

Expression of BAF57 in ovarian cancer cells and drug sensitivity

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The SMARCE1 (SWI / SNF-related, matrix-associated, and actin-dependent regulator of chromatin, subfamily e, member 1) encodes BAF57 protein. Previously, we reported that BAF57 is a predictive marker of endometrial carcinoma. In this study, we investigated BAF57 expression in ovarian cancer cell lines and their sensitivities to cisplatin, doxorubicin, paclitaxel, and 5-fluorouracil. BAF57 expression was strongly correlated with sensitivities to cisplatin, doxorubicin, and 5-fluorouracil in 10 ovarian cancer cell lines. Paclitaxel sensitivity was also correlated with BAF57 expression, but without significance. In A2780 ovarian cancer cells, knockdown of BAF57 using specific siRNA increased cell cycle arrest at G₁ phase and the sensitivities to these anticancer agents. cDNA microarray analysis of A2780 cells transfected with BAF57 siRNA showed that 134 genes were positively regulated by BAF57, including ATP-binding cassette, sub-family G (*WHITE*), member 2 (*ABCG2*) encoding breast cancer resistance protein (BCRP). We confirmed that knockdown of BAF57 decreased BCRP expression in ovarian cancer cells by Western blot analysis, and that *ABCG2* gene expression might be regulated transcriptionally. These results suggested that BAF57 is involved in ovarian cancer cell growth and sensitivity to anticancer agents, and that BAF57 may be a target for ovarian cancer therapy.

Among gynecological cancers, ovarian cancer is the leading cause of mortality, accounting for approximately half of all deaths.⁽¹⁾ Epithelial ovarian cancer treatments use various combinations of anticancer agents including: cyclophosphamide and doxorubicin; cyclophosphamide, doxorubicin, and cisplatin; cyclophosphamide and cisplatin; paclitaxel and cisplatin; paclitaxel and carboplatin; and docetaxel and carboplatin.^(2–4) Because the age of ovarian cancer onset is lower than that of other cancers, the possibility of recurrence is relatively high.^(5–7) Therefore, even with continued treatment by anticancer agents, these agents become ineffective. Thus, it is important to identify new molecular targets in ovarian cancer.

The SMARCE1 (SWI / SNF-related, matrix-associated, and actin-dependent regulator of chromatin, subfamily e, member 1) encodes BAF57 protein. BAF57 is a core subunit of the mammalian SWI/SNF chromatin-remodeling complex and can alter DNA-nucleosome topology.⁽⁸⁾ This function induces chromatin remodeling involved in transcriptional activation and repression of genes. It has been reported that BAF57 mediates direct interactions with estrogen and androgen receptors to regulate their transcriptional activity.⁽⁹⁾ Previously, we reported that BAF57 is a predictive marker of endometrial carcinoma, and that inhibition of BAF57 activity may be a target for endometrial cancer therapy.⁽¹⁰⁾ However, it is unknown whether BAF57 is associated with sensitivities to anticancer agents. BAF57 has a high mobility group (HMG) box that

binds to DNA.⁽¹¹⁾ Recently, we reported that mitochondrial transcription factor A (mtTFA) containing two HMG boxes might be a prognostic factor for serous ovarian cancer,⁽¹²⁾ and that mtTFA can translocate into the nucleus and transcriptionally regulate *BCL2L1* and *BIRC5* gene expression.^(12,13)

In this study, we investigated the correlation between the expression of BAF57 and sensitivities to anticancer agents, and found a BAF57-regulated gene involved in drug efflux pumping.

Materials and Methods

Cell culture and antibodies. Ten human ovarian cancer cell lines (A2780, MCAS, NIH OVCAR-3, OVK18, OVK18#102, OVMG1, PA-1, RMG1, SKOV3, and TYK-nu) were obtained from ATCC (Manassas, VA, USA) and cultured in RPMI. PC3 human prostate cancer cells were kindly provided by Dr. M. Nakagawa (Kagoshima University, Kagoshima, Japan) and cultured in Eagle's minimum essential medium. All media were purchased from Nissui Seiyaku (Tokyo, Japan) and supplemented with 10% FBS. The cell lines were maintained in a 5% CO₂ atmosphere at 37°C. Anti-breast cancer resistance protein (BCRP) (MAB4146) and anti-β-actin (AC-15) antibodies were purchased from Merck Millipore (Darmstadt, Germany) and Sigma (St. Louis, MO, USA), respectively. The polyclonal antibodies against human BAF57,⁽¹⁰⁾ high mobility group

box 1 (HMGB1),⁽¹⁴⁾ and mtTFA^(12,15) used in this study have been described previously.

