of 2 (for MCF-7 cells) or 1 μ M (for A431 cells) inhibited cell growth approximately by 30% after 4-day treatment (data not shown) but did not show any significant reversing effects of BCRP-mediated drug resistances in these cells (Fig. 2B).

Effects of LY294002 on BCRP expression. Since LY294002 treatment reversed BCRP-mediated multidrug resistance, BCRP expression levels in the transfectants were investigated by western blot analysis. Total BCRP levels in MCF-7/BCRP and A431/BCRP cells were not affected by the treatment with up to 5 or 3 μ M of LY294002, respectively (Fig. 3).

Effects of LY294002 on cellular topotecan uptake in BCRP-transfected cells. Next, effects of LY294002 on cellular accumulation of topotecan were evaluated by flow cytometric analysis. Cellular topotecan uptake increased in the parental MCF-7 and A431 cells in a dose-dependent manner, but not in the BCRP-transfected cells, in the absence of the reagents (Figs. 4 and 5). Treatment with LY294002 increased cellular topotecan uptake in MCF-7/BCRP in a dose-dependent manner (Fig. 4). The effects were somewhat weaker than those of gefitinib but were stronger than estrone, both known to be BCRP inhibitors (19,25). Treatment of A431/BCRP cells with 10 μ M LY294002 in the culture medium involving 10 μ M topotecan revealed only marginal increase in cellular topotecan uptake, to the degree similar to estrone (Fig. 5A). However, LY294002 treatment at the concentrations of 10–20 μ M clearly demonstrated increased cellular topotecan uptake in a dose-dependent manner in the medium containing 20 μ M of topotecan (Fig. 5B). The effects were weaker than those of gefitinib but were stronger than estrone similarly to our observations in MCF-7/BCRP cells.

FACS analysis of cell surface BCRP expression. Based on previous reports, we expected to observe BCRP translocation in the BCRP-transfected cells after the initiation of

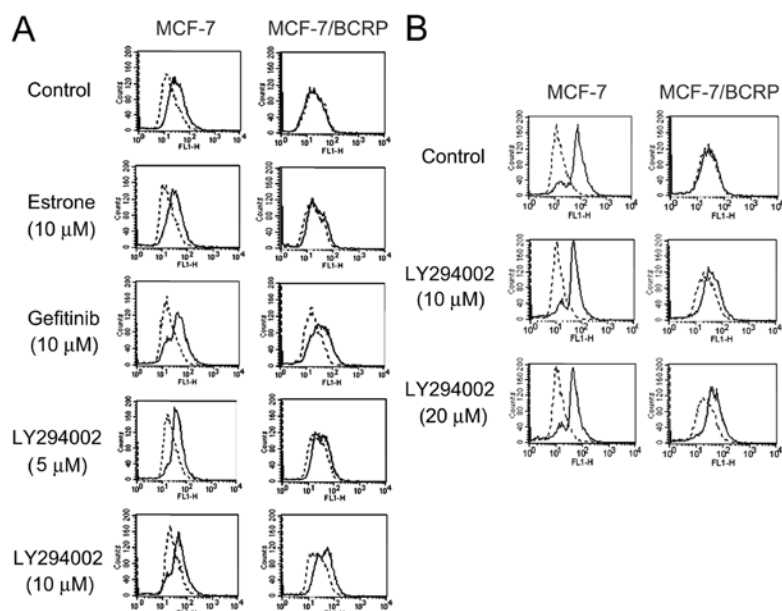


Figure 4. Effects of LY294002 on cellular topotecan uptake of MCF-7/BCRP cells. Trypsinized cells (5×10^5) were pre-incubated with indicated concentrations of reagents for 60 min. The cells were then incubated with (solid line) or without (dotted line) (A) $20 \mu\text{M}$ or (B) $40 \mu\text{M}$ of topotecan for 30 min in the presence of the reagents. Finally, cellular topotecan uptake was measured by flow cytometry. The data are representative of three independent experiments.

