

# Characterization of SN38-Resistant T47D Breast Cancer Cell Sublines Overexpressing BCRP, MRP1, MRP2, MRP3, and MRP4

Hee-Jeong Lee

Chosun University Hospital

Cheol-Hee Choi (✉ [chchoi@Chosun.ac.kr](mailto:chchoi@Chosun.ac.kr))

Chosun University School of Medicine: Chosun University College of Medicine

---

## Research article

**Keywords:** Breast cancer, T47D, SN-38, multidrug resistance, breast cancer resistance protein, epigenetic silencing, chemosensitizer

**Posted Date:** January 12th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-141966/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at BMC Cancer on April 23rd, 2022. See the published version at <https://doi.org/10.1186/s12885-022-09446-y>.

# Abstract

## Background

Although several novel resistant breast cancer cell lines have been established, only a few resistant breast cancer cell lines overexpress breast cancer resistance proteins (BCRP). The aim of this study was to establish new resistant breast cancer cell lines overexpressing BCRP using SN38 (7-ethyl-10-hydroxycamptothecin), an active metabolite of irinotecan and was to discover genes and mechanisms associated with multidrug resistance.

## Methods

SN38-resistant T47D breast cancer cell sublines were selected from the wild-type T47D cells by gradually increasing SN38 concentration. The sensitivity of the cells to anti-cancer drugs was assessed by 3-(4,5-methylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Expression profiles of the resistance-related transporters were examined using the resistance diagnostic kit (Drugsposter<sup>®</sup>), real time RT-PCR, and western blot analysis. Intracellular drug accumulation in the resistant cells was determined using flow cytometry.

## Results

The SN38-resistant T47D breast cancer cell sublines T47D/SN120 and T47D/SN150 were established after long-term exposure (more than 16 months) of wild-type T47D cells to 120 nM and 150 nM SN38, respectively. T47D/SN120 and T47D/SN150 cells were more resistant to SN38 (14.5 and 59.1 times, respectively), irinotecan (1.5 and 3.7 times, respectively), and topotecan (4.9 and 12 times, respectively), than the wild-type parental cells. Both T47D/SN120 and T47D/SN150 sublines were cross-resistant to various anti-cancer drugs. These resistant sublines overexpressed mRNAs of MRP1, MRP2, MRP3, MRP4, and BCRP. The DNA methylase inhibitor 5-aza-2'-deoxycytidine and the histone deacetylase inhibitor trichostatin A increased the expression levels of BCRP, MRP1, MRP2, MRP3, and MRP4 transcripts in T47D/WT cells. Drug accumulation was found to be lower in T47D/SN120 and T47D/SN150 cells, compared to that in T47D/WT cells. However, treatment with known chemosensitizers increased the intracellular drug accumulation and sensitivity of anti-tumor agents.

## Conclusion

T47D/SN120 and T47D/SN150 cells overexpressed MRP1, MRP2, MRP3, MRP4, and BCRP due to the suppression of epigenetic gene silencing via DNA hypermethylation and histone deacetylation. Although these resistant cells present a higher resistance to various anti-cancer drugs than their parental wild-type cells, multidrug resistance was overcome by treatment with chemosensitizers. These SN38 resistant T47D breast cancer cell sublines expressing resistance proteins can be useful for the development of new chemosensitizers.

# Background

Breast cancer is not only a frequently diagnosed cancer worldwide, it is also a leading cause of cancer-related death in women (1). The treatment of breast cancer is largely classified into two categories: local treatment including surgery, radiation, or both modalities; and systemic treatment including cytotoxic chemotherapy, endocrine therapy, biological therapy, or a combination of these. Systemic treatment, which is used in adjuvant, neoadjuvant, and palliative settings, plays an important role in the treatment of breast cancer at various stages. Indeed, systemic agents are effective at the beginning of therapy in 69-95% of primary breast cancers (2-6) and 50% of metastatic cancers (7-9). However, cancer progression often occurs after a variable duration of chemotherapy, owing to the development of chemotherapy resistance, which is divided into primary resistance and acquired resistance. Primary resistance includes cases that are unresponsive despite the use of appropriate initial chemotherapy, with continued tumor growth during the treatment (10). Acquired resistance involves cases where the tumor cells initially seem to respond well to the chemotherapy but acquire resistance to the anti-cancer drugs due to repeated exposure (10); in this case, cells are resistant to some agents of the same class but sensitive to drugs of different classes. However, eventual cross-resistance to multiple anti-cancer drugs of apparently different structures and functions is observed; this phenomenon is known as multidrug resistance (MDR) (11).

The mechanisms and pathways of MDR are complicated and multi-factorial. One of the mechanisms of MDR is associated with the alteration of anti-cancer drug transporter. One of the classic MDR mechanisms involves decreased drug accumulation due to increased expression of drug efflux pump in the tumor cell membrane (12). This MDR phenotype is mediated by ATP (adenosine triphosphate)-binding cassette (ABC) transporters (12), including P-glycoprotein (13-15) and members of the multidrug resistance protein (MRP) family (16-18). Another novel transporter, breast cancer resistance protein (BCRP), has been identified as an ABC half-transporter and is distributed in the placenta and various cancer types (19-22). Meanwhile, it was reported that a redistribution of the anti-cancer drug from the nucleus to the cytoplasm is related to non-ABC transporters-mediated MDR (23). Lung resistance protein is involved in the nucleo-cytoplasmic transport and cytoplasmic sequestration of anti-cancer drugs (24-26). Another mechanism of MDR is associated with alterations in topoisomerase (27-31).

Based on comprehensive knowledge about the mechanisms of drug resistance, recent studies on the mechanism of resistance to anticancer drugs in breast cancer have been reported. (32). Moreover, exploring the mechanism of MDR and finding molecular targets for drug resistance is important for the development of new therapeutic agents to treat cancer. Thus, further studies are vital for a better understanding of MDR mechanisms in breast cancer and development of various types of resistant cancer cell lines.

Recently, several novel resistant breast cancer cell lines have been established. Notably, although BCRP expression is observed in primary breast cancer as well as in normal breast tissue (33-35), only a few resistant breast cancer cell lines overexpress BCRP (36-39). Tumor cell lines resistant to the camptothecin-derived topoisomerase I inhibitor topotecan have been shown to overexpress BCRP and

display a significant cross-resistance to CPT11, SN38 (7-ethyl-10-hydroxycamptothecin), and 9-aminocamptothecin (28, 40-42). SN38, an active metabolite of irinotecan, possesses a much stronger cytotoxicity against tumor cells than irinotecan (43, 44) through the inhibition of DNA topoisomerase I (45). Among breast cancer cell lines, MDA-MB 231 is estrogen receptor (ER)-negative, while T47D and MCF-7 are ER-positive (46). Recently, SN-38-resistant MCF-7 and MDA-MB-231 sublines have been generated (47). However, no SN38-resistant breast cancer cell T47D sublines have been developed so far. Therefore, the specific aim of this study was to establish SN38-resistant breast cancer cell sublines, anticipating the overexpression of transporters, such as BCRP. Herein, we describe two SN38-resistant breast cancer cell lines, T47D/SN120 and T47D/SN150, characterized by MRP1, MRP2, MRP3, MRP4, and BCRP overexpression. These resistant cancer cell sublines will be used to develop chemosensitizers that can reverse the resistance.

## Methods

### Cell culture

The human breast cancer cell line T47D was purchased from the Cancer Research Center in Seoul National University (Seoul, South Korea). The cells were cultured in RPMI-1640 (Gibco BRL Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA). The cells were allowed to adhere to the culture dish and form a monolayer. The cells were sub-cultured once they reached confluence.

### Establishment of the SN38-resistant breast cancer cell sublines

The SN38-resistant breast cancer cell sublines T47D/SN120 and T47D/SN150 were established by gradually increasing the concentration of SN38 from 15 nM ( $IC_{50}$  value) to final concentrations of 120 nM ( $IC_{50} \times 8$ ) and 150 nM ( $IC_{50} \times 10$ ), respectively in the parental T47D cells. Stable T47D/SN120 and T47D/SN150 cell lines were obtained in a timeframe of 16 months.

