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Original Article

Low-dose LBH589 increases the sensitivity of cisplatin to cisplatin-resistant ovarian cancer cells

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Abstract

Objective: There is a need to develop alternative therapeutic strategies to overcome cisplatin-associated resistance in patients with ovarian cancer. Histone deacetylation (HDAC) associated with inactivation of genes has been implicated in the epigenetic silencing of tumor suppressor genes affecting critical biological activities in cancer cells and may be an important factor in acquired cisplatin-associated resistance. In this report, we tested a combination of cisplatin and LBH589 (histone deacetylation inhibitor) in cisplatin-resistant ovarian cancer cells to explore the reversal effect of cisplatin resistance and changes of gene expression.

Materials and Methods: To detect the synergistic effects of antiproliferation between cisplatin and LBH589 in ovarian cancer cells, we performed a cell viability assay and a clonogenic assay. To investigate the differences of gene expression between cells treated by cisplatin alone and cotreated with cisplatin and LBH589, a microarray mRNA analysis was performed.

Results: In the presence of low-dose LBH589, the inhibition concentration value of cisplatin for A2780-cp70 cells was much lower than with cisplatin treatment alone. Gene expression profiles identified that a total of 354 genes had been significantly upregulated and a total of 63 genes been downregulated with LBH589 cotreatment.

Conclusion: We hypothesized that combination of cisplatin and LBH589 can override cisplatin-associated resistance in ovarian cancer cells. These results provide initial evidence for testing this combination in clinical use.

Keywords: Cisplatin; Drug resistance; LBH589; Ovarian cancer; Sensitivity

Introduction

Cisplatin or carboplatin-based regimens have been introduced into clinical practice as the first-line chemotherapy for ovarian cancer after debulking surgery [1]. Intrinsic or acquired drug resistance, however, remains a major obstacle resulting in a poor prognosis in patients receiving therapy [2]. Therefore, there is a need to develop alternative therapeutic strategies to overcome cisplatin-associated resistance for ovarian cancer patients. In the last two decades, reversal of cisplatin resistance in cancer cells had raised great interest for some clinicians and scientists. Several chemicals and drugs have been evaluated as cisplatin sensitizers, including 12-O-tetradecanoylphorbol-13-acetate [3,4], 2-propylpentanoic acid (valproic acid) [5], 2′-deoxy-5-azacytidine (AZAC) [6],

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hepatocyte growth factor [7], tamoxifen [8], mifepristone [9], and cyclooxygenase inhibitors [10]. However, at present, there is no widely accepted standard application clinically effective for delaying tumor progression in platinum-resistant disease. Because cisplatin-induced toxicity is dose limiting, to discover a method to reduce the working dosage of cisplatin will be a great contribution to patients with advanced disease.

Drug resistance of cancer cells resulting from gene transcription, through complex mechanisms of genetic and epigenetic modifications, is a significant cause of chemotherapy failure in patients with advanced cancers [11,12]. DNA methylation and histone deacetylation (HDAC) are two major mechanisms of epigenetic modification, which play an important role in regulating gene expression [13]. In many forms of cancer, abnormal transcriptional activation and inactivation of genes frequently occurs, and HDAC has been implicated in epigenetic silencing of tumor suppressor genes affecting critical biological activities in cancer cells [14]. Moreover, aberrant HDAC expression in tumors was found to be associated with poor response to chemotherapy, but the mechanisms leading to drug resistance are not fully known [15]. Treatment with inhibitors of HDAC (HDACi) has shown to have suppressive effects against various tumor cells by inhibiting cell proliferation and inducing cell cycle arrest and apoptosis. Previous studies have also shown that HDACi can alter gene expression of cancer cells with respect to DNA damage response, mitosis, angiogenesis, and signal transduction [16,17]. Therefore, HDACi have been used clinically as promising anticancer drugs. LBH589 (Panobinostat), a potent hydroxamic acid-derived pan-HDAC inhibitor [18], has been effectively used for treating cutaneous T-cell lymphoma [19,20]. At present, there are more than 40 clinical trials using LBH589 for the treatment of hematological and solid malignancies (http://clinicaltrials.gov/).