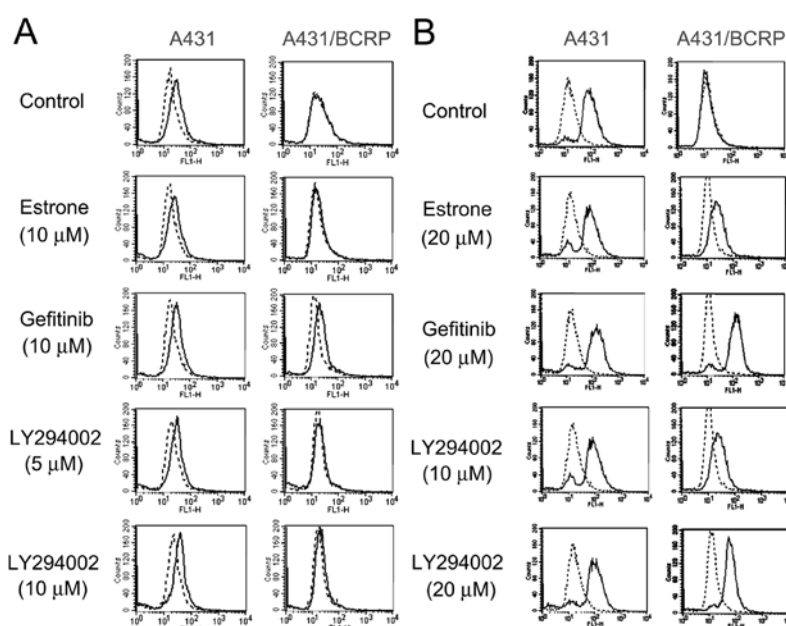


Figure 5. Effects of LY294002 on cellular topotecan uptake of A431/BCRP cells. Trypsinized cells (5×10^5) were pre-incubated with indicated concentrations of reagents for 60 min. The cells were then incubated with (solid line) or without (dotted line) (A) $10 \mu\text{M}$ or (B) $20 \mu\text{M}$ of topotecan for 30 min in the presence of the reagents. Finally, cellular topotecan uptake was measured by flow cytometry. The data are representative of three independent experiments.

LY294002 treatment. BCRP localization on the cell surface after LY294002 treatment was confirmed by FACS. Although endogenous BCRP was scarcely detected by western blot analysis under the reducing conditions (Fig. 1A), considerable amounts of BCRP were detected on the cell surface of MCF-7 and A431 cells by FACS analysis (Fig. 6). It was also confirmed by FACS that BCRP-transfected cells expressed significantly greater amounts of BCRP on the cell surface than the corresponding parental cells. However, contrary to our

expectation, amounts of BCRP expressed on the cell surface were similar with and without LY294002 treatment in both parental and BCRP-transfected cells. This surprising result suggested that trafficking problem was not a main reason for the loss of BCRP function mediated by LY294002.

Immunofluorescence analyses of the subcellular BCRP localization. We next observed BCRP expression in the cells with or without LY294002 treatment by fluorescence