Plasmid preparation. To prepare the BCRP luciferase reporter plasmid, genomic DNA was amplified using the following primers (restriction enzyme sites are underlined): 5'-AGATCTGGGTGCGAGCAGCGCTTG-3' (forward) and 5'-AAGCTTAATAGAGCTCGGTCTTAACC-3' (reverse). The PCR product containing the BCRP promoter (putative nucleotides -451 to 472) was ligated into the *Bg/II-HindIII* site of the pGL3 basic vector (Promega, Madison, WI, USA). Construction of the pEB-BCRP promoter-luciferase + IdH2K^K + IdEmGFP + hygromycin B plasmid is described in Figure S1. To prepare the BAF57 expression plasmid, cDNA was amplified using the following primers (start and stop codons are underlined): 5'-ATGTCAAAAAGACCATCTTATGCCACC-3' (forward) and 5'-TTATTCTTTTTTCTCATCTTCTGGTATGG-3' (reverse). To construct the pcDNA-Flag-BAF57 plasmid, Flag-tagged BAF57 cDNA fragments were cloned into the pcDNA3 expression vector (Invitrogen, Life Technologies, Carlsbad, CA, USA).

Knockdown analysis using siRNA. Knockdown by siRNA was carried out as described previously.^(15,16) Small interfering RNAs used for knockdown were: SMARCE1-HSS185993 (si-BAF57 #2), SMARCE1-HSS110022 (siBAF57 #3), and SMARCE1-HSS185992 (siBAF57 #4) for BAF57; and ABCG2HSS114013 (siBCRP #13), ABCG2HSS114014 (siBCRP #14), and ABCG2HSS114015 (siBCRP #15) for BCRP.

Preparation of protein and Western blotting. Preparation of whole cell lysates was described previously.⁽¹⁷⁾ Membrane fraction was obtained by the Minute Plasma Membrane Protein Isolation Kit (Invent Biotechnologies, Eden Prairie, MN, USA) according to the manufacturer's instructions. Western blot analysis with indicated amounts of protein was carried out as described previously.⁽¹⁷⁾ For preparation of membrane fraction, they were not boiled. Protein expression was quantitated using Multi Gauge version 3.0 software (Fujifilm, Tokyo, Japan).

Reverse transcription and quantitative real-time PCR analysis. Total RNA was purified from cells using the RNeasy Mini kit (Qiagen, Valencia, CA, USA), and used for RT and real-time PCR analysis as described previously.⁽¹⁸⁾ Briefly, quantitative real-time RT-PCR analysis was carried out using the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using the primer sets Hs01060665_g1 for β -actin and Hs01053790_m1 for ABCG2. The comparative cycle time ($\Delta\Delta$ CT) method was used to quantify the gene expression. Values were normalized to those for human β -actin. All samples were run in duplicate in each experiment.

Cell proliferation assay. The cell proliferation assay used in this study has been described previously.⁽¹⁶⁾ Briefly, siRNA-transfected ovarian cancer cells were seeded into 12-well plates at a density of 1×10^4 cells per well. Twenty-four hours after transfection was set as time 0. The cells were harvested by trypsinization and counted every 24 h with a Coulter-type cell size analyzer (CDA-500; Sysmex, Kobe, Japan). The population doubling time was calculated from the approximate growth curve.

Cell viability assessment by water-soluble tetrazolium salt-8 assay. The cell viability assay has been described previously.⁽¹⁹⁾ Briefly, ovarian cancer cells (1×10^3) were seeded into 96-well plates. The following day, the indicated concentrations of anticancer agents were applied to the cells. After 72 h, the surviving cells were stained with TetraColor ONE (Seikagaku, Tokyo, Japan) for 2–3 h at 37°C according to the manufacturer's instructions. The absorbance was then mea-

sured at 450 nm. For knockdown analysis, transfectants (2×10^3 /well) were seeded into 96-well plates. To measure the IC₅₀ in each experiment, we used CalcuSyn software (Bio-soft, Cambridge, UK; www.biosoft.com).

Flow cytometry. The flow cytometric analysis has been described previously.⁽¹⁶⁾ A2780 cells (2.5×10^5 /well) were seeded in 6-well plates, transfected with siRNA, and cultured for 72 h. The cells were harvested, washed twice with ice-cold PBS containing 0.1% BSA, and resuspended in 70% ethanol. After washing twice with ice-cold PBS, the cells were resuspended in PBS containing 0.1% BSA, incubated with RNase (Sigma), and then stained with propidium iodide (Sigma). The cells were analyzed using an EpicsXL-MCL flow cytometer (Beckman Coulter, Tokyo, Japan).

cDNA microarray analyses. The microarray procedure was carried out as described previously.⁽²⁰⁾ In brief, total RNA as extracted from A2780 cells transfected with BAF57 siRNA#2 and #4. Eight GeneChips (Affymetrix, Santa Clara, CA, USA) were used for the analyses. The microarray analysis was carried out using BRB Array Tools software version 3.3.0 (<http://linus.nci.nih.gov/BRB-ArrayTools.html>).

