3-Oxoacid CoA transferase 1 as a therapeutic target gene for cisplatin-resistant ovarian cancer

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Received January 18, 2017; Accepted April 3, 2017

DOI: 10.3892/ol.2017.7560

Abstract. Ovarian cancer (OC) is the second leading cause of mortality from gynecological malignancies and has the highest mortality rate worldwide. As it is commonly asymptomatic during the early stages of the disease, >70% of patients with OC are diagnosed at advanced stages with metastasis. Despite treatment methods, including optimal debulking surgery and chemotherapy with the platinum-based drug cisplatin, OC recurrence is often inevitable, with an overall 5-year survival rate of 45%, mostly due to the steady development of cisplatin resistance. To identify genes involved in cisplatin resistance, the present study determined the half-maximal inhibitory concentrations of eight different OC cell lines and classified them into two groups (sensitive and resistant). mRNA expression was analyzed with GeneChip Human Gene 1.0 ST Arrays, and DNA methylation profiles were evaluated with the HumanMethylation450 BeadChip. Using an integrated approach of analyzing gene expression levels and DNA methylation profiles simultaneously, 26 genes were selected that were differentially expressed and methylated between the resistant and sensitive groups. Among these 26 genes, 3-oxoacid CoA transferase 1 (*OXCT1*), which was demonstrated to be downregulated and hypermethylated at promoter CpGs in the cisplatin-resistant group compared with the cisplatin-sensitive group, was selected for further investigation. Treatment with a DNA methyltransferase inhibitor restored hypermethylation-mediated gene silencing of *OXCT1* in the cisplatin-resistant group, but not in the cisplatin-sensitive group. Furthermore, overexpression of *OXCT1* conferred sensitivity to cisplatin in OC cells. The results of the present study suggest that *OXCT1* serves an important role in conferring cisplatin sensitivity, and may provide a potential therapeutic target for cisplatin chemotherapy in patients with recurrent OC.

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Abbreviations: OC, ovarian cancer; *OXCT1*, 3-oxoacid CoA transferase 1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; 5-aza-dC, 5-aza-2'-deoxycytidine; FBS, fetal bovine serum; P/S, penicillin/streptomycin; DEGs, differentially expressed genes; CGI, CpG islands; TSS, transcription start site

Key words: 3-oxoacid CoA transferase 1, ovarian cancer, DNA methylation, cisplatin, integrated analysis

Introduction

Ovarian cancer (OC) is an intractable cancer with the highest mortality rate among all types of female cancers worldwide (1). Its incidence is continually increasing along with the prevalence of westernized lifestyles, the use of hormone replacement therapy and aging population in Asian regions (2). Early OC is frequently asymptomatic; accordingly, >70% of patients are reportedly diagnosed with OC when it has reached an advanced stage (stage 3 or higher). Furthermore, recurrence or metastasis occurs in >75% of patients within the first 2 years following initial treatment (3,4). In the majority of patients, initial responses can be achieved with debulking surgery and treatment with taxanes in combination with platinum-based chemotherapy; however, >75% of those responders eventually relapse, resulting in chemoresistant and fatal disease (5,6).

Epigenetic alterations, which are closely associated with ovarian tumorigenesis, are defined as heritable alterations in gene expression without changes to the DNA sequence; these include histone modification, DNA methylation and post-transcriptional gene regulation by microRNAs (7). Among the various epigenetic mechanisms that affect gene expression, DNA methylation is the most extensively studied. Specific DNA methyltransferases catalyze DNA methylation by transferring a methyl group, using S-adenosyl methionine as the methyl donor, to the cytosine residues of CpG dinucleotides (8). CpG methylation in the promoter regions of specific genes leads to physical obstruction of transcription factor binding, and recruitment of methyl-CpG-binding domain proteins and histone deacetylases that are associated with gene silencing and the formation of inactive heterochromatin (9). DNA methylation is therefore an important mechanism underlying gene silencing and inactivation, and the methylation status at

promoter CpG sites serves a pivotal role in the regulation of gene expression (10).

Recent studies have reported that aberrant DNA methylation may affect the sensitivity of cells to anticancer drugs by altering the expression of genes that are crucial for drug response. A number of studies have demonstrated that DNA hypermethylation is involved in generating drug-resistant phenotypes by inactivating genes that are required for cytotoxicity (11-14).