### Calculation of cellular population doubling time

The cells were seeded into 24-well culture plates at a density of  $5 \times 10^4$  cells per well and incubated at 37°C for 24 h. The cell numbers were counted every 24 h for 4 days. The cell doubling time ( $T_d$ ) was calculated using the formula:  $T_d = T \times \log 2 / (\log N_t - \log N_0)$ , where  $N_0$  and  $N_t$  represent the number of cells at the beginning and end of the culture during time  $T$ , respectively.

### Chemosensitivity test using MTT assay

The 3-(4,5-methylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma-Aldrich) assay was performed to assess the sensitivity of T47D/SN120 and T47D/SN150 cells to anti-cancer drugs. 50% inhibitory concentration ( $IC_{50}$ ) was defined as the drug concentration that causes a 50% reduction in the number of cells compared to the untreated control. The  $IC_{50}$  values were determined directly from the

dose-response curves. Resistance factor (RF) was calculated from the ratios of the IC<sub>50</sub> values of T47D/SN120 and T47D/SN150 to T47D/WT cells.

### **RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) assay**

Total RNA was extracted from the cells using the RNeasy mini kit (Qiagen, Hilden, Germany). mRNA expression was determined by RT-PCR and normalized to  $\beta$ -actin mRNA expression level. Genes as well as the conventional and real-time primer pairs are listed in Table 1.

RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, USA) and an oligo (dT) primer for 1 h at 37°C. The synthesized cDNA was diluted 1:5 with water and amplified using 2.5 units of Taq DNA polymerase (TaKaRa, Tokyo, Japan) and 10 pmol of each primer, under defined PCR conditions, using a GeneAmp PCR system 9600 (Perkin-Elmer-Cetus, Waltham, MA, USA). After the final cycle, all the PCR products were subjected to a final extension at 72°C for 5 min. The PCR products were electrophoresed on agarose gel. The endpoint used in PCR quantification (Ct) was defined as the PCR cycle number that crosses an arbitrarily placed signal threshold.

### **Western blot analysis**

Cells were washed with phosphate-buffered saline (PBS) and lysed in 50 mM Tris-HCl (pH 7.4), 250 mM NaCl, 0.5% Triton X-100, 10% glycerol, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (Pierce Biotechnology, Rockford, IL, USA). The cell lysates were centrifuged and then resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Western blotting was performed using a slight modification of the method described previously (48). The membrane was incubated with primary rabbit polyclonal antibodies against MRP1 (1:1000; Invitrogen, Carlsbad, CA, USA), MRP2 (1:5000; Sigma-Aldrich), MRP3 (1:50; Abcam, Cambridge, England), MRP4 (1:50; Abcam), BCRP (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and  $\beta$ -actin (1:2500; Santa Cruz Biotechnology). The membrane was then washed and incubated with horseradish peroxidase-conjugated secondary antibodies (1:2500 for BCRP and  $\beta$ -actin) for 1 h. The signal was then detected using the ECL detection kit (Amersham, Piscataway, NJ, USA).

### **Drug accumulation assay**

Cell suspensions ( $5 \times 10^5$  cells) in PBS were exposed to 1  $\mu$ M rhodamine 123, 50 nM calcein-AM, and 20  $\mu$ M mitoxantrone at 37°C for 1 h. Additionally, cells were incubated in the presence of each fluorescent substrate with 5  $\mu$ M PSC833, 5 mM probenecid, 100  $\mu$ M genistein, and 2 mM cyanide in PBS at 37°C for 1 h. After incubation, cellular drug accumulation was determined using a flow cytometer (FACSCalibur, Becton Dickinson, MA, USA), which detected drug fluorescence. A focused argon laser beam (488 nm) excited the cells in a laminar sheath flow, following which the fluorescence emissions at 530 nm (for rhodamine 123 and calcein-AM) and 670 nm (for mitoxantrone) were detected to generate the histogram.

## Screening of chemosensitizers

IC<sub>50</sub> values of SN38 were obtained in the presence and absence of chemosensitizers for SN38-resistant T47D sublines and the ratios were defined as chemosensitizing index.

## Statistical analysis

Statistical significance of the data was determined using student's t-test. P values less than 0.05 were considered significant.

# Results

## Establishment and characterization of resistant cell lines

SN38-resistant T47D/SN120 and T47D/SN150 cell sublines were established from the wild-type T47D cells following long-term exposure (of more than 16 months) to 120 nM and 150 nM SN38, respectively. Microscopic observation showed some distinct features in the SN38-resistant T47D sublines compared to their parental cell line. In monolayer, T47D/WT cells were relatively consistent in size and shape, while the resistant cells presented a spindle-shaped morphology and were smaller in size (data not shown). Moreover, the doubling times of T47D/SN120 and T47D/SN150 cells were 35.5 h and 32.8 h, respectively, different from that of T47D/WT cells (69.2 h).

## Cross-resistance to other anti-cancer drugs of resistant cell lines

MTT assay showed that T47D/SN120 and T47D/SN150 cells were more resistant to SN38 (14.5 and 59.1 times, respectively), irinotecan (1.5 and 3.7 times, respectively), and topotecan (4.9 and 12 times, respectively) as compared to the wild-type drug-sensitive parental cells. In addition to topoisomerase I inhibitors, both T47D/SN120 and T47D/SN150 sublines were cross-resistant to various anti-cancer drugs that are used in breast cancer treatment, including microtubule inhibitors (paclitaxel and vinblastine), anti-metabolites (5-fluorouracil and methotrexate), topoisomerase II inhibitors (doxorubicin and mitoxantrone), estrogen receptor blockers (tamoxifen and endoxifen), an alkylating agent (cisplatin), and a tyrosine kinase inhibitor (gefitinib) (Table 2).

As shown in Table 2, both T47D/SN120 and T47D/SN150 sublines were highly resistant to microtubule inhibitors, including paclitaxel and vinblastine. On the other hand, compared to the T47D/SN120 subline, the T47D/SN150 subline presented weak resistance to gefitinib, 5-FU, and irinotecan, and was sensitive to methotrexate and endoxifen.

## Expression profiles of 24 transporters in the T47D/SN sublines

Expression profiles of resistance-related transporters were examined in T47D/WT, T47D/SN120, and T47D/SN150 cells using a resistance diagnostic kit (Drugsposter<sup>®</sup>). Drugsposter<sup>®</sup> assays for 24 transporter genes and one house keeping gene *GAPDH* (49). Among the 24 transporter genes, only MRP2

mRNA showed a concentration-independent increase, from about two-fold to ten-fold in T47D/SN120 and T47D/SN150 cells treated with 3.5-90 nM SN38. After increasing the concentration to 120 nM and 150 nM, a high level of BCRP, MRP1, MRP2, MRP3, and MRP4 mRNA expression was detected in T47D/SN120 and T47D/SN150 cells. There was no difference in the mRNA expression of other genes between the T47D/WT cells and the SN38-resistant T47D sublines. Real-time RT-PCR confirmed that T47D/SN120 and T47D/SN150 cells overexpressed MRP1 (7-fold and 11-fold, respectively), MRP2 (795-fold and 1,061-fold, respectively), MRP3 (57-fold and 96-fold, respectively), MRP4 (204-fold both), and BCRP (536-fold and 3,083-fold, respectively), compared to T47D/WT cells (Fig. 1).

Expression levels of MRP1, MRP2, MRP3, MRP4, and BCRP were determined by western blot analysis. Fifty micrograms of protein was used to detect MRP1, MRP2, MRP3, and MRP4. MRP1, MRP2, MRP3, and MRP4 expression levels in T47D/SN150 cells were compared with those in T47D/SN120 cells. Compared to T47D/SN120 cells, T47D/SN150 cells overexpressed MRP1 (1.2-fold), MRP2 (1.1-fold), MRP3 (1.1-fold), and MRP4 (2.2-fold) proteins (Fig. 2).

Compared to T47D/WT cells, which expressed a trace amount of BCRP, T47D/SN120 and T47D/SN150 cells displayed 85.3-fold and 327.5-fold higher BCRP protein expression, respectively. T47D/SN150 cells displayed 4-fold higher expression of BCRP, as compared to T47D/SN120 cells (Fig. 2).