Reporter assays. PC3 cells were transfected with the pEB-BCRP promoter-luciferase + IdH2K^K + IdEmGFP + hygromycin B plasmid (Fig. S1). This plasmid is capable of replication in PC3 cells by *EBNA1* and *OriP* gene expression (Wako Pure Chemical Industries, Tokyo, Japan). PC3 cells transfected with this plasmid were separated from untransfected cells by the MACSelect K^k System (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) and cultured for 3–4 weeks with 0.4 μ g/mL hygromycin B. Before transfection of siRNA or the expression plasmid, over 80% of the cells contained the BCRP-luciferase reporter plasmid as evaluated by EmGFP expression. PC3 cells (1×10^5) transfected with the BCRP-promoter luciferase gene were seeded in 12-well plates. After 24 h, siRNA or the expression plasmid were transfected into the cells. For knockdown assays, control siRNA (siCtrl) or an siRNA against BAF57 (siBAF57#2) were transfected into the cells with Lipofectamine RNAiMAX (Invitrogen). For overexpression assays, the indicated amount of pcDNA-Flag-BAF57 was transfected into cells with X-tremeGENE 9 (Roche Applied Science, Penzberg, Germany). At 36 h post-transfection, luciferase activity was detected using a Picagene kit (Toyooki, Tokyo, Japan) and measured with a luminometer (Luminescencer JNII RAB-2300; ATTO, Tokyo, Japan). Results were normalized to the protein concentration determined by the Bradford method and are representative of at least three independent experiments.

Statistical analysis. Pearson's correlation was used for statistical analysis with significance set at 5%.

Results

BAF57 expression is correlated with sensitivities to anticancer agents. First, we confirmed the specificity of the anti-BAF57 antibody. As shown in Figure S2 and legend, the anti-BAF57 antibody recognized a ~57-kDa protein, and this signal was completely abolished by BAF57 peptide. This antibody also recognized Flag-BAF57 immunoprecipitated by an anti-Flag antibody (Fig. S2b). These results indicated that our antibody recognized BAF57 protein. Using this antibody, we investigated the expression of BAF57 in 10 ovarian cancer cell lines. As shown in Figure 1(a), the expression levels of BAF57 were different in each cell line. To confirm whether BAF57 expression is correlated with sensitivities to anticancer agents, we

