Cross-validation of genes potentially associated with overall survival and drug resistance in ovarian cancer

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Abstract. Ovarian cancer is the leading cause of death among malignancies of the female reproductive system. The 5-year survival rates of ovarian cancer (OC) patients are very poor as a result of recurrent disease and emergence of drug resistance; thus, studies to find predictive markers and factors for drug resistance are ongoing. In the present study, based on the microarrays from The Cancer Genome Atlas (TCGA), and the Gene Expression Omnibus (GEO) profiles covering 1648 OC patients, 11 out of 136 genes that were found to be significantly dysregulated in OC were associated with overall survival (OS) in 489 OC patients of the TCGA cohort. Of these genes, *CRISP3*, *LYVE1*, *OVGP1* and *BCHE* were identified as independent prognostic factors, with decreased expression of the first three genes predicting shorter OS, and decreased *BCHE* predicting longer OS. *OVGP1*, *BCHE* and further two genes, *CKAP2* and *CLDN10*, were consistently and remarkably associated with OS when the number of patients increased from 489 to 1583, with increased *CKAP2* and decreased *CLDN10* predicted shorter OS; combining the four genes provided better predictions. Associations among the four genes with OS in subgroups of OC were further verified. Downregulation of *OVGP1* was significantly associated with shorter OS in all subgroups of OC patients, including subgroups of 752 patients treated with chemotherapy regimens containing taxol, 763 with both platin and taxol, 1364 with platin, 371 patients with grade 1-2 disease, 968 with grade 3 disease, 1148 with stage III-IV disease, and 439 with TP53 mutations. In addition, *CKAP2* expression was significantly associated with shorter OS in 515 OC patients who had low CA125 levels. Furthermore, comprehensive analyses that including RT-qPCR, bioinformatics analysis and clinical data revealed an association of *CKAP2*, *BCHE*, *CLDN10* and *OVGP1* with drug resistance in OC. The genes identified in the present study might be prognostic factors as well as potential therapeutic targets in the treatment of OC.

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Introduction

Ovarian cancer is the most lethal cancer among gynecologic malignancies (1). Early-stage ovarian cancer is frequently asymptomatic and difficult to detect, and thus diagnosis usually occurs after the disease has disseminated beyond the ovaries (2). The current standard treatment for advanced ovarian cancer is surgical debulking followed by platinum/taxane-based chemotherapy (3). Although this standard treatment significantly reduces the mortality rates and prolongs the survival time, the majority of patients will eventually relapse (4). The main obstacle to a successful treatment for ovarian cancer is the development of drug resistance that finally leads to fatal disease (5), and 5-year survival rates of ovarian cancer are less than 40%, with only modest improvement over the past 40 years (2). Therefore, there is a consistent and urgent need to understand the mechanism of drug resistance and to identify useful biomarkers for overall survival in ovarian cancer (6).

The present study was based on profiles of 1648 ovarian cancer patients, in microarrays from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO). We identified several genes significantly associated with overall survival (OS); thus, these genes are potential biomarkers for prognosis in OC. We also found that these genes were potentially involved in regulation of drug resistance in OC.

Materials and methods

Cell culture. The human epithelial OC cell lines SKOV3 and A2780 were maintained in our laboratory and propagated *in vitro* by serial passage in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The cisplatin-resistant cell line SKOV3-DDP and the carboplatin-resistant cell line A2780-CBP were established by sequential exposure of cells to increasing concentrations of cisplatin and carboplatin, respectively (7).

Real-time quantitative polymerase chain reaction analysis. Total RNA was isolated from the cell lines SKOV3, SKOV3-DDP, A2780 and A2780-CBP, using TRIzol reagent (Life Technologies, Grand Island, NY, USA). The quantity and quality of the RNA were measured using a Thermo Scientific NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). cDNA was synthesized from 2 μg of RNA using the SuperScript III First-Strand synthesis system (Life Technologies). mRNA expression was measured using real-time quantitative polymerase chain reaction (RT-qPCR) and the Power SYBR-Green PCR Master Mix (Applied Biosystems, Waltham, MA, USA). Data were collected with the Applied Biosystems StepOne RT-PCR system in accordance with the manufacturer's instructions. The RT-qPCR gene-specific primers were: *CAKP2*: forward primer, 5'-AA GCCACAAAACCTCAGCCT-3' and reverse primer, 5'-CA TGAGGCCCTTTCCGGATT-3'. *BCHE*: forward primer, 5'-GGCCTGTCTTCAAAAGCACTG-3' and reverse primer, 5'-TCCTGCTTTCCACTCCCATTC-3'. *CLDN10*: forward primer, 5'-GGAGGCTCCGATAAAGCCAA-3' and reverse primer, 5'-GTGGCCCCGTTGTATGTGTA-3'. *OVGP1*: forward primer, 5'-AGCGAAGAAGCACTGGATTGA-3' and reverse primer, 5'-ATTCACAGCAGATGACAGCCA-3'. For *GAPDH*, used as the control, the forward primer was 5'-GA GGTGAAGGTCGGAGT-3' and the reverse primer 5'-GAA GATGGTGATGGGATTT-3'.