### **Sensitivity of SN38-resistant T47D sublines to SN38 in the presence of various chemosensitizers**

The effects of several chemosensitizers, including Pgp [verapamil, PSC833, and 3,4,5-trimethoxyflavone (TMF)], MRP (probenecid), and BCRP (genistein) inhibitors, were assessed on SN38-resistant T47D sublines. Among the Pgp inhibitors, only TMF showed chemosensitizing effects on SN38 in a concentration-dependent manner. Probenecid and genistein also sensitized SN38-resistant T47D sublines to SN38 in a concentration-dependent manner (Fig. 3). The chemosensitizing effects of TMF and genistein to SN38 were similar between T47D/SN120 and T47D/SN150 cells. As shown in Table 3, the chemosensitizing index was calculated by dividing the  $IC_{50}$  value in the absence of the chemosensitizer with that in its presence. Chemosensitizing indices of probenecid (5 and 50  $\mu$ M) and genistein (1 and 10  $\mu$ M) were 1.3 - 2.0 and 1.2 - 10.8, respectively, in both the cell lines, whereas those for verapamil (1 and 10  $\mu$ M) and PSC833 (5 and 50 nM) were less than 1.0, with the exception of PSC833 in the T47D/SN120 cell line, which displayed a chemosensitizing index of 1.3.

### **Involvement of epigenetic gene silencing of MRP1, MRP2, MRP3, MRP4, and BCRP in T47D/WT cells**

Next, we investigated whether MRP1, MRP2, MRP3, MRP4, and BCRP could be epigenetically induced in T47D/WT cells following treatment with 2.5  $\mu$ M 5-aza-2'-deoxycytidine for 96 h or 100 ng/mL trichostatin A (TSA) for 48 h. RT-PCR indicated that 5-aza-2'-deoxycytidine induced the mRNA expression of MRP2, MRP3, MRP4, and BCRP, while TSA induced the mRNA expression of MRP1, MRP2, MRP4, and BCRP in T47D/WT cells (Fig. 4).

### **Decreased drug accumulation in T47D/SN120 and T47D/SN150 cells**

Drug accumulation was assayed to estimate the functional activity of transporters in T47D/SN120 and T47D/SN150 cells. Calcein AM and mitoxantrone were used as fluorescent substrates for MRP and BCRP, respectively, and detected using flow cytometry. Accumulation of both the substrates decreased in T47D/SN120 and T47D/SN150 cells, compared to that in T47D/WT cells. However, upon treatment with the inhibitors probenecid and genistein, there was an increase in the accumulation of both the substrates (Figs. 5-6).

Contrarily, intracellular levels of rhodamine 123, a substrate of Pgp, were not affected following treatment with PCS833, a Pgp inhibitor in T47D/SN120 and T47D/SN150 cells.

## Discussion

Although there is continuous development of new strategies for breast cancer prognosis and treatment, chemotherapy resistance is frequently encountered and remains a major obstacle in the management of breast cancer. Eventually, MDR develops in most systemic recurrent or initially metastatic breast cancer patients. Overcoming MDR could significantly improve the efficacy of chemotherapy.

The aim of this study was to discover MDR-associated genes in refractory breast cancer and establish new resistant breast cancer cell lines that could aid the development of novel chemosensitizers to eventually overcome MDR.

We established two new resistant breast cancer cell sublines, T47D/SN120 and T47D/SN150. Both sublines were highly resistant to various anti-cancer drugs, including paclitaxel and vinblastine, as well as SN38. Additionally, T47D/SN150 cells showed a high resistance to mitoxantrone, topotecan, and doxorubicin. Generally, the levels of resistance are classified as high (RF >20x), moderate (RF 5-15x), and low or no resistance (RF <5x) (50).

Compared to their parental cell line, T47D/SN120 and T47D/SN150 cells displayed faster proliferation and a shorter doubling time. These features were not consistent with other resistant cell lines such as MCF-7/TAX (51), BEL-7402/5-FU (52), MCF-7/Doc, and MCF-7/Adr (36). This may be a result of the short incubation period. Most cells follow an S-shaped pattern of growth; however, we calculated the doubling time at a time point prior to the S-shaped growth curve.

The mRNA expression profiles of the transporter genes in T47D/SN120 and T47D/SN150 cells were compared to those in the T47D/WT cell line. We found that T47D/SN120 and T47D/SN150 cells overexpress MRP1, MRP2, MRP3, MRP4, and BCRP. Moreover, T47D/SN150 cells obtained following exposure to a higher concentration of SN38 expressed a greater amount of transporter genes associated with MDR. In other words, T47D/SN150 cells expressed a greater amount of MDR-related genes and showed a higher resistance to anti-cancer drugs. This suggests that MDR is related to the drug concentration used as well as the presence of MDR-related genes.

MTT assays were performed to assess the sensitivity of SN38-resistant T47D sublines to SN38 in the presence of inhibitors (Pgp inhibitors: verapamil, PSC833, and TMF; MRP inhibitor: probenecid; BCRP inhibitor: genistein). TMF, probenecid, and genistein showed a concentration-dependent chemosensitizing effect. Although MRP and BCRP expression in T47D/SN150 cells was higher than that in T47D/SN120 cells, the chemosensitizing effects of TMF and genistein were similar in T47D/SN120 and T47D/SN150 cell lines. It suggests that the concentrations of TMF and genistein used in this study were enough to occupy all transporter-binding sites. Interestingly, administration of the well-known Pgp inhibitors, verapamil and PSC833 did not sensitize the cells to SN38; however, the sensitivity was recovered upon administration of TMF. In addition, the sensitivity recovery pattern observed in SN38-resistant breast cancer cells treated with TMF was similar to that observed in SN38-resistant breast cancer cells treated with genistein. This suggests that TMF not only functions as a Pgp inhibitor, but also affects other mechanisms, reversing MDR. Further studies need to be carried out to determine the functions of TMF.

Next, experiments were designed to identify the relationship between the expression of MRP1, MRP2, MRP3, MRP4, and BCRP and epigenetic gene silencing. Generally, alteration of gene function in cancer is attributed to either genetic alterations (such as mutations or deletions) or epigenetic alterations, which alter the gene expression status (53, 54). Epigenetic alterations include promoter methylation and chromatin remodeling, such as histone modification without an alteration in the DNA sequence (55, 56). Changes in gene expression caused by epigenetic alterations in cancer can be divided into three categories, transcriptional regression by methylation of the promoter CpG islands (lesions in the genome rich in sequences consisting of a cytosine preceding a guanine), increased gene expression by hypomethylation, and decreased gene expression associated with histone deacetylase (HDAC)(57-59). Aberrant DNA methylation in normally unmethylated gene promoter CpG islands results in a decrease in gene expression. Hypermethylation of promoter CpG islands in tumor suppressor genes is a hallmark of all human cancers (60-62). Moreover, global hypomethylation of the DNA contributes to carcinogenesis by inducing genomic instability (63-65). DNA hypomethylation is associated with activation of proto-oncogenes (66). Lastly, activated HDAC induces promoter DNA methylation and represses gene expression (53, 59, 67). Consequently, epigenetic gene silencing occurs by DNA hypermethylation and histone deacetylation. Therefore, DNA methyltransferase inhibitors and HDAC inhibitors, which inhibit DNA methylation and histone deacetylation, induce the expression of genes that were abnormally suppressed. Thus, they have been used as therapeutic agents to treat cancer caused by epigenetic gene silencing. In this study, expression of MRP2, MRP3, MRP4, and BCRP were found to be significantly increased in T47D/WT cancer cells treated with the DNA methyltransferase inhibitor, 5-aza-2'-deoxycytidine. Similarly, treatment of T47D/WT cancer cells with the HDAC inhibitor, trichostatin A significantly increased the expression of MRP1, MRP2, MRP4, and BCRP. In other words, contrary to what occurs in cancer induced by epigenetic gene silencing, overexpression of MRP1, MRP2, MRP3, MRP4, and BCRP is caused by DNA demethylation and histone acetylation. These results suggest that the expression of MRP1, MRP2, MRP3, MRP4, and BCRP is induced by the suppression of epigenetic gene silencing. These findings are consistent with reports indicating that epigenetic gene silencing is involved in irinotecan sensitivity in colorectal cancer cells (68-70). Intracellular irinotecan is inactivated by UGT1A1 or

pumped out by transporters such as ABCB1 (Pgp), ABCC1 (MRP1), and ABCC2 (MRP2) (71, 72). Since silencing of UGT1A1 and transporters occurs via DNA methylation, DNA methyltransferase inhibitors can restore gene expression and thereby, enhance SN38 inactivation (69). ABC transporter gene silencing occurs through histone deacetylation(68, 70) . In this study, surprisingly, the expression of MRP3 decreased after treatment with TSA; however, the underlying mechanism remains to be elucidated and is difficult to explain because studies on the epigenetic profile of the ABC loci are still incomplete.