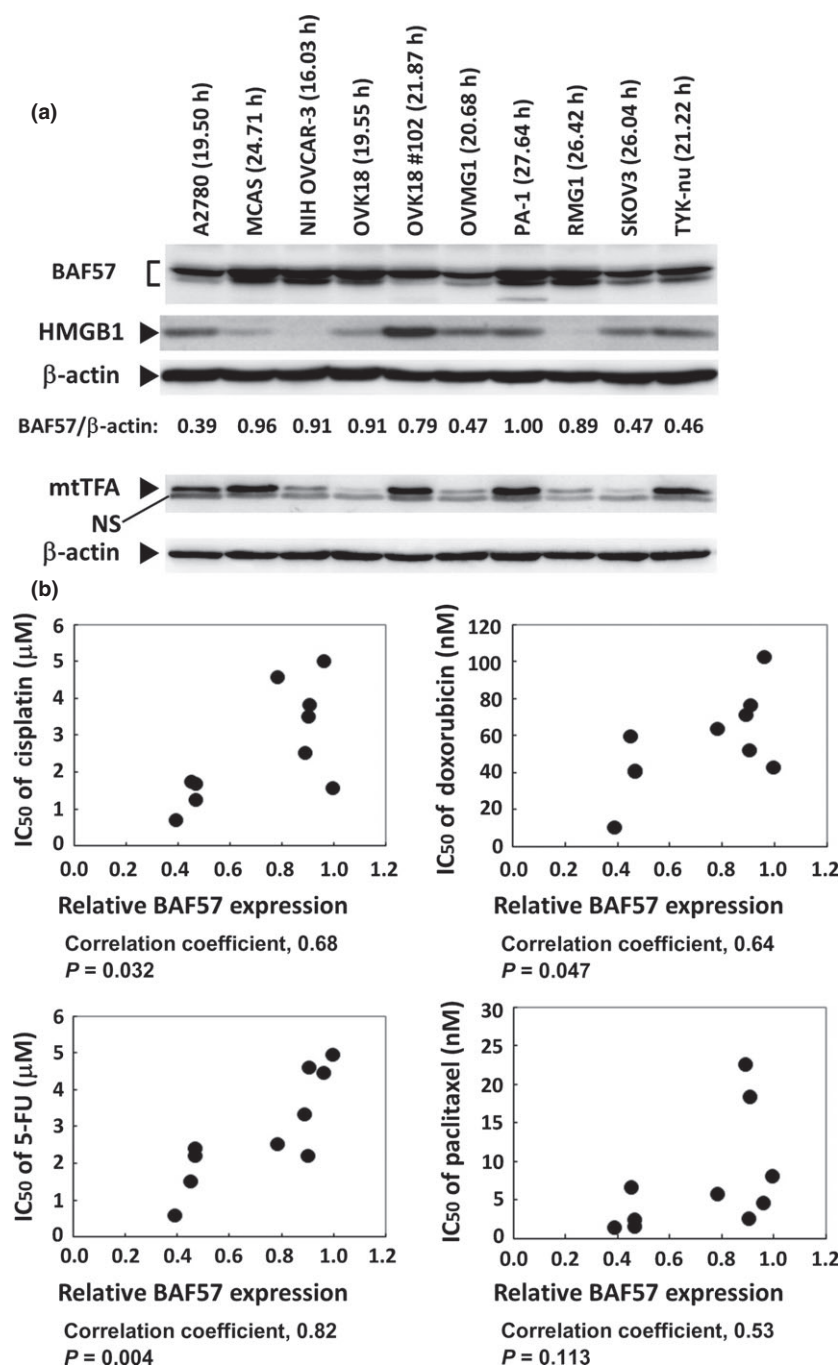


Fig. 1. Correlation between the IC₅₀ values of anticancer agents and BAF57 expression. (a) Lysates (50 μg) of 10 human ovarian cancer cell lines (A2780, MCAS, NIH OVCAR-3, OVK18, OVK18#102, OVMG1, PA-1, RMG1, SKOV3, and TYK-nu) were subjected to Western blot analysis with anti-BAF57, anti-high-mobility group protein B1 (HMGB1), anti-mitochondrial transcription factor A (mtTFA), and anti-β-actin antibodies. Signal intensity ratios (BAF57/β-actin) are shown in the middle. Non-specific signals (NS) were confirmed by siRNA analysis.⁽¹²⁾ (b) Each IC₅₀ value was calculated by concentration–response curves of the anticancer agents, and scatter plots were constructed for BAF57 expression and IC₅₀ values. 5-FU, 5-fluorouracil.

evaluated the IC₅₀ values of cisplatin, doxorubicin, 5-fluorouracil (5-FU), and paclitaxel in each cell line by concentration–response curves constructed with CalcuSyn software (Table 1). As shown in Figure 1(b), the IC₅₀ values of cisplatin, doxorubicin, and 5-FU were significantly correlated with BAF57 expression. Correlations were observed in the IC₅₀ values of paclitaxel and expression of BAF57, but were not significant. Expression of HMGB1 and mtTFA containing two HMG boxes did not correlate with the IC₅₀ values of these anticancer agents (Fig. 1a, Table 2).

Knockdown of BAF57 expression sensitizes cells to anticancer agents. To confirm whether BAF57 expression is involved in the sensitivities to anticancer agents, we used the RNA interference technique using specific siRNAs against BAF57. As shown in Figure 2(a), two siRNAs (#2 and #4) strongly

decreased BAF57 protein expression to approximately 20%. Using these siRNAs, we evaluated IC₅₀ values in A2780 cells. As shown in Figure 2(b), the IC₅₀ values of cisplatin, doxorubicin, 5-FU, and paclitaxel were significantly decreased by knockdown of BAF57 expression.

BAF57 transcriptionally induces BCRP expression. To elucidate the correlation between BAF57 expression and sensitivities to anticancer agents, we carried out cDNA microarray analysis. Compared with control siRNA, the expression of 134 genes was decreased by at least 0.5-fold after transfection of BAF57 siRNA#2 and #4 (Table S1). Few genes were associated with drug resistance, but we identified the *ABCG2* gene that encodes the BCRP protein. First, we evaluated the expression of BCRP in the 10 ovarian cancer cell lines. As shown in Figure 3, BCRP expressions of protein and mRNA were strong in

Table 1. Cytotoxicity of anticancer agents against ovarian cancer cell lines

Cell line	5-FU, μM IC ₅₀ (SD)	Cisplatin, μM IC ₅₀ (SD)	Paclitaxel, nM IC ₅₀ (SD)	Adriamycin, nM IC ₅₀ (SD)
A2780	0.556 (0.080)	0.680 (0.025)	1.168 (0.007)	9.333 (1.082)
MCAS	4.436 (0.448)	4.997 (1.003)	4.441 (1.419)	101.723 (4.323)
NIH OVCAR-3	4.619 (0.458)	3.793 (0.027)	18.141 (0.124)	76.137 (11.614)
OVK18	2.180 (0.179)	3.468 (0.333)	2.369 (0.142)	51.424 (2.300)
OVK18 #102	2.504 (0.395)	4.538 (0.494)	5.597 (1.313)	60.700 (15.079)
OVMG1	2.309 (0.070)	1.076 (0.068)	1.425 (0.108)	40.094 (5.205)
PA-1	4.924 (0.410)	1.531 (0.070)	10.026 (2.308)	41.922 (3.564)
RMG1	3.281 (0.407)	2.488 (0.136)	22.382 (0.466)	70.352 (0.058)
SKOV3	2.374 (0.129)	1.665 (0.007)	2.317 (0.296)	41.203 (5.661)
TYK-nu	1.475 (0.018)	1.719 (0.109)	6.457 (0.709)	58.783 (5.088)

5-FU, 5-fluorouracil.

Table 2. Correlation coefficients (CC) between protein expressions and IC₅₀ values of 10 ovarian cancer cell lines

Protein	5-FU CC (<i>P</i> -value)	Cisplatin CC (<i>P</i> -value)	Paclitaxel CC (<i>P</i> -value)	Doxorubicin CC (<i>P</i> -value)
BAF57	0.82 (0.004)	0.68 (0.032)	0.53 (0.113)	0.64 (0.047)
HMGB1	-0.28 (0.427)	0.11 (0.759)	-0.42 (0.230)	-0.22 (0.537)
mtTFA	0.24 (0.498)	0.29 (0.418)	-0.16 (0.659)	0.20 (0.587)

Bold values indicate significance. HMGB1, high mobility group box 1; mtTFA, mitochondrial transcription factor A.