Gene expression profiles. Gene expression data and survival information of the 1648 OC patients were deposited in the KM plotter, which was established based on the microarrays from GEO and TCGA (8). Twelve independent microarrays from GEO profiles covered 1083 patients, including GSE14764 (n=80), GSE15622 (n=36), GSE18520 (n=63), GSE19829 (n=28), GSE23554 (n=28), GSE26193 (n=107), GSE26712 (n=195), GSE27651 (n=49), GSE30161 (n=58), GSE3149 (n=166), GSE51373 (n=28) and GSE9891 (n=285); the TCGA ovarian cohort covered 565 patients. Detailed information of gene expression and clinical characteristics of the 565 OC patients in TCGA cohort were retrieved from the cBioPortal for cancer genomics (http://cbioportal.org) (9,10).

Microarray GDS3754 (Human U133 plus 2.0 GeneChips; Affymetrix, Inc., Santa Clara, CA, USA) was deposited in GEO Profiles (11), and used for the analyses of gene expression in drug-resistant OC cells. In this analysis, in the case of two probe sets that target one gene, only the probe set with significant variability was retained. In the case of more than three probe sets that target one gene, the set exhibiting the most divergent expression was excluded and the set with significant variability was retained (12).

The TCGA ovarian cohort and GEO profiles are well known publicly available cohorts that can be downloaded by researchers for further analysis. No changes were made to mRNA expression values used in the analysis.

Bioinformatics analysis. Protein/gene interaction network was generated using the GeneMANIA (http://www.genemania.org/), which is a web-based database and a tool for the prediction of gene functions on the basis of multiple networks derived from different genomic or proteomic data/sources (13). It is fast enough to predict gene functions with great accuracy using GeneMANIA because hundreds of datasets from GEO, BioGRID, Pathway Commons and I2D, as well as organismspecific functional genomics data sets have been collected in this software (14).

Biological process annotation was performed using the Coremine Medical (http://www.coremine.com/medical/), which is a gene/protein database and a web-based tool for text mining. It carries out automated extraction of experimental and theoretical biomedical knowledge from publicly available gene and text databases to create a gene-to-gene co-citation network for millions of named human genes by automated analysis of titles and abstracts in over 10 million MEDLINE records (15).

Data analysis. The data were analyzed using SPSS 20.0 software. The probability of survival and significance were calculated using the Kaplan-Meier method and KM plotter (8). The correlation between gene expression and the clinicopathological characteristics was evaluated by Pearson's χ^2 test (two-sided). Gene expression was dichotomized into high and low values using the median as a cut-off in all above analyses, in accordance with previous studies (16). Gene mRNA expression levels are shown as mean ± SD. Homogeneity of the variance was analyzed using the t-test. $P<0.05$ was considered significant. The desired Affymetrix ID for CKAP2, BCHE, CLDN10, and OVGP1 is 218252_at, 205433_at, 205328_at, and 205432_at, respectively.

Results

Determination of genes associated with OS in OC. We previously reported that 60 genes were upregulated, and 126 genes were downregulated by at least 4-fold in 594 ovarian serous cystadenocarcinomas compared with 8 normal ovaries, respectively, according to TCGA ovarian statistics data deposited in the Oncomine database (17). Of these, we selected 136 genes (38 upregulated and 98 downregulated genes) for which we found ≤2 articles in PubMed using a search with (ovarian[Title]) and 'gene'[Title/Abstract], for further analysis of their relationships with OS. The mRNA expression data of the 136 genes and related clinical data for 489 OC patients in the TCGA cohort were retrieved from cBioPortal for Cancer Genomics (9,10). Gene expression was dichotomized into high and low values using the median as a cut-off in all above analyses, in accordance with a previous study (16). As shown in Table I, among all 136 genes, 11 were closely associated with OS, as determined by Kaplan-Meier analysis. *CKAP2* is upregulated, and *ATXN10*, *BCHE*, *CLDN10*, *CRISP3*, *FCGBP*, *LYVE1*, *NDNF*, *OVGP1*, *PTGIS* and *REEP1* are downregulated

Table I. Eleven genes were determined by Kaplan-Meier analysis to be closely associated with overall survival in ovarian cancer.