BCRP, MRP1, MRP2, MRP3, and MRP4 decreased the intracellular drug accumulation. When the BCRP and MRP inhibitors genistein and probenecid were added as chemosensitizers, the intracellular drug concentrations were found to be increased. However, addition of the Pgp inhibitor PSC833 did not increase the intracellular drug accumulation, suggesting that the resistance of T47D/SN120 and T47D/SN150 cells to anti-cancer drugs is not associated with Pgp. Additionally, these inhibitors presented a synergic effect with cyanide-induced ATP depletion. As previously demonstrated by other studies, we were also able to confirm that MRP1, MRP2, MRP3, MRP4, and BCRP are ATP-dependent transporters.

In summary, two SN38-resistant breast cancer cell sublines, T47D/SN120 and T47D/SN150, were established by gradual exposure to SN38. The resistant cells overexpressed MRP1, MRP2, MRP3, MRP4, and BCRP in a SN38 concentration-dependent manner and also displayed a higher resistance to various anti-cancer drugs, when compared to their parental wild-type breast cancer cells. Intracellular substrates of anti-cancer agents were found to be lower in both SN38-resistant breast cancer cell sublines but were increased by known chemosensitizers. T47D/SN120 and T47D/SN150 cells were chemosensitized to SN38 by TMF, probenecid, and genistein. In this study, new SN38-resistant breast cancer cell sublines overexpressing MRP1, MRP2, MRP3, MRP4, and BCRP were established. These cancer cell lines can serve to be useful for the development of new chemosensitizers.

## Conclusions

T47D/SN120 and T47D/SN150 cells overexpressed MRP1, MRP2, MRP3, MRP4, and BCRP by the suppression of epigenetic gene silencing. These resistant cells presented the classical MDR phenotype, characterized by cross-resistance to various other anti-cancer drugs. This MDR phenotype decreased intracellular drug accumulation, which was reversed by known chemosensitizers, such as probenecid and genistein. Thus, these SN38-resistant T47D breast cancer cell sublines could be used for screening novel chemosensitizers.

## Abbreviations

BCRP: Breast cancer resistance protein; SN38: 7-ethyl-10-hydroxycamptothecin; MTT: 3-(4,5-methylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; MDR: Multidrug resistance; ABC: ATP (adenosine triphosphate)-binding cassette; MRP1: Multidrug resistance protein 1; MRP2: Multidrug resistance protein 2; MRP3: Multidrug resistance protein 3; MRP4: Multidrug resistance protein 4; LRP: Lung resistance

protein; RF: Resistance factor; RT-PCR: Reverse transcription-polymerase chain reaction; PBS: Phosphate-buffered saline; TMF: 3,4,5-trimethoxyflavone; Pgp: P-glycoprotein; TSA: Trichostatin A; HDAC: Histone deacetylase

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

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

### Competing interests

The authors declare that they have no competing interest

### Funding

This study was supportive by research fund form Chosun University Hospital 2018.

### Authors' contributions

HJL and CHC conceived and designed the analysis, collected the data and analyzed the data. All authors were involved in the drafting, critical review, and approval of the final manuscript and the decision to submit for publication.

### Acknowledgements

Not applicable

### Author information

Hee Jeong Lee: Department of Hemato-Oncology, Chosun University Hospital, Gwangju, 501-759, Korea

Cheol Hee Choi: Research Center for Resistant Cells and Department of Pharmacology, Chosun University Medical School, Gwangju, 501-759, Korea

## References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424.
2. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol.* 1998;16(8):2672-85.
3. Bear HD, Anderson S, Brown A, Smith R, Mamounas EP, Fisher B, et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol.* 2003;21(22):4165-74.
4. Early Breast Cancer Trialists' Collaborative G. Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. *Lancet Oncol.* 2018;19(1):27-39.
5. Heys SD, Sarkar T, Hutcheon AW. Primary docetaxel chemotherapy in patients with breast cancer: impact on response and survival. *Breast Cancer Res Treat.* 2005;90(2):169-85.
6. Hutcheon AW, Heys SD, Sarkar TK, Aberdeen Breast G. Neoadjuvant docetaxel in locally advanced breast cancer. *Breast Cancer Res Treat.* 2003;79 Suppl 1:S19-24.
7. Blohmer JU, Schmid P, Hilfrich J, Friese K, Kleine-Tebbe A, Koelbl H, et al. Epirubicin and cyclophosphamide versus epirubicin and docetaxel as first-line therapy for women with metastatic breast cancer: final results of a randomised phase III trial. *Annals of Oncology.* 2010;21(7):1430-5.
8. Nabholz J-M, Falkson C, Campos D, Szanto J, Martin M, Chan S, et al. Docetaxel and Doxorubicin Compared With Doxorubicin and Cyclophosphamide as First-Line Chemotherapy for Metastatic Breast Cancer: Results of a Randomized, Multicenter, Phase III Trial. *Journal of Clinical Oncology.* 2003;21(6):968-75.
9. Zielinski C, Beslija S, Mrsic-Krmpotic Z, Welnicka-Jaskiewicz M, Wiltschke C, Kahan Z, et al. Gemcitabine, Epirubicin, and Paclitaxel Versus Fluorouracil, Epirubicin, and Cyclophosphamide As First-Line Chemotherapy in Metastatic Breast Cancer: A Central European Cooperative Oncology Group International, Multicenter, Prospective, Randomized Phase III Trial. *Journal of Clinical Oncology.* 2005;23(7):1401-8.
10. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, et al. Drug resistance in cancer: an overview. *Cancers (Basel).* 2014;6(3):1769-92.
11. Szakacs G, Paterson JK, Ludwig JA, Booth-Gentle C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov.* 2006;5(3):219-34.
12. Dallavalle S, Dobricic V, Lazzarato L, Gazzano E, Machuqueiro M, Pajeva I, et al. Improvement of conventional anti-cancer drugs as new tools against multidrug resistant tumors. *Drug Resist Updat.* 2020;50:100682.
13. Rosenberg MF, Callaghan R, Ford RC, Higgins CF. Structure of the multidrug resistance P-glycoprotein to 2.5 nm resolution determined by electron microscopy and image analysis. *J Biol Chem.*

- 1997;272(16):10685-94.
14. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem.* 1993;62:385-427.
  15. Ferry DR, Russell MA, Cullen MH. P-glycoprotein possesses a 1,4-dihydropyridine-selective drug acceptor site which is allosterically coupled to a vinca-alkaloid-selective binding site. *Biochem Biophys Res Commun.* 1992;188(1):440-5.
  16. Riordan JR, Ling V. Genetic and biochemical characterization of multidrug resistance. *Pharmacol Ther.* 1985;28(1):51-75.
  17. Borst P, Evers R, Koel M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst.* 2000;92(16):1295-302.
  18. Muller M, de Vries EG, Jansen PL. Role of multidrug resistance protein (MRP) in glutathione S-conjugate transport in mammalian cells. *J Hepatol.* 1996;24 Suppl 1:100-8.
  19. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. *Cancer Research.* 1998;58(23):5337-9.
  20. Natarajan K, Xie Y, Baer MR, Ross DD. Role of breast cancer resistance protein (BCRP/ABCG2) in cancer drug resistance. *Biochem Pharmacol.* 2012;83(8):1084-103.
  21. Li W, Zhang H, Assaraf YG, Zhao K, Xu X, Xie J, et al. Overcoming ABC transporter-mediated multidrug resistance: Molecular mechanisms and novel therapeutic drug strategies. *Drug Resist Updat.* 2016;27:14-29.
  22. Lage H, Dietel M. Effect of the breast-cancer resistance protein on atypical multidrug resistance. *Lancet Oncol.* 2000;1:169-75.
  23. Larsen AK, Escargueil AE, Skladanowski A. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. *Pharmacol Ther.* 2000;85(3):217-29.
  24. Dalton WS, Scheper RJ. Lung resistance-related protein: determining its role in multidrug resistance. *J Natl Cancer Inst.* 1999;91(19):1604-5.
  25. Scheffer GL, Schroeijers AB, Izquierdo MA, Wiemer EA, Scheper RJ. Lung resistance-related protein/major vault protein and vaults in multidrug-resistant cancer. *Curr Opin Oncol.* 2000;12(6):550-6.
  26. Chen YL, Yang TY, Wu CL, Chen KC, Hsu SL, Hsueh CM. Mechanisms underlying lung resistance-related protein (LRP)-mediated doxorubicin resistance of non-small cell lung cancer cells. *Chin J Physiol.* 2016;59(6):331-47.
  27. Wtorek K, Dlugosz A, Janecka A. Drug resistance in topoisomerase-targeting therapy. *Postep Hig Med Dosw.* 2018;72:1073-83.
  28. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer.* 2006;6(10):789-802.