OVK18 cells but low in the other cancer cell lines including A2780. BCRP protein expression of membrane fraction (Fig. 3b) and BCRP mRNA expression (Fig. 3c) were strongly correlated (correlation coefficient, 0.92; $P = 0.0002$). However, there was no correlation between BAF57 and BCRP expression (Figs 1a,3a). To confirm whether BAF57 was involved in BCRP expression, we used Western blot analysis of cells transfected with siRNAs. The BCRP expression in OVK18 cells was completely abolished by BCRP siRNA and partially decreased by knockdown of BAF57 (Fig. 4a). To confirm whether the decrease in BCRP protein expression by BAF57 siRNAs is involved in transcriptional regulation, we used quantitative real-time PCR and a reporter assay. Knockdown of BAF57 with siRNA decreased both BCRP mRNA and BCRP-promoter activity (Fig. 4b,c), and overexpression of BAF57 increased BCRP-promoter activity in a dose-dependent manner (Fig. 4d).

Knockdown of BAF57 expression induces G₁ cell cycle arrest. It is assumed that the rate of cell division affects sensitivity to anticancer agents. In particular, cells with suppressed division might be resistant to anticancer agents. Therefore, we investigated the growth of four ovarian cancer cell lines after transfection of siRNAs against BAF57. All four ovarian cancer cell lines showed decreases in cell growth by knockdown of BAF57 expression (Fig. 5a). Flow cytometric analysis showed that knockdown of BAF57 expression induced cell cycle arrest at G₁ phase (Fig. 5b,c).

Discussion

In the present study, we found that all 10 ovarian cancer cell lines expressed BAF57 at various levels. Link *et al.*⁽²¹⁾ reported high nuclear expression of BAF57 in prostate cancer samples. Furthermore, they reported high expression of BAF57 in metastatic samples of prostate cancer. To our knowledge,

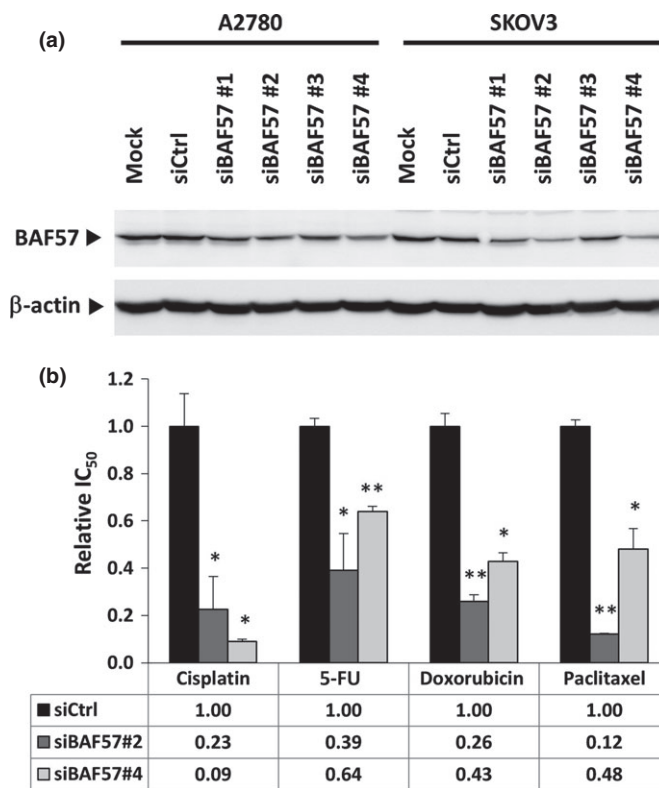


Fig. 2. Sensitization of A2780 cells to anticancer agents by knockdown of BAF57. (a) A2780 cells were transfected with the indicated siRNAs for 48 h. Cell lysates (50 μg) of each sample were subjected to Western blot analysis with anti-BAF57 and anti- β -actin antibodies. (b) A2780 cells transfected with siRNAs were seeded into 96-well plates at a density of 2×10^3 cells per well. After 24 h, the cells were treated with anticancer agents for 72 h. The surviving cells were stained with TetraColor ONE for 2–3 h. Each IC₅₀ value was calculated from the concentration–response curve. The longitudinal axis indicates the IC₅₀ value for each drug. All values represent the mean of at least two independent experiments. Significant differences are compared with each control siRNA (siCtrl). * $P < 0.05$; ** $P < 0.01$. 5-FU, 5-fluorouracil.

this is the first report showing BAF57 expression to be positively correlated with resistance to anticancer agents.