Dysregulation of genes		Estimate	Std. Error	95% CI		P-value
				Lower	Upper	
Upregulated genes						
CKAP2	H	39.900	2.482	35.035	44.765	0.024
	L	47.500	3.060	41.503	53.497	
	Overall	43.800	2.105	39.675	47.925	
Downregulated genes						
ATXN10	H	41.000	2.782	35.547	46.453	0.03
	L	45.300	2.671	40.066	50.534	
	Overall	43.800	2.105	39.675	47.925	
BCHE	H	36.300	2.283	31.826	40.774	0.000
	L	49.000	3.206	42.717	55.283	
	Overall	43.800	2.105	39.675	47.925	
CLDN10	H	48.300	1.797	44.777	51.823	0.036
	L	38.200	2.075	34.134	42.266	
	Overall	43.800	2.105	39.675	47.925	
CRISP3	H	47.700	1.795	44.181	51.219	0.043
	L	38.300	2.023	34.336	42.264	
	Overall	43.800	2.105	39.675	47.925	
FCGBP	H	41.000	2.371	36.352	45.648	0.025
	L	44.900	2.866	39.283	50.517	
	Overall	43.800	2.105	39.675	47.925	
LYVE1	H	41.500	2.221	37.148	45.852	0.032
	L	44.900	3.643	37.761	52.039	
	Overall	43.800	2.105	39.675	47.925	
NDNF	H	41.000	2.070	36.944	45.056	0.034
	L	45.100	2.086	41.012	49.188	
	Overall	43.800	2.105	39.675	47.925	
OVGP1	H	49.000	3.581	41.981	56.019	0.003
	L	37.900	1.947	34.084	41.716	
	Overall	43.800	2.105	39.675	47.925	
PTGIS	H	40.400	2.890	34.735	46.065	0.004
	L	47.500	1.975	43.629	51.371	
	Overall	43.800	2.105	39.675	47.925	
REEP1	H	38.400	2.053	34.377	42.423	0.007
	L	47.700	3.021	41.779	53.621	
	Overall	43.800	2.105	39.675	47.925	

H, high expression; L, low expression. Gene expression and survival data of 489 ovarian cancer patients in a TCGA cohort were used for the analysis. Expression values of a gene were dichotomized into high and low expression using the median as a cut-off.

in OC (17). We found that the upregulation of *CKAP2*, and downregulation of *CLDN10*, *CRISP3* and *OVGP1* were associated with shorter OS, whereas the downregulation of *ATXN10*, *BCHE*, *FCGBP*, *LYVE1*, *NDNF*, *PTGIS* and *REEP1* were associated with longer OS. To elucidate whether any of the above genes was an independent factor for predicting OS, we performed multivariate analyses of histological grade, tumor stage, residual tumor and platinum status for the 11 genes, using a Cox proportional hazards model (Table II). The results indicated that *BCHE* (P=0.026), *CRISP3* (P=0.031), *LYVE1* (P=0.014) and *OVGP1* (P=0.001) were independent prognostic factors for OS in 489 OC patients.

The associations of the 11 genes with OS were further validated using KM plotter which included gene expression data and survival information of 1287 OC patients downloaded from GEO and TCGA ovarian data in 2012 (8), and updated to

Table II. Multivariate analysis of overall survival of ovarian cancer patients using Cox proportional hazard model.

Factors	B	SE	Wald	df	Sig.	Exp(B)	95% CI	
							Lower	Upper
CKAP2	0.172	0.135	1.626	1	0.202	1.187	0.912	1.546
ATXN10	0.156	0.126	1.538	1	0.215	1.169	0.913	1.496
BCHE	0.310	0.139	4.955	1	0.026	1.363	1.038	1.790
CLDN10	-0.184	0.130	1.988	1	0.159	0.832	0.644	1.074
CRISP3	-0.282	0.131	4.647	1	0.031	0.754	0.584	0.975
FCGBP	0.196	0.128	2.330	1	0.127	1.217	0.946	1.565
LYVE1	0.324	0.132	6.004	1	0.014	1.382	1.067	1.791
NDNF	0.057	0.132	0.184	1	0.668	1.058	0.818	1.370
OVGP1	-0.439	0.135	10.577	1	0.001	0.645	0.495	0.840
PTGIS	0.030	0.142	0.045	1	0.832	1.030	0.781	1.360
REEP1	0.075	0.132	0.325	1	0.568	1.078	0.833	1.395
Grade (G2/G3)	-0.279	0.197	2.010	1	0.156	0.756	0.514	1.113
Platinum status (R/S)	1.230	0.168	53.690	1	0.000	3.420	2.462	4.753
Residual (mm) (≤10/>10)	-0.176	0.145	1.482	1	0.223	0.838	0.631	1.113
Stage (II/III-IV)	-0.636	0.406	2.449	1	0.118	0.530	0.239	1.174

R/S, platinum resistance/platinum sensitive. Gene expression and survival data of 489 ovarian cancer patients in a TCGA cohort were used for the analysis. Gene expression was dichotomized into high and low values using the median as a cut-off.