For overcoming acquired drug resistance in cancers *in vivo* or cancer cells *in vitro*, researchers have attempted to use HDACi in combination with existing chemotherapeutic agents [5,21–23]. One example is that treatment of cisplatin-resistant ovarian cancer cells with decitabine and belinostat greatly enhanced the effects on the cisplatin sensitivity of xenografts and resulted in a marked increase in the expression of epigenetically silenced MLH1 when compared with decitabine alone [23]. It has also been reported that a combination of cisplatin and valproic acid possesses HDACi activity, resensitizes cisplatin response, and increases cytotoxicity via phosphorylation of the ataxia telangiectasia mutation (ATM) in cisplatin-resistant ovarian cancer cells [5]. In this study, we treated cisplatin-resistant cell lines with a combination of cisplatin and LBH589 to explore the reversal effect of cisplatin resistance and changes of gene expression.

Materials and methods

Cell lines

The parental A2780-12 cell line was maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% antibiotic mixture (penicillin, streptomycin, and glutamine) in a humidified 5% CO_2 incubator at 37°C. The resistant cell line, A2780-cp70, was derived from the A2780-12 cell line. These cell lines were kindly provided by Dr Lin [5].

Drugs and chemicals

Cisplatin was obtained from Bristol Myers Squibb Co. (Princeton, NJ, USA), and AZAC was bought from Sigma-Aldrich Co. (St. Louis, MO) LBH589 was bought from Novartis Co., Ltd. (Taipei, Taiwan) and provided by Dr. Chang (Director, National Institute of Cancer Research, NHRI, Taiwan).

RNA extraction and purification

Total RNA of A2780-cp70 cells treated with cisplatin and LBH589 and A2780-cp70 cells treated with cisplatin alone for 24 hours were extracted by Trizol (Invitrogen, Carlsbad, CA, USA), and purified with a RNeasy Mini Kit (Qiagen, Germany). RNA purification was quantified at OD_{260nm} by a ND-1000 spectrophotometer (Nanodrop Technology, USA) and qualitated with a Bioanalyzer 2100 (Agilent Technology, Palo Alto, CA, USA).

Cell viability assay

Quantification of cell viability was done using a water-soluble tetrazolium salts-1 Kit (Biovision, CA, USA). Cells were plated and harvested in 96-well plates at 3,000 cells per well in a final volume of 200 μL/well of culture medium. At 24 hours after plating, cisplatin alone or in combination with 5 nM LBH589 or 8.8 μM AZAC was tested in various serial dilutions in 200 μL of new media for each cell line. The cancer cells were continuously incubated for 3 days with each drug concentration in triplicate wells. Next, the medium in each well, including empty wells which served as controls, was replaced with 110 μL of culture medium containing 10 μL/well water-soluble tetrazolium salts/electro-coupling solution for 2 hours, then shaken thoroughly for 1 minute on a shaker. The optical density (OD) of the wells was measured in an ELISA Microplate Reader at 450 nm and 630 nm (reference OD). The OD correlates linearly with the number of viable cells.

Clonogenic assay

Cells were cultured overnight in six-well plates in 3 mL culture medium and then treated with cisplatin and LBH589 at various concentrations. After 14 days, the cells were fixed and stained with 0.25% crystal violet and 10% ethanol for 5 minutes. The colony number (>50 cells/colony) were counted and calculated as surviving fractions by dividing by the initial number of cells in the plate. The percentage of colony number was expressed related to control.

Microarray mRNA analysis of gene expression

Microarray experiments were carried out following the manufacturer's protocols. Briefly, 0.5 μg of total RNA was amplified by a Fluorescent Linear Amplification Kit (Agilent Technologies, USA) and labeled with Cy3-CTP or Cy5-CTP (CyDye; PerkinElmer, Norwalk, CT, USA). During the *in vitro* transcription process, A2780-cp70 RNA treated with cisplatin and LBH589 was labeled with Cy5 and RNA treated with cisplatin alone was labeled by Cy3. Next, 0.825 μg of Cy-labled cRNA was fragmented to an average size of 50–100 nucleotides by incubation with fragmentation buffer (Agilent Technologies, USA) at 60°C for 30 minutes. Fragmented labeled cRNA was then pooled and hybridized to an Agilent Whole Human Genome 4×44 k oligo microarray (Agilent Technologies, USA) at 60°C for 17 hours. After washing and drying via a nitrogen gun, microarrays were scanned with an Agilent microarray scanner (Agilent Technologies, USA) at 535 nm for Cy3 and 625 nm for Cy5. Scanned images were analyzed with Feature extraction software 9.5.3 (Agilent Technologies, USA), an image analysis and normalization software used to quantify signal and background intensity for each feature, substantially normalizing the data by the rank-consistency-filtering LOWESS method.