Involvement of BAF57 in drug sensitivity. The HMG box contains three helices with irregular amino acid sequences and binds to DNA for relaxation of chromatin to regulate gene expression.^(22,23) HMGB1, mtTFA, BAF57, SRY (sex determining region Y protein), LEF1 (lymphoid enhancer binding

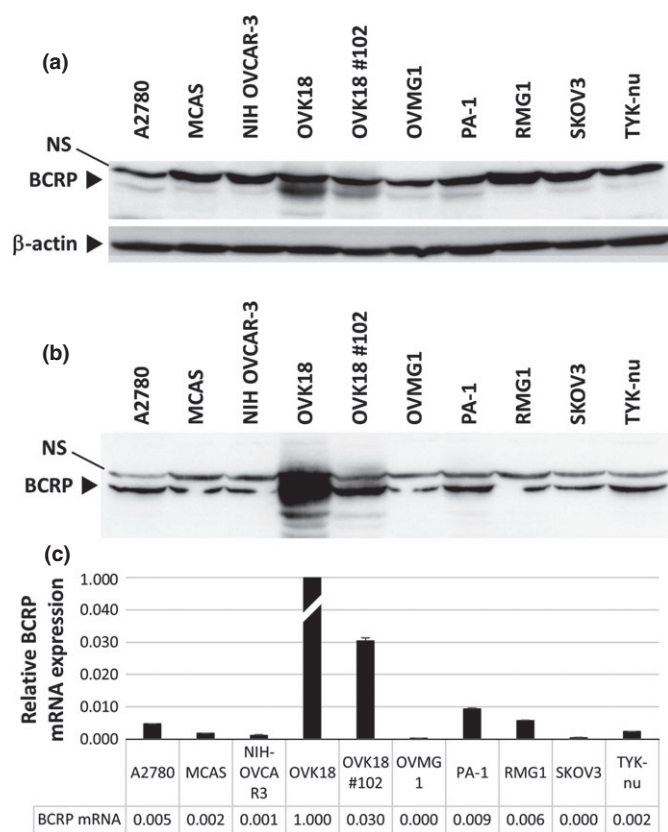


Fig. 3. Expression of breast cancer resistant protein (BCRP) in human ovarian cancer cell lines. (a) Lysates of 10 human ovarian cancer cell lines (A2780, MCAS, NIH OVCAR-3, OVK18, OVK18#102, OVMG1, PA-1, RMG1, SKOV3, and TYK-nu) were subjected to Western blot analysis with anti-BCRP (100 μ g) and anti- β -actin (20 μ g) antibodies. NS, non-specific signal. (b) Membrane fractions (50 μ g) of 10 human ovarian cancer cell lines were subjected to Western blot analysis with anti-BCRP antibody. (c) Total RNA of 10 human ovarian cancer cell lines was used to carry out RT and quantitative real-time PCR. BCRP mRNA expression of OVK18 cells was set to 1.

factor 1), TCF-1 (T-cell factor 1), and UBF 1/2 (upstream binding factor) contain a HMG box and are collectively called the HMG family. Proteins containing one or multiple HMG boxes usually recognize DNA dependently or independently, respectively.⁽²⁴⁾ Structural analyses have shown that BAF57⁽²⁵⁾ contains one HMG box, and HMGB1 and mtTFA contain two HMG boxes each.⁽²⁶⁾ In this study, we showed that BAF57 expression in 10 ovarian cancer cell lines was significantly correlated with the IC_{50} values of anticancer agents such as cisplatin, doxorubicin, and 5-FU. BAF57 was also correlated with the IC_{50} values of paclitaxel, but without significance. However, there was no correlation between the sensitivity of these anticancer agents and HMGB1 or mtTFA containing two HMG boxes. These results suggest that genes regulated by BAF57 through specific DNA-binding sequences might affect the expression of certain genes that contribute to the sensitivity of anticancer agents in ovarian cancer. To find target genes of BAF57, we carried out cDNA microarray analyses and found that expression of 134 genes decreased after transfection with BAF57 siRNA. Among them, transcription of the *ABCG2* gene that encodes the drug transporter BCRP was positively regulated by BAF57. However, there was no correlation between BCRP and BAF57 expression or sensitivities to anticancer agents. It has been reported that the HMG box may be associ-