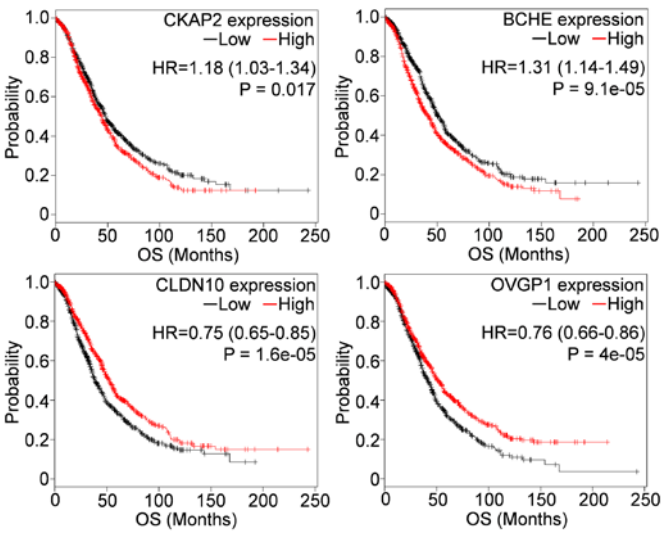

Figure 1. Relationships between gene expression and overall survival (OS) in 1583 OC patients, using KM plotter. Gene expression was dichotomized into high and low values using the median as a cut-off. HR, hazard ratio.

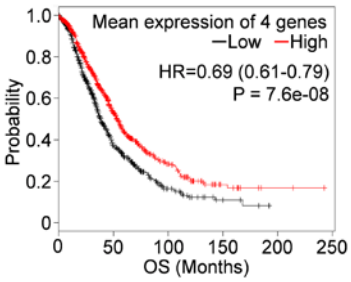

Figure 2. Relationships between mean expressions of the four genes and overall survival (OS) in 1583 OC patients, using KM plotter. Reciprocals of hazard ratio (HR) values (i.e., HR^{-1}) for *CKAP2* and *BCHE* were used. Gene expression was dichotomized into high and low values using the median as a cut-off.

1648 patients for the 2015 version. As shown in Fig. 1, among the 11 genes, *CKAP2*, *BCHE*, *CLDN10* and *OVGP1* were consistently associated with OS when the number of patients increased from 489 in TCGA cohort to 1583 in KM plotter (Table I and Fig. 1). Of these, upregulation of *CKAP2*, and downregulation of *CLDN10* and *OVGP1* were associated with shorter OS, whereas downregulation of *BCHE* was associated with longer OS. When the four genes were combined, we found that their mean expression in combination was greatly more associated with OS than any of the genes used separately (Fig. 2).

Verification of genes associated with OS in OC patients treated with different chemotherapy regimens. As shown in Table III, in subgroups of patients treated with different regimens, we found that the high expression of *CKAP2* was associated with

Table III. Four genes were associated with overall survival in subgroups of ovarian patients treated with different chemotherapy regiments, as determined by KM plotter.

	Chemotherapy containing (no. of patients)					
	Platin (1364)		Taxol (780)		Platin and taxol (763)	
Gene	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
CKAP2	1.09 (0.94-1.26)	0.27	1.24 (1.02-1.5)	0.033	1.24 (1.01-1.51)	0.037
BCHE	1.26 (1.09-1.46)	0.0017	1.19 (0.97-1.44)	0.088	1.19 (0.97-1.45)	0.091
CLDN10	0.76 (0.66-0.88)	0.00029	0.82 (0.68-1)	0.052	0.81 (0.67-0.99)	0.042
OVGP1	0.77 (0.66-0.89)	0.00037	0.74 (0.61-0.91)	0.0031	0.74 (0.61-0.91)	0.0035

Gene expression was dichotomized into high and low values using the median as a cut-off. HR, hazard ratio.

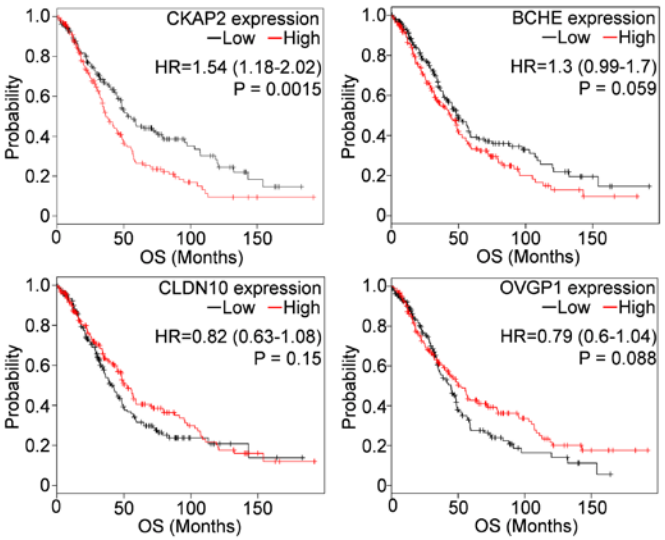

Figure 3. Relationships between gene expression and overall survival (OS) in 515 OC patients with average CA125 below lower quartile, using the KM plotter. Expression values of genes were dichotomized into high and low expressions using the median as a cut-off. HR, hazard ratio.