Based on previous studies, we hypothesized that epigenetically regulated genes involved in drug resistance may serve as promising novel targets for the effective treatment of cisplatin-resistant OC. To identify the genes involved in cisplatin resistance, the cytotoxicities of eight different OC cell lines were determined, and the cell lines were classified into two groups (sensitive and resistant). mRNA expression levels were analyzed with GeneChip Human Gene 1.0 ST Arrays, and DNA methylation profiles were determined using the Human Methylation450 BeadChip. Using an integrated approach of analyzing gene expression level and DNA methylation profiles simultaneously, 26 genes were selected that were differentially expressed and methylated between the resistant and sensitive groups. Among these 26 genes, 3-oxoacid CoA transferase 1 (*OXCT1*) was selected for further investigation. OXCT1 protein has been identified as a homodimeric mitochondrial matrix enzyme involved in ketone body utilization via the reversible transfer of coenzyme A from succinyl-CoA to acetoacetate (15); however, the involvement of this gene in the drug response has not yet been reported. Epigenetic silencing of *OXCT1* via the hypermethylation of promoter CpGs was revealed in the present study, and was shown to be associated with cisplatin resistance in OC. Furthermore, the overexpression of *OXCT1* restored chemosensitivity to cisplatin, indicating that *OXCT1* acts as a suppressor of cisplatin resistance in OC. The results of the present study offer novel insight into the function of *OXCT1* in chemoresistant OC.

Materials and methods

Cell culture. SK-OV-3, PA-1, Caov-3, TOV-21G, TOV-112D, OV-90 and OVCAR-3 human OC cell lines studied were purchased from the American Type Culture Collection (Manassas, VA, USA), and the human OC A2780 cell line was purchased from the European Collection of Cell Cultures (London, UK). All cell lines were initially cultured using the medium and supplements recommended by the suppliers. Table I summarizes components of the culture media for individual cell lines. All eight cell lines were grown as monolayers and attached cells were fully disaggregated by trypsinization between passages. The cell lines were maintained in a 95% humidified and 5% CO_2 atmosphere at 37˚C.

Cisplatin sensitivity assay. The cisplatin sensitivities of the eight human ovarian cell lines (SK-OV-3, PA-1, Caov-3, TOV-21G, TOV-112D, OV-90, OVCAR-3 and A2780) were determined using MTT assays (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Briefly, $2x10^4$ cells were seeded onto 96-well plates and incubated at 37˚C overnight. The medium was exchanged with fresh medium supplemented with various cisplatin concentrations (0-100 μM). Following incubation for 48 h, 20 μl 2.5 mg/ml MTT solution was added to each well and the plates were further incubated for 2 h at 37˚C. Dimethyl sulfoxide (100 μl; Sigma-Aldrich; Merck KGaA) was added to solubilize the MTT formazan product through a 10 min oscillation at 37˚C. Absorbance at 540 nm was determined using a microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA). Dose-response curves were plotted as the percentage of the control, which was obtained from the sample with no drug exposure. Half-maximal inhibitory concentration (IC_{50}) was evaluated as the concentration of cisplatin that reduces cell growth by 50% under the experimental conditions. The eight human ovarian cell lines were classified into two groups: Sensitive and resistant cell lines.

Total RNA isolation and mRNA microarray. Total RNA was extracted from the eight human OC cell lines using the RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA) and amplified and labeled according to the Affymetrix GeneChip Whole Transcript Sense Target Labeling protocol (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The resulting labeled cDNA was hybridized to Affymetrix GeneChip Human Gene 1.0 ST Arrays (Thermo Fisher Scientific, Inc.). The scanned raw expression values were background-corrected, normalized and summarized using the Robust Multiarray Averaging approach in the Bioconductor 'affy' package (Bioconductor; http://www.bioconductor.org/). The resulting log2-transformed data were used for further analyses. To identify differentially expressed genes (DEGs), moderated t-statistics were applied based on an empirical Bayesian approach (16). Significantly upregulated and downregulated DEGs were defined as those with ≥1.5-fold difference in expression level between the cisplatin-resistant and -sensitive groups following correction for multiple testing [Benjamini-Hochberg false discovery rate (BH FDR)-adjusted P<0.01] (17).

Genomic DNA isolation and CpG methylation microarray. Genomic DNA was extracted from the eight human OC cell lines using the QIAmp Mini kit (Qiagen, Inc.), according to the manufacturer's instructions. For genome-wide screening of DNA methylation, the Illumina HumanMethylation450 BeadChip (Illumina, Inc., San Diego, CA, USA) was used, which targets 450,000 specific CpG sites. DNA methylation values were described by β-values, which were determined by subtracting the background obtained from negative controls on the array and calculating the ratio of the methylated signal intensity to the sum of the methylated and unmethylated signals. β-values ranged from 0 (completely unmethylated) to 1 (fully methylated) on a continuous scale for each CpG site. To identify differentially methylated CpG sites, the difference in mean β-value (Δβ; mean β-value in resistant group-mean β-value in sensitive group) was determined. If the absolute difference in mean β-values (|Δβ|) was >0.3, the sites were defined as differentially methylated CpG sites. CpG sites or genes were described as hypermethylated if Δβ>0.3 and as hypomethylated if Δβ<-0.3.