29. Chrencik JE, Staker BL, Burgin AB, Pourquier P, Pommier Y, Stewart L, et al. Mechanisms of camptothecin resistance by human topoisomerase I mutations. *J Mol Biol.* 2004;339(4):773-84.
30. Gongora C, Vezzio-Vie N, Tuduri S, Denis V, Causse A, Auzanneau C, et al. New Topoisomerase I mutations are associated with resistance to camptothecin. *Mol Cancer.* 2011;10:64.
31. Ganapathi RN, Ganapathi MK. Mechanisms regulating resistance to inhibitors of topoisomerase II. *Front Pharmacol.* 2013;4:89.
32. Ji X, Lu Y, Tian H, Meng X, Wei M, Cho WC. Chemoresistance mechanisms of breast cancer and their countermeasures. *Biomed Pharmacother.* 2019;114:108800.
33. Burger H, Foekens JA, Look MP, Meijer-van Gelder ME, Klijn JG, Wiemer EA, et al. RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: correlation with chemotherapeutic response. *Clin Cancer Res.* 2003;9(2):827-36.
34. Wang M, Wang X, Yuan J, Guo L. Expression of the breast cancer resistance protein and 5fluorouracil resistance in clinical breast cancer tissue specimens. *Mol Clin Oncol.* 2013;1(5):853-7.
35. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, et al. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res.* 2001;61(8):3458-64.
36. Li WJ, Zhong SL, Wu YJ, Xu WD, Xu JJ, Tang JH, et al. Systematic expression analysis of genes related to multidrug-resistance in isogenic docetaxel- and adriamycin-resistant breast cancer cell lines. *Molecular Biology Reports.* 2013;40(11):6143-50.
37. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells.[Erratum appears in *Proc Natl Acad Sci U S A* 1999 Mar 2;96(5):2569]. *Proceedings of the National Academy of Sciences of the United States of America.* 1998;95(26):15665-70.
38. Liu F, Fan D, Qi J, Zhu H, Zhou Y, Yang C, et al. Co-expression of cytokeratin 8 and breast cancer resistant protein indicates a multifactorial drug-resistant phenotype in human breast cancer cell line. *Life Sciences.* 2008;83(13):496-501.
39. Robey RW, Medina-Perez WY, Nishiyama K, Lahusen T, Miyake K, Litman T, et al. Overexpression of the ATP-binding cassette half-transporter, ABCG2 (Mxr/BCrp/ABCP1), in flavopiridol-resistant human breast cancer cells. *Clin Cancer Res.* 2001;7(1):145-52.
40. Schellens JH, Maliepaard M, Scheper RJ, Scheffer GL, Jonker JW, Smit JW, et al. Transport of topoisomerase I inhibitors by the breast cancer resistance protein. Potential clinical implications. *Ann N Y Acad Sci.* 2000;922:188-94.
41. Beretta GL, Perego P, Zunino F. Mechanisms of cellular resistance to camptothecins. *Curr Med Chem.* 2006;13(27):3291-305.
42. Zander SA, Sol W, Greenberger L, Zhang Y, van Tellingen O, Jonkers J, et al. EZN-2208 (PEG-SN38) overcomes ABCG2-mediated topotecan resistance in BRCA1-deficient mouse mammary tumors. *PLoS One.* 2012;7(9):e45248.

43. Kaneda N, Nagata H, Furuta T, Yokokura T. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. *Cancer Res.* 1990;50(6):1715-20.
44. Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Research.* 1991;51(16):4187-91.
45. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *Journal of Biological Chemistry.* 1985;260(27):14873-8.
46. Engel LW, Young NA. Human breast carcinoma cells in continuous culture: a review. *Cancer Res.* 1978;38(11 Pt 2):4327-39.
47. Jandu H, Aluzaita K, Fogh L, Thrane SW, Noer JB, Proszek J, et al. Molecular characterization of irinotecan (SN-38) resistant human breast cancer cell lines. *BMC Cancer.* 2016;16:34.
48. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. 1979. *Biotechnology.* 1992;24:145-9.
49. Lee T-B, Choi C-H. Detection of drug transporter expression using a 25-multiplex RT-PCR assay. *Biotechnology Letters.* 2009;31(10):1485-92.
50. Snow K, Judd W. Characterisation of adriamycin- and amsacrine-resistant human leukaemic T cell lines. *British Journal of Cancer.* 1991;63(1):17-28.
51. Chen SY, Hu SS, Dong Q, Cai JX, Zhang WP, Sun JY, et al. Establishment of paclitaxel-resistant breast cancer cell line and nude mice models, and underlying multidrug resistance mechanisms in vitro and in vivo. *Asian Pac J Cancer Prev.* 2013;14(10):6135-40.
52. Gu W, Fang FF, Li B, Cheng BB, Ling CQ. Characterization and resistance mechanisms of a 5-fluorouracil-resistant hepatocellular carcinoma cell line. *Asian Pac J Cancer Prev.* 2012;13(9):4807-14.
53. Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG. Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. *Hum Mol Genet.* 2001;10(7):687-92.
54. Baylin SB, Jones PA. Epigenetic Determinants of Cancer. *Cold Spring Harb Perspect Biol.* 2016;8(9).
55. Gibney ER, Nolan CM. Epigenetics and gene expression. *Heredity (Edinb).* 2010;105(1):4-13.
56. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis.* 2010;31(1):27-36.
57. Park JY. Promoter hypermethylation in prostate cancer. *Cancer Control.* 2010;17(4):245-55.
58. Yelina N, Diaz P, Lambing C, Henderson IR. Epigenetic control of meiotic recombination in plants. *Sci China Life Sci.* 2015;58(3):223-31.
59. Hernandez-Rosas F, Hernandez-Oliveras A, Flores-Peredo L, Rodriguez G, Zarain-Herzberg A, Caba M, et al. Histone deacetylase inhibitors induce the expression of tumor suppressor genes Per1 and Per2 in human gastric cancer cells. *Oncol Lett.* 2018;16(2):1981-90.
60. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet.* 2002;3(6):415-28.

61. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. *Adv Cancer Res.* 1998;72:141-96.
62. Bilgrami SM, Qureshi SA, Pervez S, Abbas F. Promoter hypermethylation of tumor suppressor genes correlates with tumor grade and invasiveness in patients with urothelial bladder cancer. *Springerplus.* 2014;3:178.
63. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature.* 1983;301(5895):89-92.
64. Roberts LR, Gores GJ. Hepatocellular carcinoma: molecular pathways and new therapeutic targets. *Semin Liver Dis.* 2005;25(2):212-25.
65. Lee HS, Kim BH, Cho NY, Yoo EJ, Choi M, Shin SH, et al. Prognostic implications of and relationship between CpG island hypermethylation and repetitive DNA hypomethylation in hepatocellular carcinoma. *Clinical Cancer Research.* 2009;15(3):812-20.
66. Calvisi DF, Ladu S, Gorden A, Farina M, Lee JS, Conner EA, et al. Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. *J Clin Invest.* 2007;117(9):2713-22.
67. Rountree MR, Bachman KE, Herman JG, Baylin SB. DNA methylation, chromatin inheritance, and cancer. *Oncogene.* 2001;20(24):3156-65.
68. Huo H, Magro PG, Pietsch EC, Patel BB, Scotto KW. Histone methyltransferase MLL1 regulates MDR1 transcription and chemoresistance. *Cancer Res.* 2010;70(21):8726-35.
69. Gagnon JF, Bernard O, Villeneuve L, Tetu B, Guillemette C. Irinotecan inactivation is modulated by epigenetic silencing of UGT1A1 in colon cancer. *Clinical Cancer Research.* 2006;12(6):1850-8.
70. Hauswald S, Duque-Afonso J, Wagner MM, Schertl FM, Lubbert M, Peschel C, et al. Histone deacetylase inhibitors induce a very broad, pleiotropic anticancer drug resistance phenotype in acute myeloid leukemia cells by modulation of multiple ABC transporter genes. *Clin Cancer Res.* 2009;15(11):3705-15.
71. Iyer L, King CD, Whittington PF, Green MD, Roy SK, Tephly TR, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest.* 1998;101(4):847-54.
72. Innocenti F, Kroetz DL, Schuetz E, Dolan ME, Ramirez J, Relling M, et al. Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. *J Clin Oncol.* 2009;27(16):2604-14.