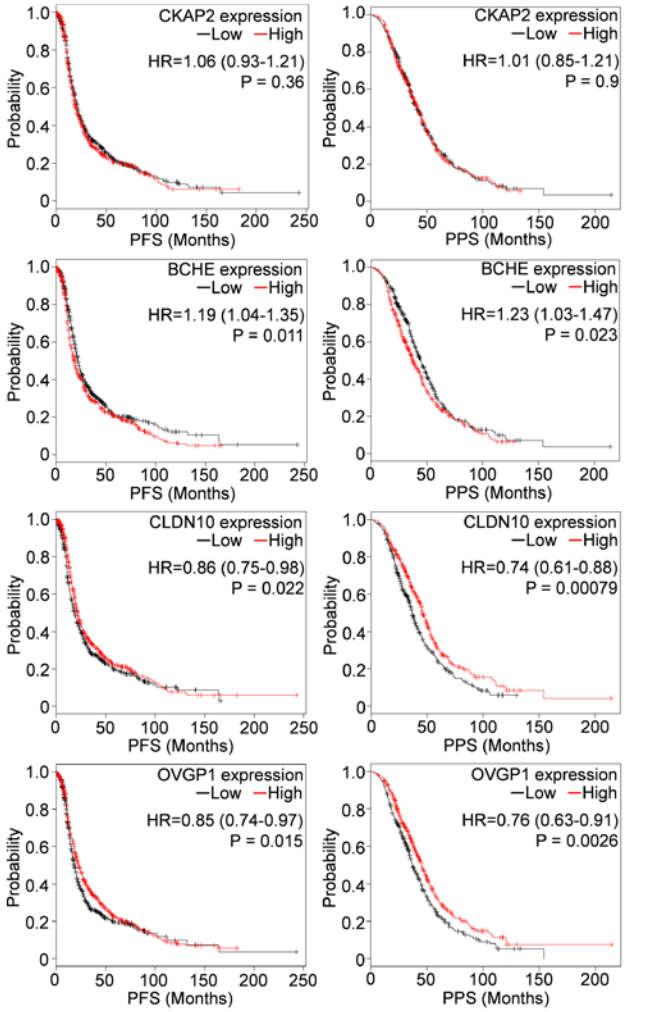

Figure 4. Relationships between gene expression in OC patients and progression-free survival (PFS; n=1307) or post-progression survival (PPS; n=708), as determined by KM plotter. Gene expression was dichotomized into high and low values using the median as a cut-off. HR, hazard ratio.

shorter OS in 752 patients treated with taxol-based regimens, and in 763 treated with regimens containing both platin and taxol, but the association was not significant in 1364 patient treated with only platins; downregulation of *BCHE* predicts a longer OS in patients treated with platin, but not in patients treated with taxols or taxols with platin; downregulation of *CLDN10* was associated with shorter OS in patients treated with platin or platin with taxol, but not in the taxol-only group, and downregulation of *OVGP1* predicted a shorter OS in all three subgroups of ovarian patients treated with different regimens.

Verification of genes associated with OS in subgroups of OC patients. The relationships between dysregulation of the four genes (*CKAP2*, *BCHE*, *CLDN10* and *OVGP1*) with OS in subgroups of OC patients with different CA125 levels, grades, stages and *TP53* mutation status were further investigated. We revealed that the high *CKAP2* expression significantly associated with shorter OS in 515 OC patients having low CA125 levels (the average CA125 below lower quartile) (Fig. 3). Besides, as shown in Table IV, all four genes were significantly associated with OS in 968 OC patients with grade 3 disease, but only *BCHE* and *OVGP1* were associated with OS in 371 patients with grade 1-2 disease. *CLDN10* and *OVGP1* were

Table IV. The four genes were associated with overall survival in subgroups of ovarian patients with different grades, stages and *TP53* mutation status, as determined by KM plotter.

Subgroup	No. of patients	*CKAP2*		*BCHE*		*CLDN10*		*OVGP1*	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
Grade									
I-II	371	1.22 (0.91-1.64)	0.18	1.73 (1.28-2.34)	0.00026	0.78 (0.58-1.04)	0.091	0.58 (0.43-0.78)	0.00027
III	968	1.21 (1.02-1.43)	0.032	1.20 (1.01-1.42)	0.04	0.75 (0.63-0.89)	0.00088	0.80 (0.67-0.95)	0.011
Stage									
I-II	133	1.36 (0.61-3.01)	0.45	1.04 (0.47-2.31)	0.92	0.90 (0.41-1.97)	0.78	0.70 (0.32-1.55)	0.38
III-IV	1148	1.1 (0.94-1.29)	0.22	1.13 (0.97-1.32)	0.12	0.84 (0.72-0.98)	0.024	0.73 (0.62-0.85)	8.0e-5
TP53									
Wild	86	0.99 (0.55-1.78)	0.97	2.0 (1.09-3.66)	0.023	0.78 (0.43-1.39)	0.4	0.8 (0.44-1.43)	0.45
Mutation	439	1.02 (0.79-1.31)	0.9	1.12 (0.87-1.44)	0.37	0.85 (0.66-1.09)	0.2	0.75 (0.58-0.96)	0.024

Gene expression was dichotomized into high and low values using the median as a cut-off. HR, hazard ratio.