Integrated analysis of DNA methylation and gene expression. To identify genes that had expression regulated by epigenetic alteration in the cisplatin-resistant group, the global DNA methylation profiling data was integrated with the mRNA

expression profiles using stringent selection criteria (|Δβ|>0.3, expression fold change >1.5). Expression of candidate genes was considered to be upregulated (fold change >1.5) by hypomethylation (Δβ<-0.3) at promoter CpG sites and downregulated (fold change >1.5) by hypermethylation (Δβ>0.3) at promoter CpG sites in the cisplatin-resistant group compared with the cisplatin-sensitive group.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA (1 μg) was converted to cDNA using Superscript II Reverse Transcriptase and oligo-$(dT)_{12-18}$ primers (both from Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. RT-qPCR was performed in a 20 μl reaction mixture containing 1 μl cDNA, 10 μl SYBR Premix Ex Taq (Takara Bio, Inc., Otsu, Japan), 0.4 μl Rox reference dye (x50; Takara Bio, Inc.), and 200 nM primers for each gene. The primer sequences were as follows: *OXCT1* forward, 5'-GGGTCCATATCCACGACAACA-3'; *OXCT1* reverse, 5'-GACGTGTCCACCTCTAATCATTG-3'; *GAPDH* forward, 5'-AATCCCATCACCATCTTCCA-3'; and *GAPDH* reverse, 5'-TGGACTCCACGACGTACTCA-3'. The reactions were run on a 7500 Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 3 sec and 60˚C for 30 sec, and a single dissociation cycle of 95˚C for 15 sec, 60˚C for 60 sec and 95˚C for 15 sec. All reactions were performed in triplicate, and the specificity of the reaction was determined using melting curve analysis at the dissociation stage. Comparative quantification of each target gene was performed based on the cycle threshold (Cq) normalized to GAPDH using the $2^{-\Delta\Delta Cq}$ method (18).

5-aza-2'-deoxycytidine (5-aza-dc) treatment. To demethylate methylated CpG sites, the eight human ovarian cell lines were treated with 10 μM 5-aza-dc (Sigma-Aldrich; Merck KGaA) for 3 days at 37˚C . Each day, the medium was exchanged with fresh medium supplemented with 10 μM of 5-aza-dc.

Transient transfection. To establish a transient expression system, SK-OV-3 cells were transfected with pCMV-SPORT6-OXCT1 (KRIBB, Daejeon, Korea) or pEGFP-N3 (Clontech Laboratories, Inc., Mountainview, CA, USA) plasmids using Lipofectamine 2000® (Invitrogen; Thermo Fisher Scientific, Inc.). Briefly, the cells were plated at a density of $6x10^5$ cells/well in 6-well plates and allowed to grow overnight at 37˚C. In total, 2 μg of each plasmid DNA and 5 μl Lipofectamine 2000 were diluted separately in Opti-MEM (Gibco; Thermo Fisher Scientific, Inc.) to a total volume of 250 μl. The diluted plasmid DNAs and Lipofectamine 2000 were mixed and incubated at room temperature for 20 min to generate the transfection mixtures. The cells were washed with serum-free McCoy's 5A medium, and subsequently the transfection mixtures were added to each well of the 6-well plates containing complete growth medium, and incubated at 37˚C for 24 h in a 5% CO_2 incubator. The sensitivity to cisplatin of the transfected cells was determined using the MTT assay, as described for the aforementioned 'cisplatin sensitivity assay'.

Statistical analysis. Data are expressed as the mean ± standard deviation of ≥3 independent experiments. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). The unpaired Student's t-test was used to perform statistical analysis between two groups. P<0.05 was considered to indicate a statistically significant difference.

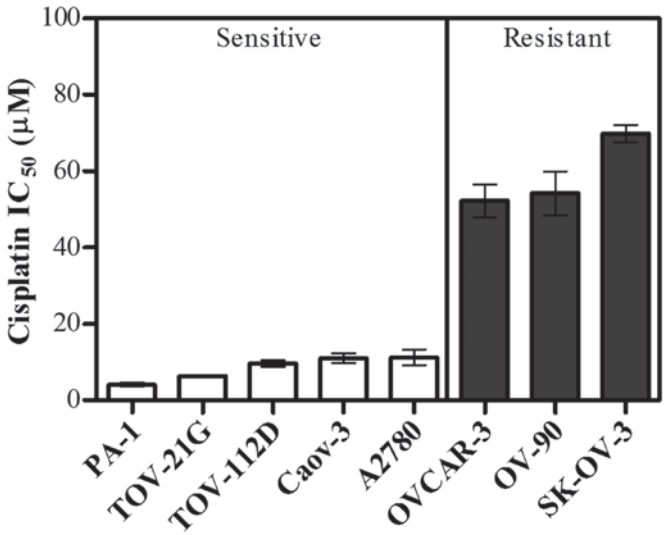

Figure 1. The IC_{50} values of eight human ovarian cancer cell lines. Cell viability was assessed using MTT assays following exposure to cisplatin for 48-h. The error bars indicate the mean ± standard deviation of triplicate experiments. IC_{50}, half-maximal inhibitory concentration.