Results

Enhancement of cisplatin sensitivity in A2780-cp70 cells by 5 nM LBH589

To detect the synergistic effects of growth inhibition and antiproliferation between cisplatin and LBH589 in cancer cells, we performed cell a viability assay and a clonogenic assay. The parental and resistant cells were treated with increasing concentrations of cisplatin in combination with either fixed LBH589 (5 nM) or AZAC (8.8 μM) concentration over a period of 72 hours. In cell viability assays, the combined treatment had a greater inhibition effect in A2780-cp70 cells than in A2780-12 cells, as shown in Fig. 1A. LBH589 and AZAC both enhanced cisplatin sensitivity in A2780-cp70 cells, and treatment with LBH589 and AZAC together exhibited a superior effect in overcoming acquired cisplatin resistance in A2780-cp70 cells. In A2780-cp70 cells treated with cisplatin and LBH589, the inhibition concentration (IC_{50}) value by cisplatin was approximately 15.7 μM; and for cells treated with cisplatin alone, it was about 33.2 μM (data not shown). The combination of LBH589 (5 nM) and cisplatin in resistant cells resulted in enhancement of growth inhibition, whereas the potentiation of growth inhibition was absent in parental cells, that is, A2780-12 (Fig. 1B). Overall, in the presence of low-dose LBH589, the IC_{50} of A2780-cp70 was lower than with cisplatin treatment alone.

Clonogenic assays showed that LBH589 can resensitize A2780-cp70 cells to cisplatin. Low-nanomolar concentrations of LBH589 and cisplatin were sufficient to kill cisplatin-resistant cells, that is, A2780-cp70. The concentration of cisplatin resulting in 50% cell death (EC_{50}) was approximately

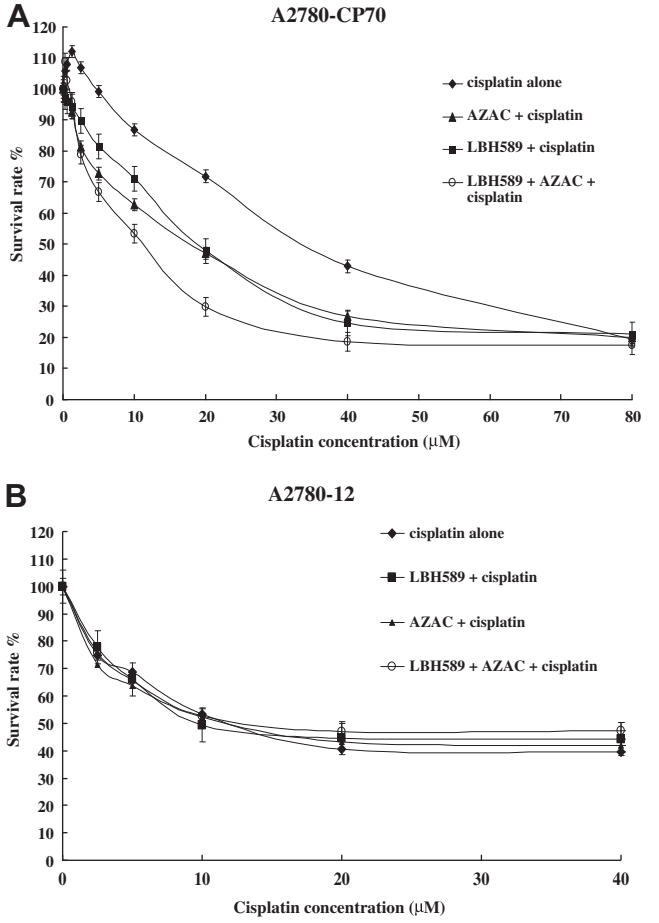

Fig. 1. (A and B) Effects of LBH589 sensitize cisplatin-mediated cytotoxicity on ovarian cell lines. A2780-cp70 cell (A) and A2780-12 (B) were plated and harvested in 96-well plates at 3,000 cells per well; cisplatin alone or combination with 5 nM LBH589 or 8.8 μM AZAC were tested in various concentrations after 24 hours. Quantification of cell viability was done by using WST-1 and performed in independently triplicate experiments. AZAC = 2'-deoxy-5-azacytidine; bars = standard error; points = average of three independent experiments.