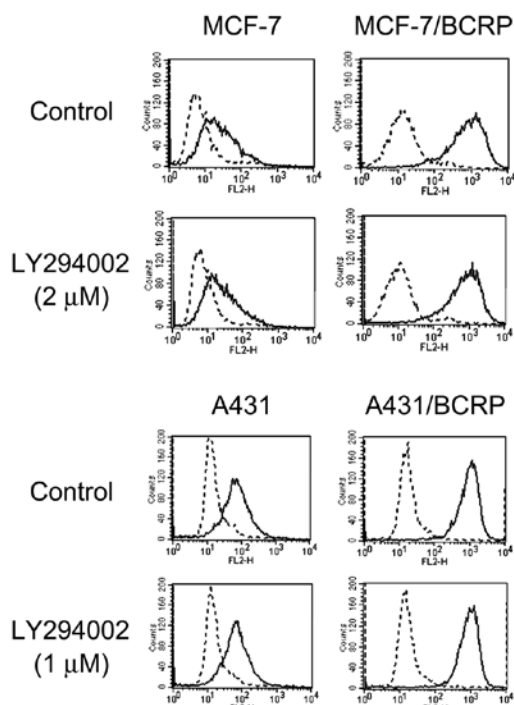


Figure 6. FACS analysis of BCRP expression on the cell surface. Cells were incubated for 12 h with or without 2 μ M (MCF-7/BCRP and MCF-7) or 1 μ M (A431/BCRP or A431) LY294002. Trypsinized cells (5×10^5 – 10^6) were incubated with either biotinylated mouse IgG or anti-human ABCG2, 5D3, washed and incubated with R-phycoerythrin-conjugated streptavidin. Bold lines indicate fluorescence in the presence of 5D3 and dotted lines indicate control fluorescence in the presence of mouse IgG.

immunocytochemistry. The *BCRP*-transfected cells were found to express BCRP mainly on the plasma membrane, but translocation of BCRP was not observed in the cells treated with LY294002 in this experimental setting (Fig. 7). Since LY294002 at the concentrations used in these experiments reversed BCRP-mediated drug resistance to a significant degree (Fig. 2A), these results clearly demonstrated that LY294002 can reverse BCRP-mediated drug resistance without BCRP translocation and suggest its role as a competitive inhibitor.

Discussion

For the past decade, there is growing evidence that alterations of the oncogenic signaling pathways can affect cellular responses to anticancer drugs. PI3K enzymes are regulated by growth factors and serve to phosphorylate phospholipids at the plasma membrane. Activated PI3K phosphorylates phosphatidylinositol biphosphate (PIP₂) and generates phosphatidylinositol trisphosphate (PIP₃), which acts as a second messenger. Akt interacts with PIP₃, subsequently translocates to the plasma membrane, and plays a critical role in controlling the balance between survival and apoptosis. In addition to that, PI3K/Akt inhibitors have been known to cause more favorable outcomes when co-administered with usual anticancer drugs. Several mechanisms have been postulated, such as alterations of intracellular trafficking of anticancer drugs and/or the DNA repair system, and deregulation of drug transporters. In this study, we demonstrated that the PI3K inhibitor LY294002

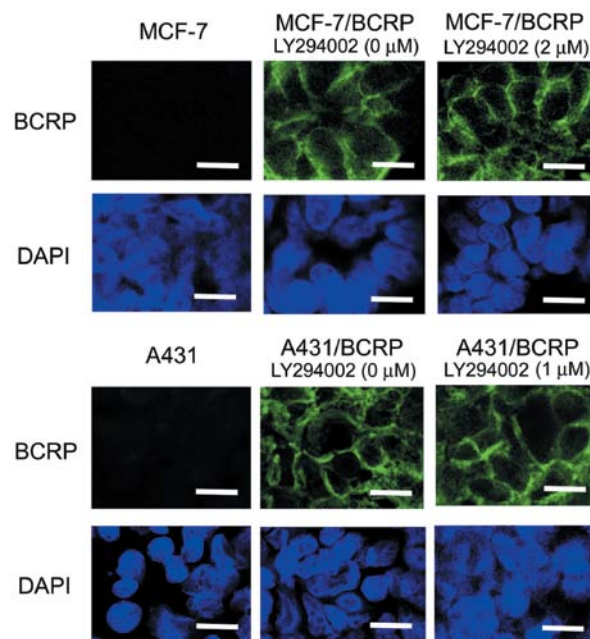


Figure 7. Immunofluorescence analyses of the subcellular BCRP localization. Cells were treated with 2 μ M (MCF-7/BCRP) or 1 μ M (A431/BCRP) LY294002 for 12 h, collected as a cell block, formalin-fixed, paraffin-embedded, and processed. BCRP is presented as green fluorescence, while cell nuclei are indicated by purple fluorescence. Scale bar, 30 μ m.