Results

Determination of cisplatin resistance in eight human OC cell lines. The sensitivity to cisplatin of the eight human ovarian cell lines (SK-OV-3, PA-1, Caov-3, TOV-21 G, TOV-112D, OV-90, OVCAR-3 and A2780) was determined using the MTT cytotoxicity assay. The highest IC_{50} value for cisplatin was observed in SK-OV-3 cells (69.8 μM), and the lowest IC_{50} value was observed in PA-1 cells (4.1 μM). The IC_{50} values of the eight human OC cell lines increased in the order PA-1, TOV-21 G, TOV-112D, Caov-3, A2780, OVCAR-3, OV-90 and SK-OV-3, as presented in Fig. 1. Based on the IC_{50} values for cisplatin, the cell lines were classified into two groups as sensitive (PA-1, TOV-21G, TOV-112D, Caov-3 and A2780) and resistant (OVCAR-3, OV-90, and SK-OV-3).

Identification of differentially expressed genes between cisplatin-resistant and cisplatin-sensitive groups. To identify DEGs, the present study applied moderated t-statistics based on an empirical Bayesian approach (16). Significantly upregulated and downregulated DEGs were defined as genes with ≥1.5-fold difference in expression level between cisplatin-resistant and -sensitive groups based on the microarray, following correction for multiple testing (BH FDR-adjusted P<0.01) (17). Using this criterion, the expression levels of 376 genes were altered in the cisplatin-resistant group compared with in the cisplatin-sensitive group.

Identification of differentially methylated CpG sites between cisplatin-resistant and cisplatin-sensitive groups. To identify differentially methylated CpG sites, the difference in Δβ

Table I. Components of culture media for the human ovarian cancer cell lines studied.

Cell line	Components of culture media[a]
SK-OV-3	McCoy's 5a + 10% FBS + 1% P/S
PA-1	MEM α + 10% FBS + 1% P/S
Caov-3	DMEM (1.5 g/l sodium bicarbonate) + 10% FBS + 1% P/S
TOV-21G	MCDB 105 (1.5 g/l sodium bicarbonate) and Medium 199 (2.2 g/l sodium bicarbonate) 1:1 mix + 10% FBS + 1% P/S
TOV-112D	MCDB 105 (1.5 g/l sodium bicarbonate) and Medium 199 (2.2 g/l sodium bicarbonate) 1:1 mix + 10% FBS + 1% P/S
OV-90	MCDB 105 (1.5 g/l sodium bicarbonate) and Medium 199 (2.2 g/l sodium bicarbonate) 1:1 mix + 10% FBS + 1% P/S
OVCAR-3	RPMI-1640 (25 mM HEPES) + 10% FBS + 1% P/S
A2780	RPMI-1640 (25 mM HEPES) + 10% FBS + 1% P/S

[a]All media, reagents and supplements were provided by Gibco/BRL (Rockville, MD, USA) except DMEM and RPMI-1640. DMEM and RPMI-1640 were purchased from Welgene (Gyeongsan-si, Gyengsanbuk-do, Republic of Korea). FBS, fetal bovine serum; P/S, penicillin/streptomycin; DMEM, Dulbecco's modified Eagle's medium; MCDB, molecular cell developmental biology; MEM, minimum essential medium.

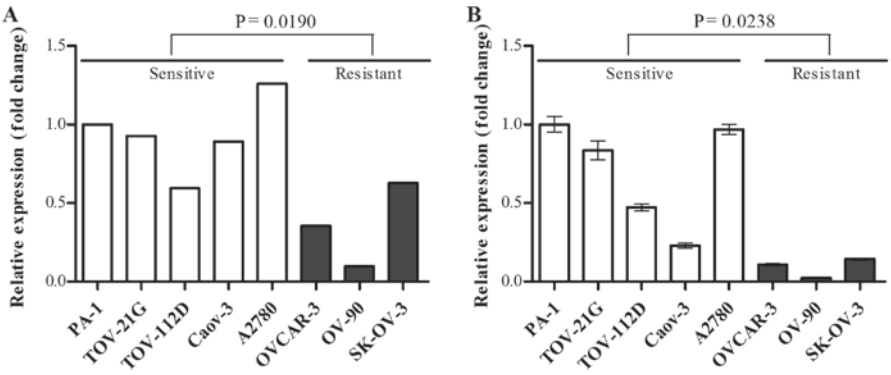

Figure 2. Downregulation of *OXCT1* expression in cisplatin-resistant cell lines. *OXCT1* mRNA expression was determined by (A) gene expression microarray and (B) reverse transcription-quantitative polymerase chain reaction. Data are presented as the mean ± standard deviation of triplicate evaluations. Statistical analyses were performed using a t-test. *OXCT1*, 3-oxoacid CoA transferase 1.