1.37 μM in combination with 5 nM of LBH589, whereas the EC_{50} for the cells treated with cisplatin alone was approximately 4.78 μM (Fig. 2). The reversal index of EC_{50} of cotreatment with LBH589 and cisplatin was approximately 3.5-fold more potent than cisplatin treatment alone. Base on these results, LBH589 can enhance the cytotoxicity effects of cisplatin in A2780-cp70 cells. However, no significant difference was not observed when LBH589 was added first, concomitantly, or subsequently to the cisplatin-treated cells (data not shown).

Effects of LBH589 on gene expression in A2780-cp70 cells with cisplatin treatment

As mentioned above, the combined results of cell the viability and clonogenic assays revealed that low-dose LBH589 increased the effectiveness of cisplatin to inhibit the proliferation of A2780-cp70 cells in comparison with cisplatin treatment alone. To investigate the differences of

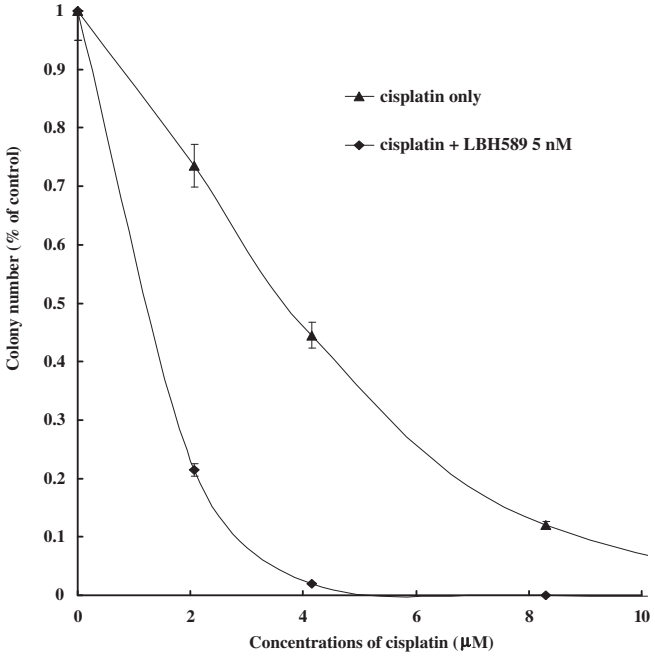

Fig. 2. Clonogenic assay of A2780-cp70 cells cotreated with LBH589 and cisplatin. The concentration of cisplatin resulting in 50% cell death (EC_{50}) was about 1.37 μM in combination with 5 nM of LBH589, whereas the EC_{50} for the cells treated with cisplatin alone was about 4.78 μM. Cells were cultured overnight in six-wells plates in 3 mL culture medium and then treated with cisplatin and LBH589 at various concentrations. After 14 days, the cells were fixed and stained. The colony number (>50 cells/colony) were counted and calculated as surviving fractions by dividing the initial number of cells in the plate. The percentage of colony number was expressed related to control. Points = average of three independent experiments; bars = standard error.

gene expression in cells treated with cisplatin and LBH589 or cisplatin alone, a microarray mRNA analysis was performed. As shown in Table 1, cotreatment with LBH589 and cisplatin in A2780-cp70 cells, as compared with treatment with cisplatin alone, was effective in increasing or decreasing the expression levels of a variety of genes associated with ion/channel/pore class transporter activity, hydrolase activity, nucleotide binding/cellular physiological process, nucleotide binding/telomerase activity, transferase activity, transcription factor activity, immune response/receptor activity, immune response/receptor binding, nucleic acid binding, and other uncategorized functions. Overall, more genes were activated than suppressed. Gene expression profiles of A2780-cp70 cells altered by LBH589 treatment identified that a total of 354 genes had been significantly upregulated (cDNA expression of ≥2-fold) and a total of 63 genes had been significantly downregulated (cDNA expression of ≤0.5-fold). The number of genes showing a ≥2- or ≤0.5-fold change in expression was approximately 417. Among them, 85% were upregulated, whereas only 15% were downregulated. The additional treatment of LBH589 in cells with cisplatin resulted in a striking induction of TEP1 and ARNTL2 genes (up to 10-fold induction), as well as CASP8, 0 POLR3E, and AL13357 (up to 6-fold induction). On the other hand, a significant suppressed gene (down to 0.22-fold reduction) was TRPC2, and similar effects were shown in LINCR and TNRC6B (0.29- and 0.39-fold reduction).