sensitized BCRP-overexpressing cancer cells to its substrate anticancer drugs. BCRP function can be regulated through several mechanisms, including expression regulation at the RNA/protein levels and competitive block of ATP-binding or substrate transport. Previous studies reported that inhibition of the PI3K/Akt pathway caused BCRP internalization in cells. Mogi *et al* reported a reduced SP fraction in the bone marrow of Akt1-null mice (20). Enforced expression of Akt increased the SP fraction in the wild-type mouse bone marrow but not in the *Bcrp*^{-/-} bone marrow. They also showed that treatment of mouse bone marrow cells with 10 μ M of LY294002 for 30 min depleted the SP cell population. Then, after 30 min of incubation without LY294002, the SP cell fraction recovered to the untreated cell levels. Finally, they showed that treatment of SP cells with 10 μ M of LY294002 for 90 min promoted BCRP translocation from the plasma membrane to the intracellular compartment. The depletion of SP cells in the bone marrow was attributable to BCRP internalization by inhibiting the PI3K/Akt pathway.

Alteration of subcellular BCRP localization was also observed in porcine non-SP cells, LLC-PK1 (21). Treatment with 20 μ M of LY294002 for 90 min caused BCRP internalization from the cell surface. Transfection of the dominant-negative Akt plasmid resulted in internalization of a part of BCRP in LLC-PK1 cells. Similar results have been reported concerning glioma tumor stem-like cells (22). Treatment of SP cells from PDGF-induced, PTEN-deleted, glioma cells with 20 μ M of LY294002 for 16 h resulted in internalization of BCRP from the cell surface. Changes in BCRP localization after LY294002 treatment were also observed in SP cells from *BCRP*-transfected human glioma cell line U87-MG. These studies led us to hypothesize that the reversal of BCRP-mediated

drug resistance would be attributable to alteration of cellular BCRP trafficking.

However, effects of the PI3K/Akt inhibitors on BCRP function were considerably different in *BCRP*-transfected MCF-7 and A431 cells. First, LY294002 reversed BCRP-mediated drug resistances, but another PI3K inhibitor, wortmannin, did not. Considering that wortmannin has stronger pharmacological activities than LY294002, reversal by LY294002 could not be due to inhibition of the PI3K/Akt signal but by specific characteristics of the compound.

Next, although LY294002 treatment resulted in inhibition of drug efflux in *BCRP*-transfected cells, either total cellular BCRP or cell surface BCRP levels were not changed as demonstrated by western blotting and FACS analyses. The presented data are derived from experiments using 1 or 2 μ M of LY294002, but similar results were obtained from experiments using LY294002 up to 10 μ M (data not shown). In addition, immunocytochemical analyses have clearly demonstrated no recognizable effects on cellular BCRP distribution after LY294002 treatment for 12 h in the both cell lines.

Since spontaneous recovery of the cell surface BCRP distribution could not be excluded during the LY294002 treatment, we established *EGFP-BCRP*-transfected MCF-7 and A431 cells. Those cells expressed EGFP-BCRP mainly on the cell membrane and acquired resistances to BCRP-substrate anticancer drugs, which were reversed by LY294002 (data not shown). We then performed live observation of cellular EGFP-BCRP distribution of living cells in the presence of LY294002 ranging from 1 to 20 μ M for up to 48 h, and could not observe alterations in the EGFP-BCRP localization at any time (data not shown). All these results suggest that LY294002 does not affect cellular BCRP distribution in the cancer cell lines.