was used. If $|\Delta\beta|>0.3$, the sites were defined as differentially methylated CpG sites. CpG sites or genes were described as hypermethylated if $\Delta\beta>0.3$ and as hypomethylated if $\Delta\beta<-0.3$. By these criteria, the promoter methylation of 5,384 genes (12,293 CpGs) was altered in the cisplatin-resistant group compared with the cisplatin-sensitive group.

Selection of cisplatin resistance-associated genes using integrated analysis. To identify genes whose expression was regulated by DNA methylation during the development of cisplatin resistance, the global DNA methylation profiling data was integrated with the mRNA expression profiles using stringent selection criteria ($\Delta\beta>0.3$; expression fold change >1.5). Expression of candidate genes was considered to be upregulated (fold change >1.5) by hypomethylation ($\Delta\beta<-0.3$) at promoter CpG sites and downregulated (fold change >1.5) by hypermethylation ($\Delta\beta>0.3$) at promoter CpG sites in the cisplatin-resistant group compared with the cisplatin-sensitive group. Using these criteria, 26 candidate genes were selected. The candidate genes are presented in Table II.

mRNA expression level of OXCT1 was downregulated in the cisplatin-resistant group. Among the 26 cisplatin resistance-associated genes, *OXCT1* was selected as, to the best of our knowledge, its association with chemosensitivity has not been reported in previous cancer studies, and its expression level was confirmed by RT-qPCR. *OXCT1* mRNA expression level was significantly decreased in the cisplatin-resistant group compared with the cisplatin-sensitive group, in agreement with the results of gene expression microarray (Fig. 2).

OXCT1 expression was suppressed by DNA methylation in the cisplatin-resistant group. Substantial DNA methylation changes during the acquisition of chemoresistance have been widely reported in various types of cancer (19). In particular, DNA hypermethylation of CpG islands (CGIs) within promoter regions serves a prominent role in the development of drug resistance by silencing genes that are required for cytotoxicity (11). Thus, the present study investigated the DNA methylation status of CGIs within the promoter region of the *OXCT1* gene using the Illumina HumanMethylation450

Table II. Candidate genes with expression levels regulated by DNA methylation during cisplatin-resistance development.

	DNA methylation				Gene expression	
Gene symbol	Difference in β value[a]	P-value	Chromosome	CpG site	Fold change (log_2)[b]	P-value
MFSD2A	0.795151465	0.000000402	1	40420603	0.94105296	0.015471851
	0.528977905	0.000119729	1	40420537		
	0.638894305	0.000139608	1	40420635		
FKBP10	0.392134207	0.0000166	17	39968802	3.577717953	0.000795819
	0.635697744	0.000000435	17	39968772		
	0.450164388	0.00000461	17	39968804		
	0.418122533	0.000429913	17	39968600		
MARVELD1	0.470414467	0.000134843	10	99474521	1.346310667	0.000609707
HIST1H2BF	0.539404884	0.000000755	6	26199465	1.109350157	0.005487531
HIVEP2	-0.649083953	0.00000458	6	143249236	-2.267853853	0.032947151
ZNF257	-0.439560377	0.000513184	19	22235199	-2.515197001	0.001691734
	-0.556456643	0.000598785	19	22235022		
	-0.7204497	0.00000623	19	22234992		
	-0.588986337	0.000100124	19	22235281		
ZFP3	-0.699854973	0.0000295	17	4981598	-1.681825862	0.0000449
	-0.756465323	0.000142409	17	4981610		
	-0.614927003	0.00043569	17	4981403		
	-0.824812407	0.0000127	17	4981603		
INA	0.649938177	0.00020484	10	105036701	4.355589328	0.0000111
HIST1H3D	0.397435269	0.0000184	6	26199702	0.6066328	0.04004395
LEPREL2	0.31302834	0.00022931	12	6938638	0.920190572	0.003181991
	0.433342418	0.0000252	12	6938635		
OXCT1	0.550269195	0.000511071	5	41870875	1.716584541	0.013127834
	0.497255367	0.000143461	5	41870856		
	0.511680656	0.000544145	5	41870860		
	0.377481771	0.0000288	5	41869963		
NME4	0.725727483	0.0000517	16	446668	1.482274313	0.009042391
MAP3K12	0.342133404	0.0000789	12	53893000	1.81515751	0.008388664
DLG4	-0.56680592	0.000631474	17	7108468	1.410099319	0.04186927
	-0.522567633	0.000105786	17	7108653		
TMEM180	0.420509153	0.000114655	10	104221598	0.789638858	0.047135907
DCBLD1	-0.469961667	0.000118409	6	117869857	-1.955294821	0.002032113
DNAJC15	-0.545150123	0.000684898	13	43597565	-3.261718049	0.014808162
MAPRE3	0.499877566	0.000140963	2	27194315	-0.808403845	0.021186922
VSIG10L	0.564933039	0.000167731	19	51843854	0.813198578	0.007985285
HIST1H2BB	-0.65683174	0.000172726	6	26044274	-1.511985487	0.031374454
	-0.575591967	0.000262479	6	26043990		
	-0.6231011	0.00030871	6	26044220		
FAM188B	0.474266434	0.000703141	7	30810858	0.755956576	0.00432309
	0.533877189	0.000787152	7	30810882		
	0.417810231	0.000671844	7	30810864		
	0.504869284	0.000261883	7	30810870		
BAMBI	0.53068002	0.000322864	10	28965584	-2.435784283	0.016972961
NAGA	0.545561447	0.000456257	22	42466345	0.637024627	0.01300139
ST3GAL2	0.327738228	0.000518788	16	70473447	-0.880289271	0.024322723
RUNX1	-0.484414007	0.000602711	21	36421955	-1.735856425	0.019877857
ZC3HAV1L	0.661703861	0.000765107	7	138720989	1.317571487	0.048052422