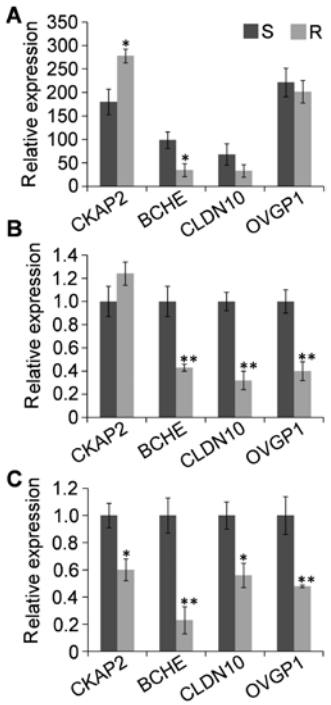

Figure 5. Gene mRNA expression in drug-sensitive and drug-resistant OC cells, using microarray data from GEO Profiles and RT-qPCR analysis. (A) mRNA expression of the four genes in drug-sensitive A2780 epithelial OC cells and cells with acquired platinum resistance, using microarray data (GDS3754). Five replicates were performed for each cell line. Normalized data were deposited in GEO Profiles (18). (B) mRNA expression of the four genes in cisplatin-sensitive and -resistant SKOV3 OC cells, as measured by RT-qPCR. Four replicates were performed for the drug-resistant cells and for the controls. (C) mRNA expression of the four genes in carboplatin-sensitive and -resistant A2780 OC cells, as measured by RT-qPCR. Four replicates were performed for the drug-resistant cells and for the controls. S, drug sensitive cells; R, drug resistant cells. *P<0.05; **P<0.01.

associated with OS in 1148 patients with stage III-IV OC, but none of the genes was associated with OS in 133 patients with stage I-II OC. In the subgroup of 439 patients with *TP53* mutation, downregulation of *OVGP1* predicted shorter OS, whereas downregulation of *CLDN1*0 predicted shorter OS in the *TP53* wild-type group. In addition, downregulation of *BCHE* was associated with longer PFS and PPS in cohorts of 1307 and 708 patients, respectively; whereas downregulation of *CLDN10* and *OVGP1* were associated with shorter PFS and PPS (Fig. 4).

Clinical importance of the analysis. The four OS-related genes, *CKAP2*, *BCHE*, *CLDN10* and *OVGP1*, with clinical characteristics such as tumor stage, histological grade, and primary therapy outcome were checked in the TCGA cohort (n=489). As shown in Table V, *BCHE* and *OVGP1* were closely associated with primary therapy outcomes. Downregulation of *BCHE* was associated with good complete response, whereas downregulation of *OVGP1* was associated with poor complete response. Besides, downregulation of *BCHE* was associated with lower tumor stage, in particular, stage II. Only *CLDN10* was associated with platinum status; its downregulation might predict drug resistance. The 4 genes were not significantly associated with histological grade in this analysis.

Possible associations with drug resistance. The four genes, *CKAP2*, *BCHE*, *CLDN10* and *OVGP1*, were dysregulated in platinum-resistant OC cells, as determined using microarray data retrieved from GEO Profiles and RT-qPCR analysis (Fig. 5). Compared with their expression in sensitive cells, expression of *CKAP2* was increased in cisplatin-resistant cells, but decreased in carboplatin-resistant cells; *BCHE*, *CLDN10* and *OVGP1* all were decreased in cisplatin- and carboplatin-resistant cells, although the changes in *CLDN10* and *OVGP1* were not significant in one analysis (Fig. 5A).

Bioinformatics analyses were performed to further explain the associations of the four genes with drug resistance in OC. As shown in Fig. 6A, text mining indicated that *BCHE* had direct associations with drug resistance and OC, and all the four genes were indirectly associated with drug resistance and OC through six biological processes (P<0.05), including cell

Table V. Correlation between gene expression and the clinicopathological characteristics of 489 patients with ovarian cancer in TCGA cohort.