Discussion

Our results showed that LBH589 at a low concentration (5 nM) increased the sensitivity of cisplatin-resistant ovarian cancer cell lines to cisplatin. HDAC catalyzed by HDACi is associated with transcriptional repression and increasingly thought to play a role in the development of human cancers [13,14]. This study aimed to investigate the influence of a combination of cisplatin and LBH589 on antiproliferative activity in cisplatin-resistant ovarian cancer cells. We also performed a microarray RNA analysis to evaluate changes of gene expression after concurrent treatment with LBH589 and cisplatin. The combination treatment had a greater inhibition effect in A2780-cp70 cells than A2780-12 cells as determined by a cell viability assay. In addition, a clonogenic assay showed remarkable reversal of cisplatin resistance by 5 nM of LBH589 in A2780-cp70 cells. Gene expression profiles were altered in A2780-cp70 cells after adding LBH589 to cisplatin treatment, and more genes were activated than suppressed. These genes may play roles in various cellular biological pathways to increase cisplatin sensitivity in A2780-cp70 cells.

In clinical practice, cisplatin and HDACi are known as antiproliferative agents and used in the treatment of a variety of malignancies [2,16,17,19,24]. Cisplatin is a platinum-based chemotherapeutic agent and induces DNA cross-links and adducts, as well as the generation of superoxide radicals. HDACi function as modifiers of histone and nonhistone proteins via inhibiting deacetylation and then trigger the reexpression of certain genes, which play important roles in antitumor activity [16,17,19]. LBH589 is a potent pan-HDACi, causing cell cycle arrest in G2/M and has been shown to kill tumor cell lines both *in vitro* and *in vivo* [18,25–28]. Using LBH589 to inhibit HDAC activity is a rational therapeutic strategy currently in clinical development. Recent studies have demonstrated synergistic antiproliferative effects in cells cotreated with a chemotherapeutic drug and HDACi [21–23,29,30]. In the present study, the phenotype of cisplatin-associated resistance in A2780-cp70 cells was observed when only cisplatin was added. The result was consistent with previous study [5]. However, when cells were cotreated with cisplatin and LBH589 at a concentration of 5 nM, greater inhibitory effects were seen in A2780-cp70 cells than in A2780-12 cells. Notably, the reversal factor of resistance was 3.5-fold. The results revealed that LBH589 may act as a sensitizing drug and can be combined with cisplatin to overcome the cisplatin resistance phenotype. Collectively, the results indicate that LBH589 can sensitize A2780-cp70 cells to cisplatin and lead to inhibition of the proliferation of tumor cells. Furthermore, because LBH589 was effective at the nanomolar level, it may have a safer toxicity profile than traditional chemotherapeutic agents. These important results may potentially expand more clinical applications of LBH589 and benefit patients with advanced or recurrent ovarian cancer by increasing cisplatin sensitivity of tumors and reducing the required cisplatin dosage to minimize its side effects.

To investigate the growth inhibition in cells treated with LBH589 and cisplatin, an inhibitor of DNA methyltransferase,

Table 1
Candidate genes possibly involved in HDACi effect to increase cisplatin sensitivity of A2780-cp70 cells by microarray mRNA analysis[a]