[a]Difference in β value=β value in the resistant group-β value in the sensitive group. [b]Fold change (log_2)=expression value in resistant group (log_2)-expression value in sensitive group (log_2).

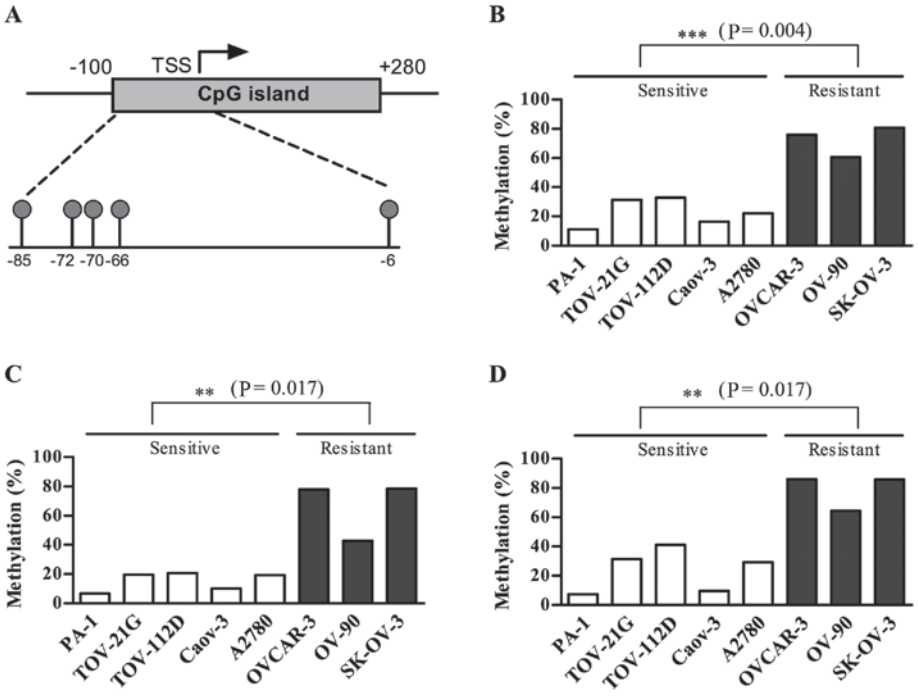

Figure 3. Hypermethylation of CGIs within *OXCT1* promoter in cisplatin-resistant cell lines. The DNA methylation status of CGI within the *OXCT1* promoter region was quantified using the Illumina HumanMethylation 450 BeadChip in eight ovarian cancer cell lines. (A) The Illumina HumanMethylation 450 BeadChip included five CpG sites within the CGI promoter region of the *OXCT1* gene, which are located at positions -85, -72, -70, -66 and -6 from the TSS. The DNA methylation status of three CpGs at positions (B) -66, (C) -70 and (D) -85 relative to TSS are shown. Statistical analyses were performed using t-tests. **P<0.01; ***P<0.001. CGI, CpG islands; *OXCT1*, 3-oxoacid CoA transferase 1; TSS, transcription start site.