## Tables

**Table 1. Primer sequences of PCR**

Gene	Primer	Conventional	Real-time
BCRP	Sense	5' CAGGTCTGTTGGTCAATCTCAC 3'	GCCTACAACCTGGCTTAGACT
	Antisense	5' CAGTGTGATGGCAAGGGAAC 3'	GATGATTGTTTCGTCCCTGCT
MRP1	Sense	5' CTAACCTGGACCTGGAAGT 3'	GGTCAGCCCAACTCTCTTG
	Antisense	5' TCAATCAACACTGTAAGCAACC 3'	ACTGAACTCCCTTCCTCCTC
MRP2	Sense	5' AACCTCATTGACGACCATCC 3'	TCAGGTTTGCCAGTTATCCG
	Antisense	5' GACCATTACCTTGTCACTGTCC 3'	TGGTTGGTGTCAATCCTCAC
MRP3	Sense	5' CTCCAAGACAGAGACAGAGGC 3'	CCTGCTACTTGCTCTACCTG
	Antisense	5' TGGCCCACGCTGAGATTCTC 3'	ACACCCAGGACCATCTTGA
MRP4	Sense	5' AACCTCTAACCGACATTCTG 3'	GGGAGAGAACCAGCACTTC
	Antisense	5' TCAACATATTACAGCCACCATC 3'	TGCTGTTTCCAAGGCATCT

**Table 2. The sensitivity of the T47D/WT, T47D/SN120 and T47D/SN150 cells to SN38 and other anticancer drugs. Drugs are sorted according to the order of relative resistance of T47D/SN120 cells to T47D/WT cells.**

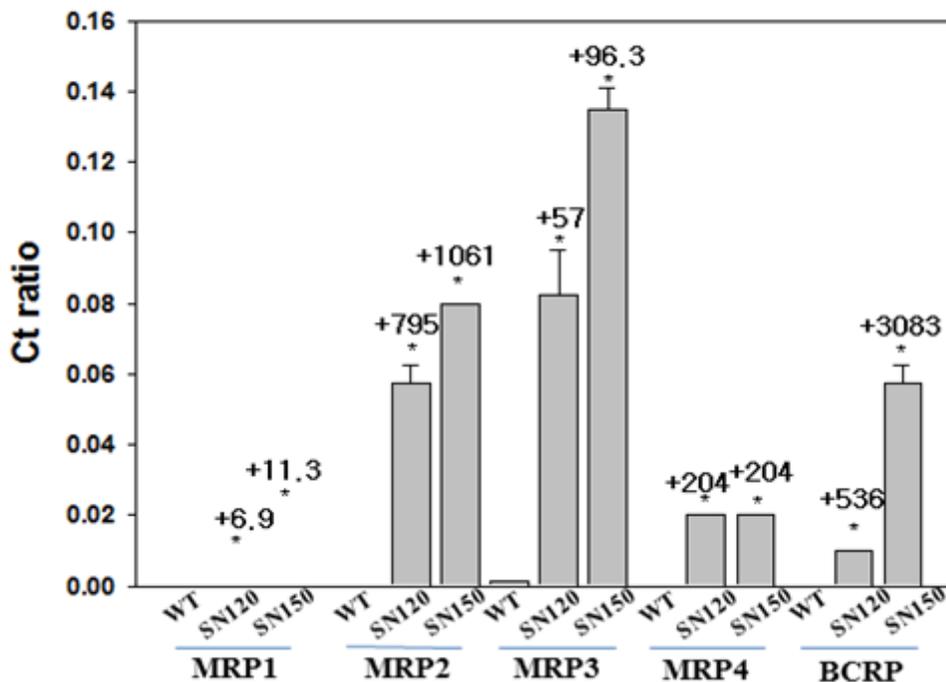
Drug	T47D/WT	T47D/SN120	T47D/SN150
	IC <sub>50</sub> µg/mL value (Relative resistance fold as compared with T47D/WT)		
Paclitaxel	0.007 ± 0.0004 (1)	15.54 ± 3.38 (2,220)	41.45 ± 1.52 (5,921)
Vinblastine	0.017 ± 0.0004 (1)	33.04 ± 1.12 (1,943)	39.96 ± 3.10 (2,350)
Doxorubicin	0.056 ± 0.0095 (1)	0.59 ± 0.08 (10.4)	1.07 ± 0.08 (19.1)
SN38	0.017 ± 0.0037 (1)	0.25 ± 0.01 (14.5)	1.00 ± 0.20 (59.1)
Topotecan	0.16 ± 0.036 (1)	0.79 ± 0.23 (4.9)	1.92 ± 0.18 (12.0)
Gefitinib	23.53 ± 0.745 (1)	97.11 ± 2.91 (4.1)	97.21 ± 2.27 (4.1)
Mitoxantrone	0.057 ± 0.032 (1)	0.20 ± 0.04 (3.4)	1.51 ± 0.22 (26.4)
Endoxifen	25.83 ± 4.318 (1)	79.34 ± 0.17 (3.1)	68.05 ± 2.09 (2.6)
5-FU	2.89 ± 0.214 (1)	8.19 ± 0.77 (2.8)	9.83 ± 0.39 (3.4)
Irinotecan	10.24 ± 0.399 (1)	14.98 ± 0.36 (1.5)	38.03 ± 2.37 (3.7)
Cisplatin	118.84 ± 27.13 (1)	159.51 ± 7.82 (1.3)	194.17 ± 8.42 (1.6)
Tamoxifen	46.06 ± 3.512 (1)	59.65 ± 2.44 (1.3)	66.64 ± 2.75 (1.4)
Methotrexate	53.91 ± 6.316 (1)	57.72 ± 2.38 (1.1)	43.48 ± 8.46 (0.8)

**Table 3. Effect of chemosensitizers on SN38-resistant T47D sublines**

	Chemosensitizing index (%) of chemosensitizers									
	verapamil ( $\mu\text{M}$ )		PSC833 (nM)		TMF ( $\mu\text{M}$ )		Probenecid ( $\mu\text{M}$ )		Genistein ( $\mu\text{M}$ )	
	1	10	5	50	5	50	5	50	1	10
SN120	0.56	0.88	0.67	1.30	3.01	7.73	1.31	1.72	1.28	3.90
SN150	0.48	0.50	0.65	0.60	15.95	10.56	2.00	1.99	7.53	10.78