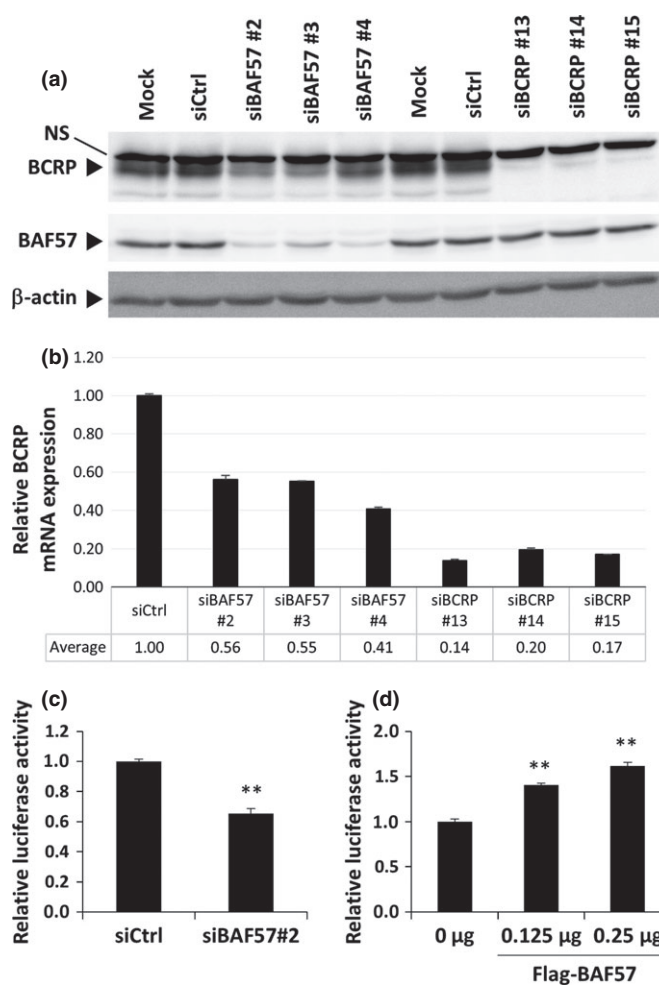


Fig. 4. Induction of breast cancer resistant protein (BCRP) expression by BAF57. (a) OVK18 cells were transfected with the indicated siRNAs, and cell lysates were subjected to Western blot analysis with anti-BCRP (100 μ g), anti-BAF57 (50 μ g), and anti- β -actin (20 μ g) antibodies. (b) Total RNA of OVK18 cells treated with indicated siRNA was used to carry out RT and quantitative real-time PCR. BCRP mRNA expression of OVK18 cells treated with control siRNA (siCtrl) was set to 1. (c) PC3 cells transfected with the BCRP-promoter luciferase gene were further transfected with BAF57 siRNA#2 (siBAF57#2). After 36 h, a luciferase assay was carried out. The results were normalized to the protein concentration and are representative of at least three independent experiments. Significant differences are compared to siCtrl. ** $P < 0.01$. (d) PC3 cells transfected with the BCRP-promoter luciferase gene were further transfected with the indicated amounts of the BAF57 expression plasmid. Luciferase assays were carried out as described in (c). Significant differences are compared with 0 μ g. *** $P < 0.01$.

ated with protein–protein interactions⁽²⁷⁾ and that BAF57 interacts with androgen receptor and estrogen receptor, and regulates their transcriptional activities.⁽⁹⁾ We hypothesize that BAF57 transcribes the *BCRP* gene by interacting with different proteins in the individual cells and that BAF57 is involved in drug sensitivities by regulating several gene expressions containing BCRP.

Breast cancer resistant protein and drug sensitivities. Human BCRP contributes to drug resistance by actively excreting several anticancer drugs from cancer cells.^(28–30) It has been reported that BCRP transports not only DNA-damaging agents such as doxorubicin, and topoisomerase inhibitors such as topotecan, but also molecular-targeting agents such as gefitinib.^(28,31) Several genes are involved in resistance to