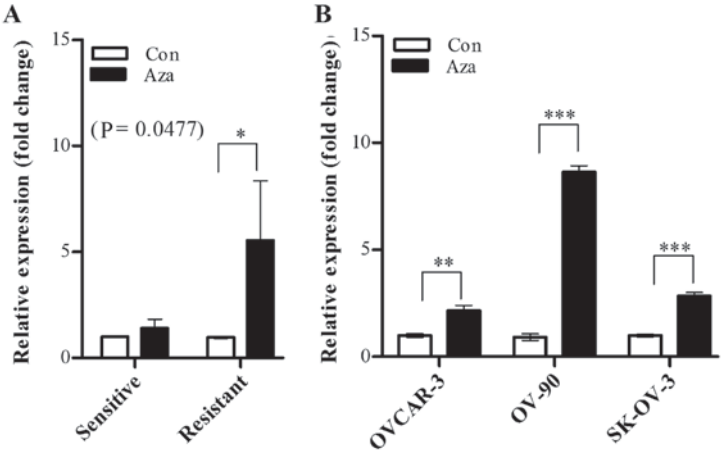

Figure 4. Restoration of *OXCT1* expression following demethylation in cisplatin-resistant cell lines. Eight ovarian cancer cell lines were treated with 5-aza-2'-deoxycytidine. Following treatment with 5-aza-2'-deoxycytidine, *OXCT1* mRNA expression levels were determined by reverse transcription-quantitative polymerase chain reaction. The *OXCT1* mRNA expression levels relative to the untreated control are shown for (A) the cisplatin-sensitive and cisplatin-resistant groups of cell lines, and (B) the individual cisplatin-resistant cell lines. The error bars indicate the mean ± standard deviation of triplicate evaluations. Statistical analyses were performed using t-tests. *P<0.05; **P<0.01; ***P<0.001. Con, control; Aza, 5-aza-2'-deoxycytidine; *OXCT1*, 3-oxoacid CoA transferase 1.

BeadChip, which included five CpG sites within the CGI promoter region of the *OXCT1* gene, located between positions 41,870,792 and 41,870,890 of chromosome 5 (human GRCh37/hg19). The five CpGs were at positions -85, -72, -70, -66 and -6, relative to the transcription start site (TSS) as presented in Fig. 3A. Among the five CGI promoter CpGs, the three CpG sites located at -85, -70 and -66 from the TSS were significantly hypermethylated in the cisplatin-resistant group compared with the cisplatin-sensitive group (Fig. 3).

Subsequently, whether *OXCT1* expression is regulated by epigenetic modification was investigated using a DNA methyltransferase inhibitor. The cisplatin-sensitive and cisplatin-resistant groups were treated with 5-aza-dc, and *OXCT1* expression was evaluated by RT-qPCR. The results demonstrated that *OXCT1* expression level was significantly increased in the cisplatin-resistant group, whereas there was no significant increase detected the in cisplatin-sensitive group (Fig. 4A). In all the cisplatin-resistant cell lines

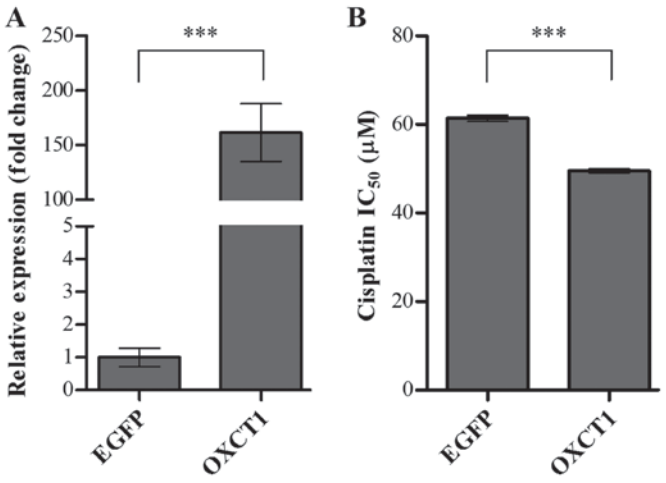

Figure 5. Sensitization of cells to cisplatin following overexpression of *OXCT1*. Cisplatin-resistant SK-OV-3 cells were transiently transfected with *EGFP* and *OXCT1* expression constructs. (A) Following 24 h of transfection, *OXCT1* transfection efficiency was confirmed by evaluating *OXCT1* mRNA expression levels by reverse transcription-quantitative polymerase chain reaction. (B) Cell viability of transfected cells was determined using MTT assay following a 48-h treatment with cisplatin. Data are presented as the mean ± standard deviation from three independent experiments. Statistical analyses were performed using t-tests. ***P<0.001. *OXCT1*, 3-oxoacid CoA transferase 1; *EGFP*, enhanced green fluorescent protein.