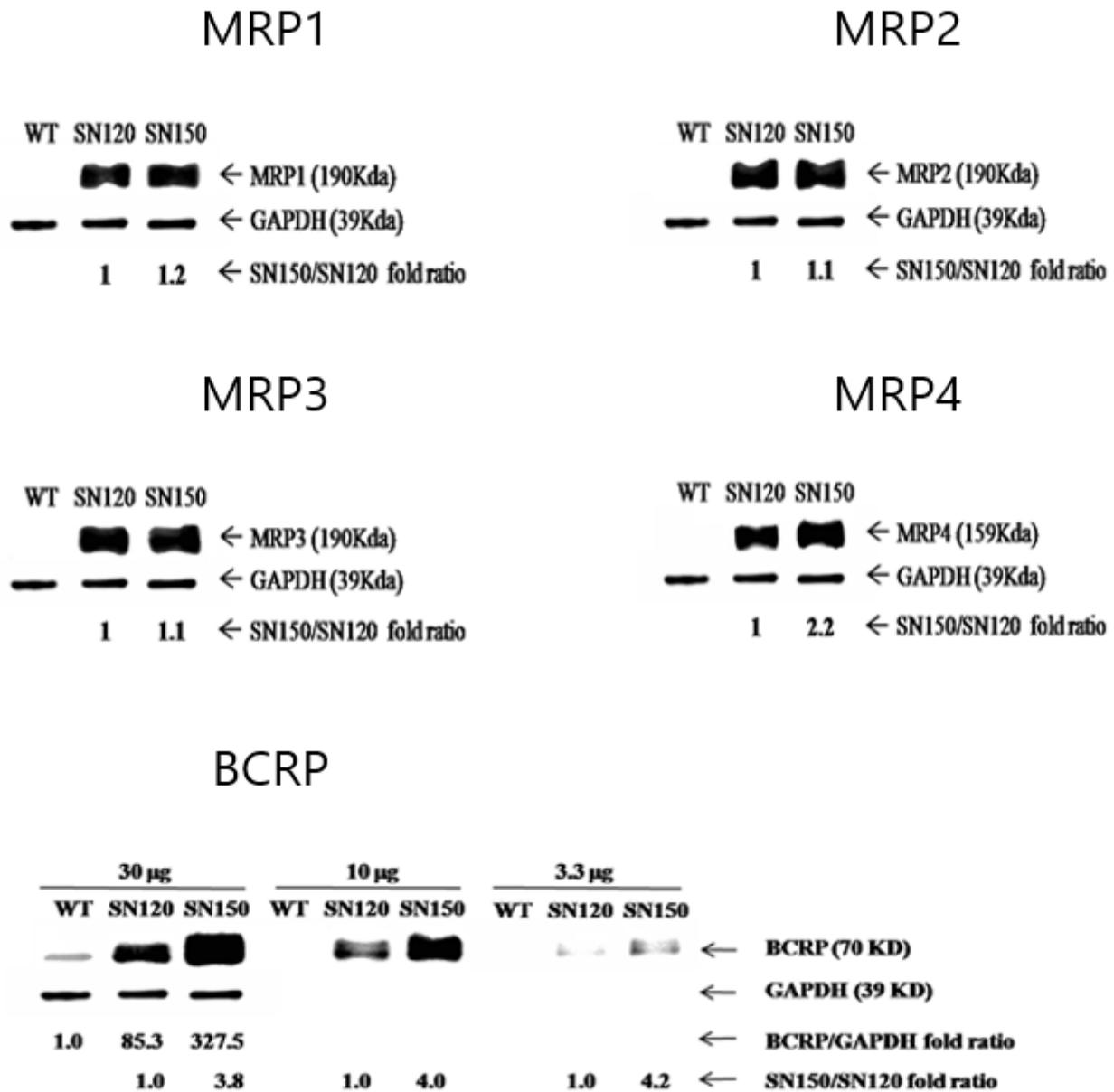
Chemosensitizing index (%) =  $\frac{\text{IC}_{50} \text{ of SN38 in the absence of chemosensitizer}}{\text{IC}_{50} \text{ of SN38 in the presence of chemosensitizer}}$

## Figures



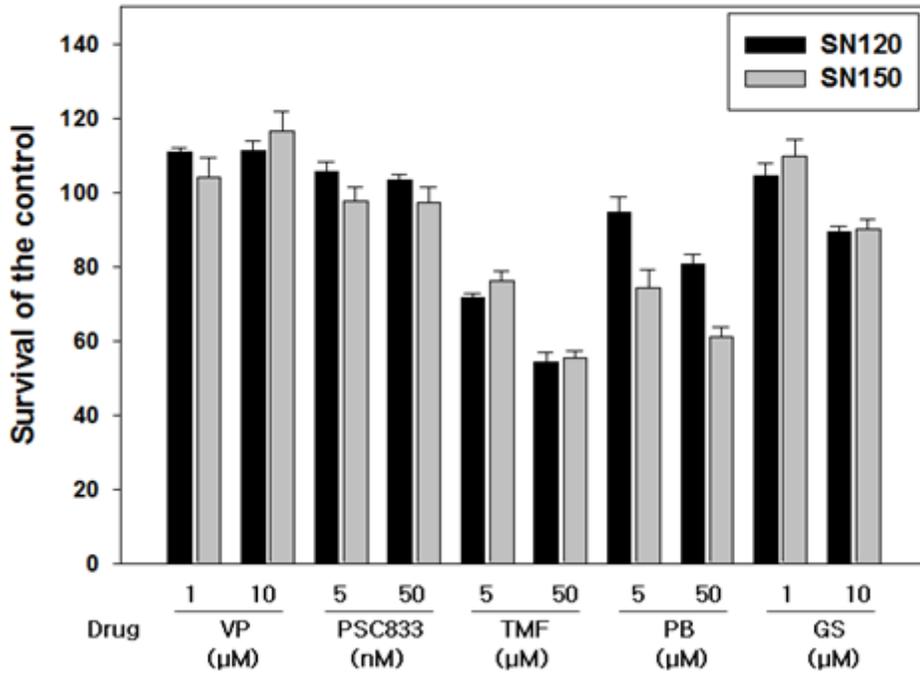
**Figure 1**

Real-time RT-PCR assay for MRP1, MRP2, MRP3, MRP4 and BCRP mRNA expression of the T-47D/WT, T-47D/SN120 and T-47D/SN150 cells. Numbers above column refer relative fold increase as compared with WT cells. \*,  $P < 0.05$  vs WT



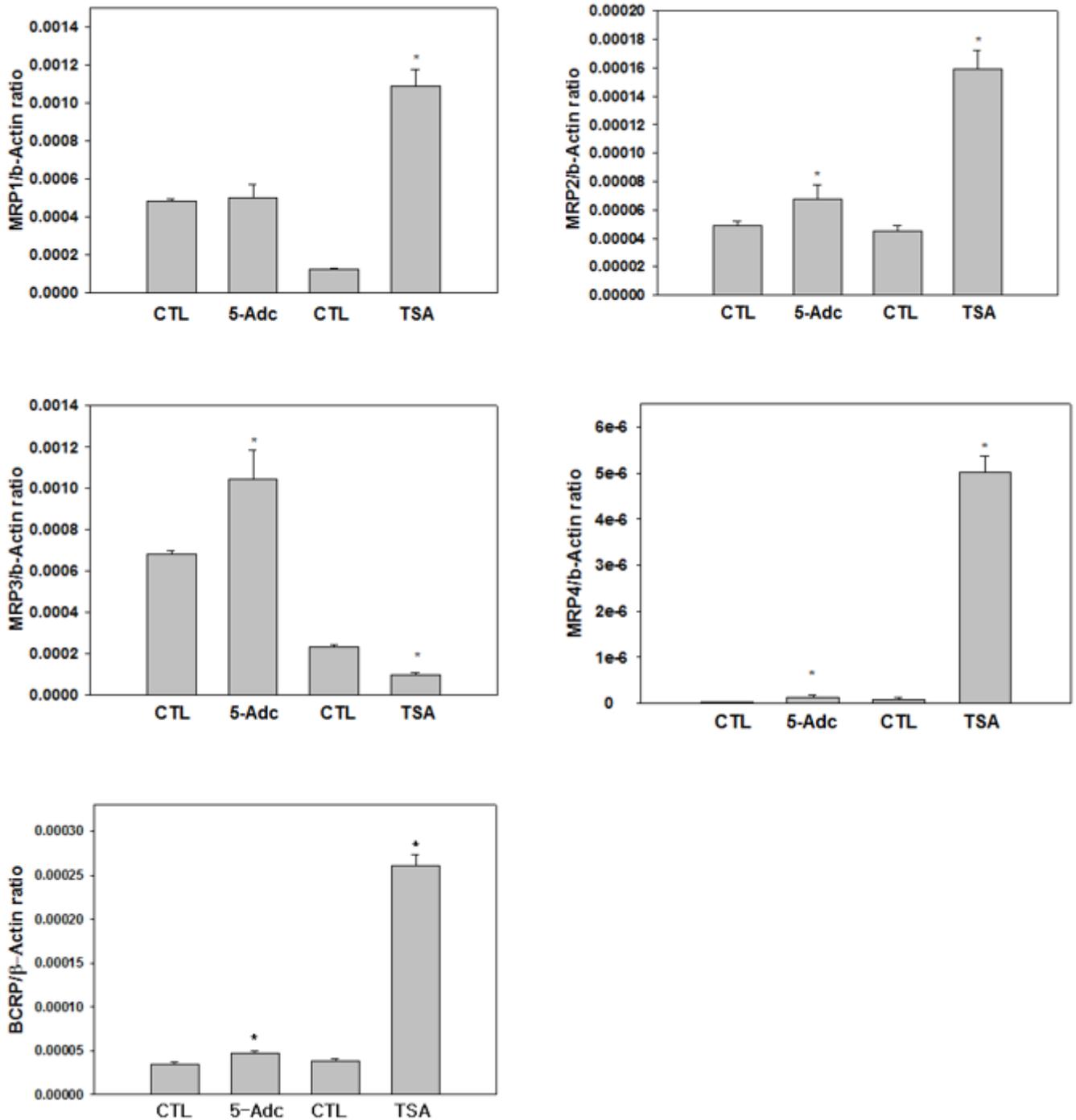
**Figure 2**

Western blot analysis for MRP1, MRP2, MRP3, MRP4 and BCRP protein of the T47D/WT, T47D/SN120 and T47D/SN150 cells.