Gene name	Fold change	Genbank	Description
Upregulated			
Ion/channel/pore class transporter activity			
SLC1A1	2.15	NM_004170	Solute carrier family 1, member 1
SLC40A1	2.08	NM_014585	Solute carrier family 40, member 1
SLC17A5	2.08	NM_012434	Solute carrier family 17, member 5
GJE1	2.12	BC038207	Gap junction protein, epsilon 1
STEAP1	2.32	NM_012449	Six transmembrane epithelial antigen of the prostate 1
SCN2B	2.04	NM_004588	Sodium channel, voltage-gated, type II, beta
KCNMB4	2.31	NM_014505	Potassium large conductance calcium-activated channel, subfamily M, beta member 4
KCNA7	2.15	NM_031886	Potassium voltage-gated channel, shaker-related subfamily, member 7
Hydrolase activity			
CASP8	6.97	NM_033355	Caspase 8, apoptosis-related cysteine peptidase
FRAS1	2.35	NM_025074	Fraser syndrome 1
PDE4C	2.04	Z46632	HSPDE4C1 gene for 3′,5′-cyclic AMP phosphodiesterase
DUSP23	2.17	NM_017823	Homo sapiens dual specificity phosphatase 23
GLB1L	2.15	NM_024506	Galactosidase, beta 1-like
KLK10	2.08	NM_002776	Kallikrein-related peptidase 10
KLK8	2.91	NM_144505	Kallikrein-related peptidase 8
SULF2	2.69	NM_018837	Homo sapiens sulfatase 2
AF086125	2.09	AF086125	Full-length insert cDNA clone ZA79D12
Nucleotide binding/cellular physiological process			
GNL3L	4.80	NM_019067	Guanine nucleotide binding protein-like 3 (nucleolar)-like
MAT1A	2.47	NM_000429	Methionine adenosyltransferase I, alpha
KIF5C	2.78	NM_004522	Kinesin family member 5C
NLRP1	2.13	BC051787	NLR family, pyrin domain containing 1
VCX	4.31	NM_013452	Variable charge, X-linked
VCX3A	4.18	NM_016379	Variable charge, X-linked 3A
Nucleotide binding/telomerase activity			
TEP1	12.63	NM_007110	Telomerase-associated protein 1
Transferase activity			
GSTA1	4.09	NM_145740	Glutathione S-transferase A1
GSTA2	3.15	NM_000846	Glutathione S-transferase A2
GSTA5	3.09	NM_153699	Glutathione S-transferase A5
NMNAT2	3.31	NM_015039	Nicotinamide nucleotide adenylyltransferase 2
AS3MT	3.92	NM_020682	Arsenic (+3 oxidation state) methyltransferase
PNMT	2.98	NM_002686	Phenylethanolamine N-methyltransferase
UST	2.05	NM_005715	Uronyl-2-sulfotransferase
POLR3E	6.83	AB040885	Homo sapiens mRNA for KIAA1452 protein, partial cds
AK125361	2.16	AK125361	Homo sapiens cDNA FLJ43371 fis, clone NTONG2005969
AL133570	6.23	AL133570	Homo sapiens mRNA; cDNA DKFZp434L201
Transcription factor activity			
ARNTL2	10.72	AF256215	Cycle-like factor CLIF
BHLHB3	2.95	NM_030762	Basic helix-loop-helix domain containing, class B, 3
ONECUT2	2.38	NM_004852	One cut domain, family member 2
PBX2	2.03	NM-002586	Homo sapiens pre-B-cell leukemia transcription factor 2
ZIC1	2.05	NM-003412	Zic family member 1
SNFT	2.43	NM_018664	Jun dimerization protein p21SNFT
STON1	2.32	NM_006873	Stonin 1
Immune response/receptor activity			
CXCR4	4.30	NM_001008540	Chemokine (C-X-C motif) receptor 4
HLA-DMA	2.65	NM_006120	Major histocompatibility complex, class II, DM alpha
HLA-DMB	2.38	NM_002118	Major histocompatibility complex, class II, DM beta
HLA-DPA1	2.00	NM_033554	Major histocompatibility complex, class II, DP alpha 1
HLA-DRB5	2.46	NM_002125	Major histocompatibility complex, class II, DR beta 5
PTPRF	2.04	NM_002840	Protein tyrosine phosphatase, receptor type, F
IFNAR2	3.45	NM_207585	Interferon alpha receptor 2
IL3RA	2.42	NM_002183	Interleukin 3 receptor, alpha
Immune response/receptor binding			
TNFSF10	2.25	NM_003810	Tumor necrosis factor (ligand) superfamily, member 10
XCL1	2.05	NM_002995	Chemokine (C motif) ligand 1
XCL2	2.20	NM_003175	Chemokine (C motif) ligand 2
C3	2.36	NM_000064	Complement component 3