Nevertheless, LY294002 was found to exert inhibitory effects on BCRP-mediated topotecan efflux, which were stronger than estrone but weaker than gefitinib. Thus, LY294002 demonstrated BCRP-inhibitory effects different from those observed in the previous studies. To explain the reasons for this discrepancy, we postulate two possible explanations. The first one is the difference of cell characters between stem cells and non-stem cells. Regulation of subcellular BCRP distribution by the PI3K/Akt pathway might be specific to a subset of cells such as stem/stem-like cells. It is known that the PI3K/Akt signaling pathway is activated and plays a role in the maintenance of pluripotency and viability of stem cells (26). BCRP is expressed on the cell surface of pluripotent stem cells but becomes downregulated with cell maturation. On the other hand, the PI3K/Akt signals have been reported to regulate activity and trafficking of some transporters and/or receptors, such as transferrin receptor, low-density lipoprotein receptor, the amino acid transporter 4F2, integrin, and Glut1 through regulating endocytosis and recycling (27,28). Therefore, the PI3K/Akt signaling pathway may be involved in mechanisms of BCRP trafficking to the cell surface and maintenance of the pluripotency of stem cells. In addition, the MEK/ERK pathway is likely to act downstream of the PI3K/Akt pathway in the embryonic stem cells (26). We previously reported that inhibition of the Ras/Raf/MEK/ERK pathway results in internalization and degradation of the BCRP protein (29). Therefore, BCRP translocation caused by inhibiting PI3K/Akt signaling may be specific to stem/stem-like cells in which the

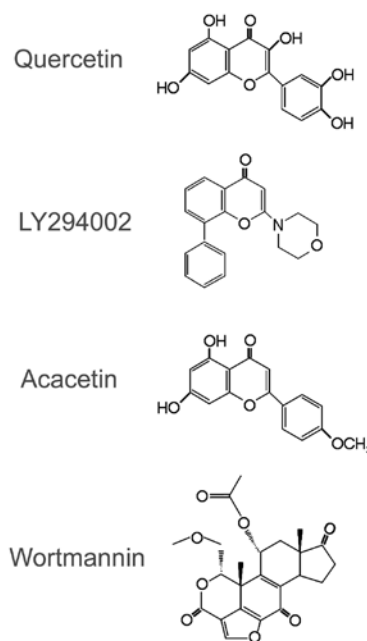


Figure 8. Chemical structures of the PI3K inhibitors and the referred flavonoids.

signal is inherently kept active. Because of a very small fraction of cancer stem cells, we might not have detected internalized BCRP in our experimental setting. Another explanation would be that some of the BCRP protein might be internalized, but expression levels of overexpressed BCRP in cancer cells are too high to discern internalized BCRP and most of BCRP would be left uninternalized. For successful internalization of the whole expressed BCRP, upregulation of proteins associated with protein trafficking might be necessary. But, we are afraid that this hypothesis cannot successfully explain the data shown in Figs. 2, 4 and 5. To explain the reversal effects, we reasoned that LY294002 would interact with the drug-binding sites of BCRP.

LY294002 was designed as a synthetic PI3K inhibitor based on quercetin, a naturally occurring flavonoid that inhibits a broad range of protein kinases (30,31). Quercetin and other flavonoids have been found to reverse BCRP-mediated drug resistance to variable degrees (32). In addition, a member of the flavonoids, genistein, has been demonstrated to be subjected to BCRP-mediated transport in its native form (32). In fact, the chemical structure of LY294002 is very similar to those of quercetin and acacetin, the strongest flavonoid that reverses BCRP-mediated multidrug resistance, but the chemical structure of wortmannin, a furanosteroid metabolite of the fungus, is quite different (Fig. 8). Since BCRP overexpression did not result in acquisition of comparable degrees of the LY294002 resistance with those of anticancer drugs (Fig. 1B), we reason that LY294002 would not be a transported substrate of BCRP, but that it would competitively interact with the drug-binding site. Further investigations are needed to clarify the interaction of LY294002 with BCRP, and we are planning further experiments.

Furthermore, it has been reported that LY294002 competitively inhibited MRP1-mediated doxorubicin transport (33). Therefore, LY294002 can inhibit both MRP-1 and BCRP,

which are two major drug transporters that cause multidrug resistance. Based on these findings, flavonoid-based PI3K inhibitors could be an attractive choice for cancer chemotherapy due to their multifunction in inhibition of drug efflux pumps and in the oncogenic signaling pathways.

In conclusion, LY294002 sensitized BCRP-overexpressing drug resistant cancer cells without affecting BCRP translocation. Due to its multifunctional effects, LY294002 can be used for developing more effective and tolerable reagents for cancer treatment.

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