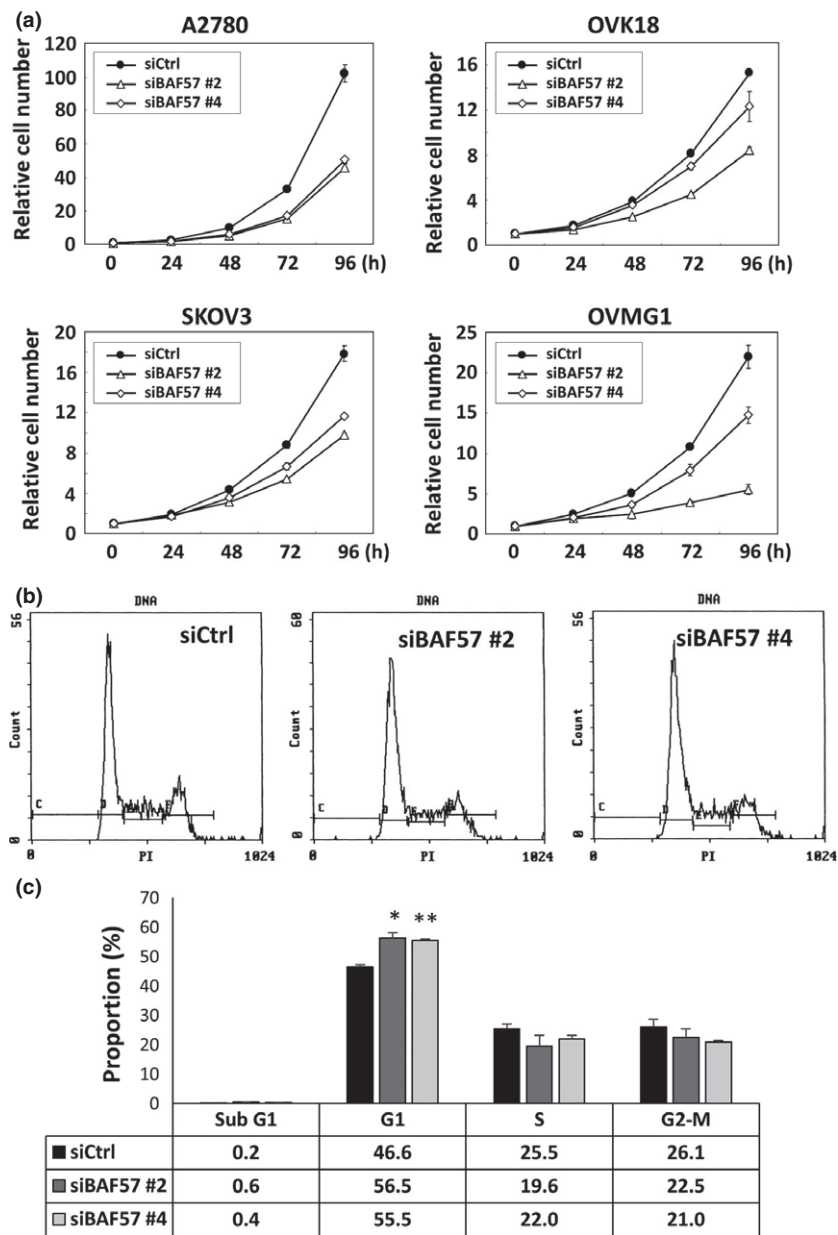


Fig. 5. Inhibition of cancer cell growth by knockdown of BAF57. (a) siRNA-transfected A2780, OVK18, SKOV3, and OVMG1 cells were seeded into 12-well plates at a density of 1×10^4 cells per well. The cell number was counted every 24 h. Twenty-four hours after transfection was set as time 0. (b) A2780 cells were transfected with or without the indicated siRNAs. Cells were harvested after 72 h and stained with propidium iodide. DNA content was then measured in single cells. siBAF #2 and #4 indicate BAF57 siRNAs #2 and #4, respectively. (c) SubG₁, G₁, S, and G₂-M cell populations were calculated from the duplicate samples in (b). Significant differences are compared with control siRNA (siCtrl). * $P < 0.05$; ** $P < 0.01$.

5-FU, such as thymidylate synthase, mismatch repair-related factors, and anti-apoptosis factors.⁽³²⁾ However, we did not detect any such genes in the cDNA microarray analysis. Yuan *et al.*⁽³³⁾ reported that a substrate of BCRP is 5-FU and that it is involved in drug resistance. There are no reports that cisplatin or paclitaxel are substrates of BCRP. However, Ceckova *et al.*⁽³⁴⁾ reported that the cytotoxicity of cisplatin is significantly lower in cells transfected with the ABCG2 transporter. It has been widely reported that cisplatin is a substrate of ABCC2 (MRP2) and ABCC6 (MRP6), whereas paclitaxel is a substrate of ABCB1 (P-gp).⁽³⁵⁻³⁷⁾ We did not investigate the expression of these transporters because there was no significant change in their mRNA expression by knockdown of BAF57. Further investigations are required to determine whether there are genes, other than *BCRP*, that are associated with resistances to anticancer agents. It is known that daunorubicin, doxorubicin, etoposide, gefitinib, imatinib, irinotecan,

methotrexate, mitoxantrone, teniposide, and topotecan are transport substrates of BCRP.⁽²⁸⁻³⁰⁾ These findings suggest that targeting both BAF57 and BCRP may sensitize resistant cells expressing BCRP.

Involvement of BAF57 in cell cycle. In this study, we showed that knockdown of BAF57 induced cell cycle arrest at G₁ phase and decreased cell growth. Hah *et al.*⁽³⁸⁾ reported that knockdown of BAF57 reduces cancer cell proliferation and growth in soft agar while promoting the accumulation of cells in G₂-M phase. This finding differs from our results, but the only difference is the cell line. In cDNA microarray analyses, we identified *BUB1* and *E2F2* genes associated with the cell cycle. *BUB1*, which is a spindle checkpoint component,⁽³⁹⁾ may be positively regulated by BAF57. Guo *et al.*⁽⁴⁰⁾ reported that knockdown of *BUB1* by specific siRNA results in increased and decreased numbers of G₁ and G₂-M cells, respectively. It is reported that BCRP also involved in the cell cycle.^(41,42) Xie *et al.*⁽⁴²⁾ reported that ABCG2 siRNA

inhibited the proliferation of the human laryngeal carcinoma cells with the increase of G₀/G₁ phase. These results indicate that BAF57 might be involved in the cell cycle through the expression of BUB1 and BCRP, which supports our results. However, knockdown of BAF57 did not repress cell growth completely, and BAF57 expression did not correlate with the population doubling time (Fig. 1a). These results indicated that BAF57 is not a major regulatory molecule of the cell cycle but is involved in its regulation.

In conclusion, BAF57 is a very attractive target for cancer therapy, because knockdown of BAF57 induced not only a decrease in cell growth, but also an increase in sensitivity to anticancer agents. In particular, combination therapy with BAF57-targeting agents and anticancer agents such as doxorubicin, etoposide, and gefitinib, which are substrates for BCRP,

might be an effective treatment strategy for ovarian cancer. Furthermore, elucidation of the molecular mechanisms of BAF57 in multidrug-resistant cells should improve our understanding of the genomic responses against anticancer agents, as well as drug resistances.

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Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Construction of breast cancer resistant protein (BCRP) reporter plasmid.

Fig. S2. Specificity of anti-BAF57 antibody.

Table S1. List of 134 genes whose expression was decreased by BAF57 siRNA