(*continued on next page*)

Table 1 (*continued*)

Gene name	Fold change	Genbank	Description
Downregulated			
Ion binding/transporter activity			
SLC25A25	0.48	NM_001006641	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25
SLC39A7	0.45	NM_006979	Solute carrier family 39 (zinc transporter), member 7
RASSF1	0.48	NM170713	Ras association (RalGDS/AF-6) domain family 1
TRPC2	0.22	NR_002720	Transient receptor potential cation channel, subfamily C, member 2
Transferase activity			
ABL1	0.47	NM_005157	v-abl Abelson murine leukemia viral oncogene homolog 1
SNRK	0.43	NM_017719	SNF-related kinase
Nucleic acid binding			
TNFAIP3	0.48	NM_006290	Tumor necrosis factor, alpha-induced protein 3
Chaperone activity			
BAG5	0.48	NM_001015049	BCL2-associated athanogene 5
Uncategorized			
HNRPCL1	0.43	NM_001013631	Heterogeneous nuclear ribonucleoprotein C-like 1
TNRC6B	0.39	NM_015088	Trinucleotide repeat containing 6B
LINCR	0.29	BC012317	Likely ortholog of mouse lung-inducible neutralized-related C3HC4 RING domain protein

[a] The genes showing a $\geq$2- or $\leq$0.5-fold changes in the ratios of cDNA expression is known as significant threshold.
BCL2 = B-cell lymphoma 2; HDACi = inhibitors of histone deacetylation; SNF = sucrose nonfermentor.

AZAC, was also used in the study. The results showed that LBH589 at a concentration of 5 nM was as effective as AZAC at a concentration of 8.8 μM in inhibiting cell growth, indicating that LBH589 combined with cisplatin could be as clinically useful as AZAC. Although combined treatment with LBH589 and AZAC revealed a synergistic effect to resensitize cisplatin resistance in A2780-cp70 cells, the toxicity of AZAC remains a clinical limitation to be considered. Moreover, the superior effect of treatment with LBH589 and AZAC together implies that the formation of acquired cisplatin resistance in ovarian cancer may result from different epigenetic mechanisms.

A whole-genome expression microarray was used to explore the possible genes involved in acquired cisplatin resistance mediated through HDAC in A2780-cp70 cells treated with LBH589 (Table 1). Previous studies have observed upregulation of cell cycle inhibitor p21 in response to LBH589 treatment [22,25,27,28,31]. However, p21 has not been universally observed to be upregulated [20,32,33]. The absence of p21 expression in A2780-cp70 may reflect a feature of the cell line. Furthermore, several genes associated with cell cycle arrest and apoptosis (e.g. ATM, CASP8, CYCS) were increased in our study, suggesting that the predominant effects of LBH589 on A2780-cp70 cells are cell cycle arrest and cell apoptosis. Since the de-expression and re-expression of other genes were observed, LBH589 may participate in controlling cell cycle regulation through the effects of gene expression. On the other hand, distinct expression changes in a variety of genes in A2780-cp70 cells following treatment with cisplatin and/or LBH589 were observed, and more genes were activated than suppressed. A unique set of genes that may have roles in biological responses, such as apoptosis and antiproliferative effects on cell growth, were observed in A2780-cp70 cells. A previous study indicated that LBH589 may attenuate the expression of Bcr-Abl protein and lead to suppression of Bcr-Abl expression in human leukemia cells [34]. Based on this study, it is plausible that the inhibition of cell proliferation in A2780-cp70 by combined cisplatin and LBH589 may at least partially be explained because of the attenuation of Bcr-Ab1 by LBH589. Therefore, these genes may play a role in mediating the resistant phenotype of cisplatin in A2780-cp70 cells, and further investigation is warranted.

On the basis of our findings, we hypothesize that the combination of cisplatin and LBH589 can override cisplatin-associated resistance in ovarian cancer cells. These results provide initial evidence for testing this combination in clinical use. Further *in vivo* research and clinical trials have to be pursued to verify the preliminary results. Whether epigenetic alterations of certain candidate genes are associated with the reversal of cisplatin resistance remains to be investigated in the future.

Acknowledgments

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