Characteristics	No. of patients (%)	*CKAP2*			*BCHE*			*CLDN10*			*OVGP1*		
		High n (%)	Low n (%)	P-value	High n (%)	Low n (%)	P-value	High n (%)	Low n (%)	P-value	High n (%)	Low n (%)	P-value
Primary therapy outcome	395 (100)			0.451			0.005			0.521			0.012
Complete response	276 (69.9)	142 (51.4)	134 (48.6)		127 (46.0)	149 (54.0)		149 (54.0)	127 (46.0)		148 (53.6)	128 (46.4)	
Partial response	57 (14.4)	23 (40.4)	34 (59.6)		37 (64.9)	20 (35.1)		25 (43.9)	32 (56.1)		20 (35.1)	37 (64.9)	
Progressive disease	37 (9.4)	20 (54.1)	17 (45.9)		26 (70.3)	11 (29.7)		18 (48.6)	19 (51.4)		12 (32.4)	25 (67.6)	
Stable disease	25 (6.3)	12 (48.0)	13 (52.0)		12 (48.0)	13 (52.0)		12 (48.0)	13 (52.0)		11 (44.0)	14 (56.0)	
Tumor stage	484 (100.0)			0.110			0.013			0.094			0.602
II	24 (5.0)	13 (54.2)	11 (45.8)		5 (20.8)	19 (79.2)		17 (70.8)	7 (29.2)		10 (41.7)	14 (58.3)	
III	381 (78.7)	198 (52.0)	183 (48.0)		197 (51.7)	184 (48.3)		184 (48.3)	197 (51.7)		193 (50.7)	188 (49.3)	
IV	79 (16.3)	31 (39.2)	48 (60.8)		38 (48.1)	41 (51.9)		41 (51.9)	38 (48.1)		37 (46.8)	42 (53.2)	
Platinum status	287 (100)			0.377			0.079			0.017			0.153
Resistance	90 (31.4)	45 (50.0)	45 (50.0)		49 (54.4)	41 (45.6)		36 (40.0)	54 (60.0)		50 (55.6)	40 (44.4)	
Sensitive	197 (69.6)	93 (47.2)	104 (52.8)		88 (44.7)	109 (55.3)		107 (54.3)	90 (45.7)		95 (48.2)	102 (51.8)	

P-value was evaluated using Pearson's χ^2 test (2-sided).

Figure 6. Bioinformatics analysis of the CKAP2, BCHE, CLDN10 and OVGP1 with drug resistance in OC. (A) Associations of the four genes/proteins with drug resistance and OC, as determined using the Coremine Medical tool. Input terms were CKAP2, BCHE, CLDN10, OVGP1, drug resistance and OC. Red line, direct interactions of the input terms; grey line, direct interactions of the input terms with annotated biological processes (P<0.05); (B) protein/gene-protein/gene interaction network of the four proteins/genes with 49 drug resistance-related proteins/genes in OC (GeneMANIA tool). The query in blue includes the targets. The types of interactions between proteins/genes are indicated in the network legend of the figure.

proliferation, apoptosis, mitotic nuclear division, phosphorylation, growth and gene expression. We further generated a protein/gene interaction network for the four genes and their products with 49 drug-resistance proteins/genes in OC, which including 15 tumor suppressors (19): BRCA1, BRCA2, CHEK2, FBXO32, MLH1, SULF1, IL24, CDKN2A, CDKN1A, TP53, TP73, PDCD4, PTEN, RASSF1 and WWOX, 25 oncoproteins (20): ACTN4, AKT1, AKT2, BAX, BCL2, MIEN1, CLU, CUZD1, DAXX, EGFR, ERBB2, FOS, JUN, IKBKE, KRAS, MET, MYC, NFKB1, NINL, NOTCH3, PIK3CA, RSF1, SRC, STAT3 and URI1, NEK2 (21), NEK11 (22), TRPC1 (12), CCL21 and SPARCL1 (23), GGNBP2 and RNASET2 (7), ALDH1A2 and ADH1B (17). As shown in Fig. 6B, CKAP2, BCHE, CLDN10 and OVGP1 had direct and

indirect interactions with all of the 49 drug-resistant proteins/genes, and directly interacted with 11, 23, 19 and 13 of those 49 proteins/genes, respectively. The four genes and their products also directly interacted with each other, with *OVGP1* and *BCHE* both directly interacting with *CKAP2* and *CLDN10*.

Discussion

Biomarkers not only have prognostic implications, but are also helpful in monitoring treatment responses, surveillance for tumor recurrence and guidance of clinical decisions (24). Long-term survival of OC patients remains very poor as a result of recurrence and emergence of drug resistance, and the 5-year survival rates is only ~40% (2). Thus, prognostic biomarkers for OC patients are particularly necessary and crucial, and there is ongoing search for predictive biomarkers. In the present study, we identified a group of genes significantly associated with OS in 489 OC patients from a TCGA cohort (Table I), of which *BCHE*, *CRISP3*, *LYVE1* and *OVGP1* were identified as independent risk prognostic factors for OS (Table II). Four genes (*CKAP2*, *BCHE*, *CLDN10* and *OVGP1*) were consistently associated with OS when the number of patients increased from 489 to 1583 (Fig. 1). Of these, upregulation of *CKAP2*, and downregulation of *CLDN10* and *OVGP1* were associated with shorter OS, whereas downregulation of *BCHE* was associated with longer OS. With the exception of *CKAP*2, the other three genes all were significantly related to PFS and PPS, with decreased BCHE expression associated with longer PFS and PPS in 1307 and 708 OC patients, respectively; and decreased expression of *CLDN10* and *OVGP1* were associated with shorter PFS and PPS (Fig. 4). In subgroups of patients with OC, we further verified that downregulation of *OVGP1* was significantly associated with shorter OS in all OC subgroups, including the 752 patients treated with chemotherapy regimens that contained taxol, 763 treated with both platin and taxol, 1364 treated with platin, 371 patients with grade 1-2 disease, 968 with grade 3 disease, 1148 with stage III-IV disease, and 439 with TP53 mutations (Tables III and IV). All these results together suggest that *CKAP2*, *BCHE*, *CLDN10* and *OVGP1*, (especially *OVGP1*), are potential biomarkers for predicting OS in OC.