**Figure 3**

Sensitivity of SN38-resistant T47D sublines to SN38 in the presence of various chemosensitizers. Vp, verapamil; TMF, 3,4,5-trimethoxyflavone; PB, probenecid; GS, genistein.



**Figure 4**

Effects of 5-aza-2'-deoxycytidine (AdC) and trichostatin A (TSA) on expression of MRP1, MRP2, MRP3, MRP4 and BCRP in T47D/WT cells. \*,  $P < 0.05$  vs CTL (n=4).

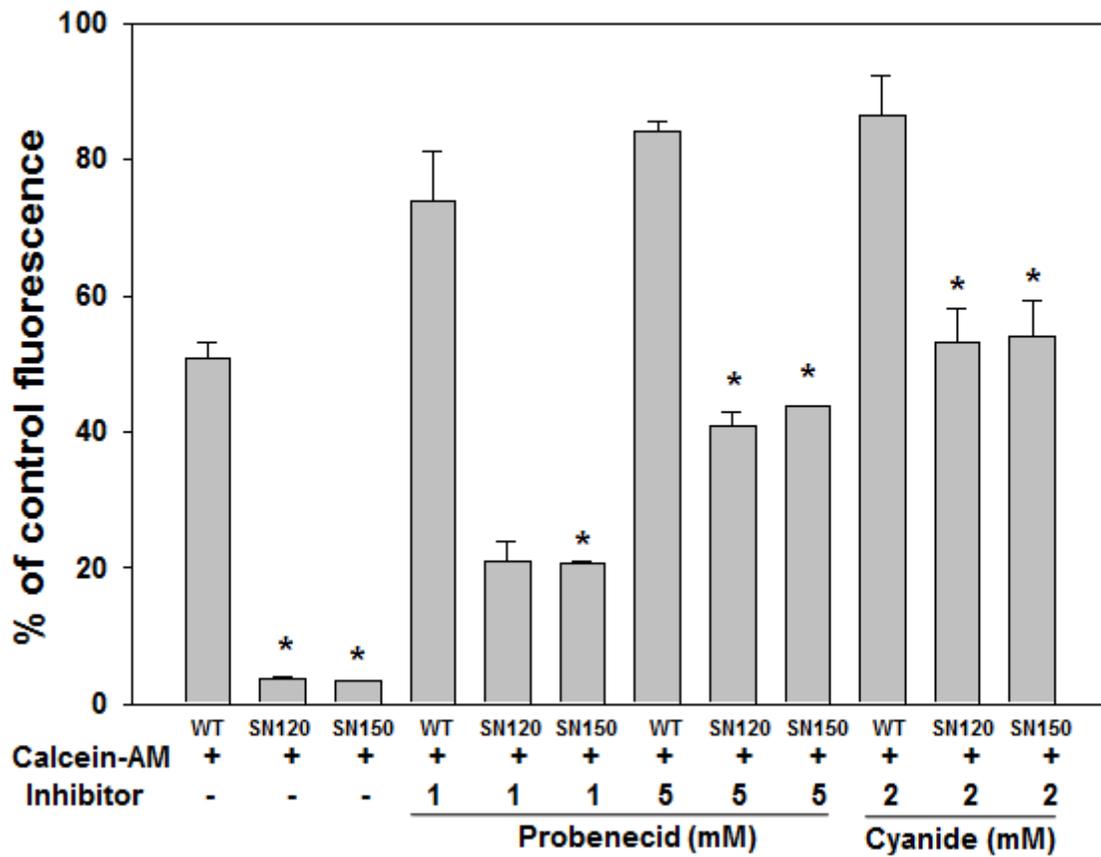


Figure 5

Intracellular calcein-AM accumulation of the T47D/WT, T47D/SN120 and T47D/SN150 cells in the presence and absence of MRP inhibitor probenecid and ATP deplete cyanide. \*,  $P < 0.05$  vs WT (n = 3).

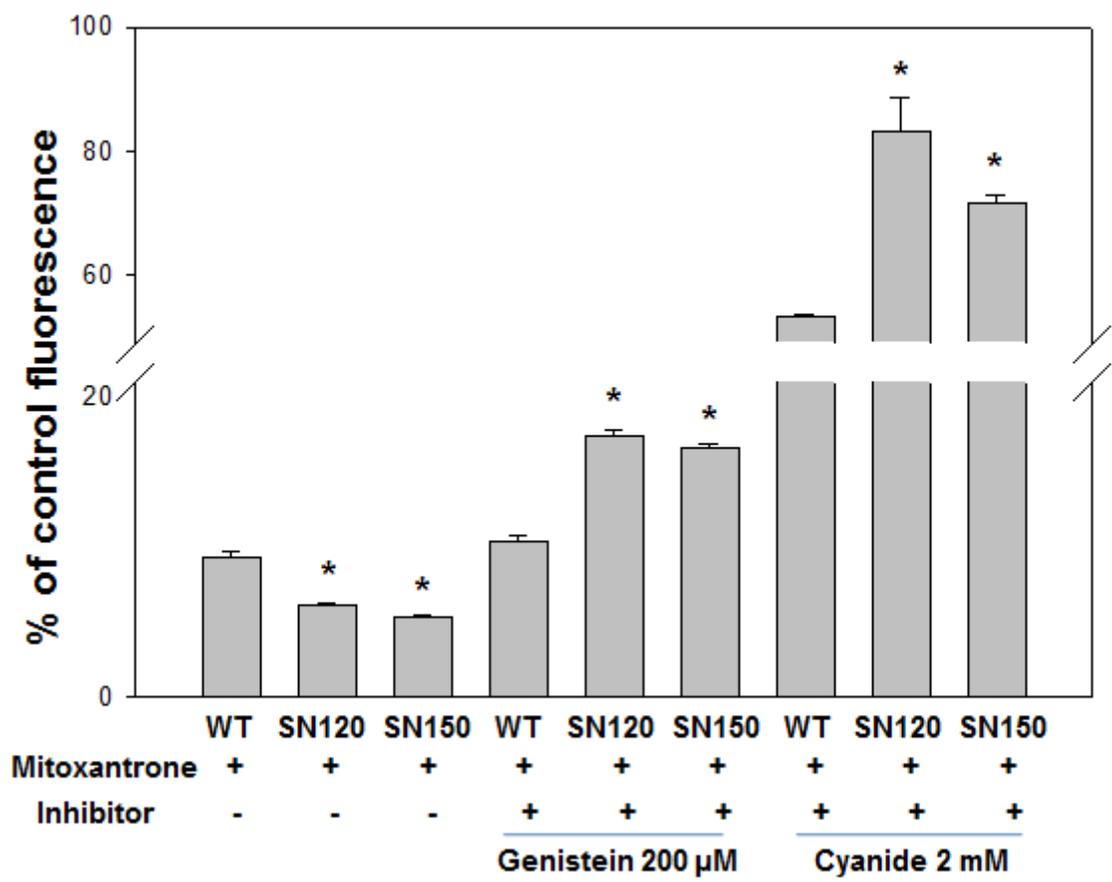


Figure 6

Intracellular mitoxantrone accumulation of the T47D/WT, T47D/SN120 and T47D/SN150 cells in the presence and absence of chemosensitizers such as BCRP inhibitor genistein and ATP deplete cyanide. \*,  $P < 0.05$  vs WT (n = 3).