(OVCAR-3, OV-90 and SK-OV-3), the *OXCT1* expression level of 5-aza-dc-treated cells was restored, in the range of 2.1-6.8-fold, compared with that of 5-aza-dc-untreated cells, which indicated that the *OXCT1* expression level was suppressed by hypermethylation in cisplatin-resistant cell lines (Fig. 4B).

Overexpression of OXCT1 enhanced sensitivity to cisplatin in the SK-OV-3 OC cell line. To determine whether *OXCT1* overexpression in a cisplatin-resistant cell line improved sensitivity to cisplatin, SK-OV-3 cells were transiently transfected with *OXCT1* or *EGFP* expression plasmid constructs. Following a 24-h transfection, the expression levels of *OXCT1* were determined by RT-qPCR. Compared with the *EGFP*-transfected control cells, the level of *OXCT1* expression was increased to 161.5-fold in *OXCT1*-transfected cells (Fig. 5A). The sensitivity to cisplatin was also determined in *EGFP*- or *OXCT1*-transfected cells using an MTT assay. IC_{50} was evaluated in SK-OV-3 cells transfected with *EGFP*- or *OXCT1*-overexpressing constructs following treatment with cisplatin at various concentrations. Overexpression of *OXCT1* significantly decreased the IC_{50} for cisplatin by ~21% compared with *EGFP*-transfected control cells (Fig. 5B). These results indicated that overexpression of *OXCT1* in the cisplatin-resistant cell line significantly attenuated resistance to cisplatin.

Discussion

The present study provides evidence that epigenetically regulated genes are involved in cisplatin resistance of OC cells. mRNA expression profiles were integrated with DNA methylation profiles to identify candidate genes for drug resistance, and the *OXCT1* gene was revealed to be a promising target for modulating cisplatin resistance.

Cisplatin is one of the most effective broad-spectrum anticancer drugs, and this platinum-based anticancer drug activates the intrinsic apoptosis pathway via formation of platinum-DNA adducts, resulting in DNA strand breaks during mitotic cell division, which induce apoptosis (20). The DNA strand break initiates multiple cellular self-defense systems, including DNA damage repair, exocytosis of toxic metal compounds and alterations in gene expression, and these responses result in chemoresistance to cisplatin (21). Therefore, effector genes responsible for cisplatin resistance may be due to a defective influx route (reduced endocytosis of cisplatin), changes to other putative proteins for cisplatin uptake, or altered expression of detoxifying enzymes. Additionally, aberrant promoter hypermethylation-mediated gene silencing is an epigenetic hallmark of drug resistance; cisplatin treatment induces promoter hypermethylation, alters gene expression profiles and renders cells cisplatin-resistant (11).

OXCT1 encodes 3-oxoacid-conenzyme A transferase 1, which is a homodimeric mitochondrial matrix enzyme that serves a central role in extrahepatic ketone body catabolism (ketone bodies to acetyl-CoA to mitochondrial tricarboxylic acid cycle entrance) (15). There is currently no direct evidence linking *OXCT1* to cisplatin sensitivity; however, a previous study demonstrated that *OXCT1* may be involved in autophagy-mediated apoptosis in epithelial cell cancer cells (22). In autophagy-mediated apoptosis, c-Jun N-terminal kinase phosphorylates B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL), disrupting the Beclin/Bcl-2 and Beclin1/Bcl-xL complexes; this process results in necroptosis in cancer cells (23).

The nature of autophagy in drug resistance is paradoxical and its role in carcinogenesis is context-dependent. Regarding tumor-suppressive mechanisms, autophagy can inhibit inflammation and genomic stability at an early stage (24). A previous study demonstrated that the loss of the autophagy-regulating Beclin gene results in poor recovery from ischemic stress with accumulation of cellular aggregates and denatured proteins. Consequently, essential cellular processes, including mitosis and centrosome functions. Are damaged leading to chromosomal instability (24). Conversely, inhibition of autophagy can sensitize cancer cells to ionizing radiation and chemotherapeutic drugs (25). Further studies are required to elucidate the precise mechanism underlying *OXCT1* in cisplatin resistance.

In conclusion, the present study demonstrated that *OXCT1* acts as a suppressor of cisplatin resistance, and its gene silencing by hypermethylation of CGI within the promoter region is associated with cisplatin resistance in OC. The results of the present study provide evidence of a potential novel therapeutic target for the treatment of chemoresistant OC.

Acknowledgements

The present study was supported by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute), funded by the Ministry of Health & Welfare, Republic of Korea (grant no. HI13C2148).

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