The relationship between these genes and prognosis in OC is poorly studied. Upregulation of chromatin *CKAP2* is an independent prognostic marker for shorter relapse-free survival in early-stage breast cancer (25), and for significantly worse prognoses in male patients with T1-2 gastric cancer (26). Downregulation of *CLDN10* indicates shorter OS in some patients with lung adenocarcinoma (27). All these reports were consistent with our findings in this study. However, low levels of preoperative serum *BCHE* were validated as an independent negative prognostic factor for prostate cancer patients who undergo radical prostatectomy (28), and a predictor of shorter OS and DFS in patients with muscle-invasive bladder cancer who undergo radical cystectomy (29). These results were contrary to our findings that downregulation of *BCHE* was associated with longer OS. The associations of the four genes with OS in OC is rare, with only one study reporting that the downregulation of *BCHE* predicts a longer OS in OC patients, which was also based on the analysis of data in the TCGA cohort (30).

Serum CA125 is widely used to distinguish malignant from benign pelvic masses, to monitor response to treatment, to assess prognosis and to detect disease recurrence. Low levels of CA125 are normally an optimistic indicator of OC progression and prognosis. For example, preoperative CA125 <65 U/ml correlates with longer survival in OC patients (31); CA125 <50 U/ml correlates with significantly longer DFS and OS in patient with borderline ovarian tumors (32). However, no data are available that further predicts OS in subgroups of patients with low levels of CA125. CKAP2 expression was significantly associated with shorter OS in 515 OC patients who had low CA125 levels (the average CA125 below lower quartile) (Fig. 3), which suggests that this gene could be used for prognosis prediction with CA125. However, this study measured tissue levels of CA125, whereas the FDA-approved test for OC is serum-based (8). Associations between *CKAP2* and OS in patients with low CA125 levels should be further studied.

The relationships of the four genes with drug resistance in cancer has not been widely reported. In the present study, we found that *CKAP2*, *BCHE*, *CLDN10* and *OVGP1* all were significantly dysregulated in cisplatin- and carboplatin-resistant cells (Fig. 5). Text mining indicated that the four genes were directly and indirectly associated with drug resistance through several biological processes. A protein/gene interaction network indicated that the four genes interacted with 49 drug-resistant proteins/genes in OC. In particular, *BCHE* and *CLDN10* directly interacted with 23 and 19 of those 49 proteins/genes (Fig. 6). Downregulation of *CLDN10* was also an indicator of drug resistance, and downregulation of *OVGP1* was significantly associated with poor complete response (Table IV). All these results imply that dysregulation of the four genes affects drug resistance in OC. Furthermore, among patients treated with different regimens, high expression of *CKAP2* was associated with shorter OS in patients treated with taxol-containing regimens, whereas downregulation of *BCHE* was associated with OS in patient treated by regimens without taxol; downregulation of *CLDN10* was associated with shorter OS in patients treated with platin, and downregulation of *OVGP1* predicted a shorter OS in patients treated with either platin or taxol (Table III). These results indicated that *CKAP2* is associated with taxol resistance, *BCHE* and *CLDN10* with platin resistance, and *OVGP1* with both platin and taxol resistance.

The roles of *CKAP2*, *BCHE*, *CLDN10* and *OVGP1* in OC have rarely been studied. We previously reported that *CKAP2* was upregulated, and *BCHE*, *CLDN10* and *OVGP1* were downregulated in OC, by a 4.26, -8.39, -7.04 and -102.95-fold changes, respectively (17). Downregulation of *OVGP1* in OC was recently confirmed in a study that showed the protein to be less abundant in high-grade serous ovarian tumor fluids (malignant) than in benign serous cystadenoma tumor fluids (33). *CLDN10* mRNA was specifically detected in five cancerous ovaries compared with three normal controls in experimental hens, which suggests that *CLDN10* is a novel biomarker for detecting OC in chickens (34); however, its association with human OC was not discussed.

In summary, we identified 11 genes significantly related to OS in 489 OC patients. Of these, *BCHE*, *CRISP3*, *LYVE1* and *OVGP1* were identified as independent risk prognostic

factors. Four genes *CKAP2*, *BCHE*, *CLDN10*, and particularly the *OVGP1*, were remarkably associated with OS in total of 1583 OC patients, and combination of these genes had much better prediction potential. Besides, comprehensive analysis indicated that *CKAP2*, *BCHE*, *CLDN10* and *OVGP1* might contribute to drug resistance in OC. This study has implicated genes that might be both prognostic markers and potential therapeutic targets to pursue in the treatment of ovarian cancer.

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