Big data-based identification of methylated genes associated with drug resistance and prognosis in ovarian cancer

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Abstract

It is imperative to further the understanding of the drug resistance mechanisms of ovarian cancer (OC) and to identify useful biological markers for prognosis prediction.

Cormine, cBioportal, and The Cancer Genome Atlas databases were used to search microarray data of gene methylation related to OC, drug resistance in OC, and prognosis, and to analyze methylated genes potentially inducing the drug resistance in OC. Fifty-five DNA-methylated genes significantly associated with drug resistance in OC were screened, and the regulatory mechanisms underlying changes in methylation levels of these genes were systematically integrated.

Enrichment and annotation of biological processes indicated that most of the above DNA-methylated genes were significantly associated with cell proliferation and cell cycle. In addition, pathway enrichment demonstrated that the above DNA-methylated genes were significantly associated with PI3K-AKT and P53 signaling pathways. Among the 55 genes, 4 were significantly associated with OC prognostic disease-free survival, namely bromodomain containing 4, PDZ domain containing 1 (*PDZK1*), phosphatase and tensin homolog, and TNF receptor superfamily member 10c; 5 were significantly related to overall survival, namely bromodomain containing 4, *PDZK1*, *PIK3C2B*, Rh associated glycoprotein, and *DYRK*; among them, the degree of methylation of TNF receptor superfamily member 10c, *PDZK1*, and Rh associated glycoprotein genes was significantly correlated with mRNA expression. Furthermore, *PDZK1*, Rh associated glycoprotein, and TNF receptor superfamily member 10c genes showed significant hypomethylation in drug-resistance tissues of OC, and their mRNAs had significantly high expression.

The association between the methylation of these 55 genes and OC and drug resistance in OC, in addition to bioinformatics analyses clarify the important mechanisms of gene methylation in the development, progression, and drug resistance of OC.

Abbreviations: BRD4 = bromodomain containing 4, CD44 = cluster of differentiation 44, CDK2 = cyclin dependent kinase 2, CTSL = cathepsin L, DFS = disease-free survival, DYRK2 = dual specificity tyrosine phosphorylation regulated kinase 2, ESR1 = estrogen receptor 1, ETS1 = ETS proto-oncogene 1, GNA13 = G protein subunit alpha 13, HOXA11 = homeobox A11, MDK = midkine, MDM2 = MDM2 proto-oncogene, MDR = multidrug resistance, OC = ovarian cancer, OS = overall survival, PDZK1 = PDZ domain containing 1, PIK3C2B = phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta, PTEN = phosphatase and tensin homolog, RASSF1 = Ras association domain family member 1, RHAG = Rh associated glycoprotein, TCGA = The Cancer Genome Atlas, TMEM158 = transmembrane protein 158, TNFRSF10C = TNF receptor superfamily member 10c.

Keywords: DNA methylation, multidrug resistance, ovarian cancer, prognosis

1. Introduction

Ovarian cancer (OC) is a common malignant tumor that poses a serious threat to women's health. The main type of OC is epithelial malignant tumor, which accounts for 85% to 90% of all ovarian malignancies and has the highest mortality rates among female malignant tumors of genital tract.[1] Over the past decade, significant advances have been made in the research of risk factors and molecular pathways of OC, resulting in numerous opportunities for prevention, detection, early diagnosis, prognostic prediction, and treatment of OC. Nevertheless, the 5-year survival rate of patients with OC remains around 50%.[2] In 2018, the Clinical Practice Guidelines by the American Cancer Society pointed out that there were approximately 22,240 newly diagnosed patients with OC in the United States each year, among whom approximately 14070 would die within 5 years.[2] Under these circumstances, it is of utmost importance to further the understanding of the mechanisms of progression, development, and drug resistance in OC, and to explore effective biomarkers and therapeutic targets.

Over recent years, the genome sequencing and biotechnology have flourished, greatly enhancing the potential of cancer genome

research. The biomedical studies have reported lots of information, including mRNA expression, DNA methylation, microRNA expression, etc., thus providing a reliable basis for a variety of new studies. Gene function prediction based on bioinformatics analysis is a potential, feasible, and valuable method for exploring gene functions, and related networks.[3] The Cancer Genome Atlas (TCGA) is an unprecedented, data-disclosed multi-digit data set of cancer genome co-founded by the National Cancer Institute and the National Human Genome Research Institute of National Institutes of Health[4] that provides researchers with new opportunities to study cancer development and progression. Data from TCGA can be used to improve diagnostic methods, develop new treatment standards and novel targeted therapies.

In the present study, a group of genes associated with the progression and prognosis of OC were identified using DNA methylation, mRNA expression, and clinical data extracted from the TCGA cohort.

2. Methods

Ethical approval and informed consent is not applicable in this study.

The data in this study were obtained from the Coremine Medical (http://www.cormine.com), cBioportal (http://cbioportal.org), and TCGA (The Cancer Genome Altas) databases, including micro-array data on DNA-methylated genes of specimens obtained during operation from 97 platinum-resistant patients with OC and 190 platinum-sensitive patients with OC, mRNA expression data, and clinical data related to the prognosis of OC. The cBioPortal platform was employed for its suitability to analyze complex cancer genomics and clinical profiles such as the TCGA database.[5] Before inclusion in the cBioPortal platform, all methylation data were submitted to checksum standardization.[6]

SPSS 22.0 software was used for data analyses. Independent samples t-test with Benjamini-Hochberg correction, with a threshold false discovery rate (FDR) of 0.1, was used to analyze the methylation degree of DNA-methylated genes in drug-sensitive and drug resistant patient groups, while 55 methylated genes significantly associated with the drug resistance in OC were screened.

DAVID and String online software were used for enrichment analysis and gene/protein interaction analysis of biological process for differentially methylated genes in drug-resistant tissues in 55 OC genes. The common biological pathways and related genes were screened by a corrected $P < .05$. In addition, the Benjamini method was applied for P value adjustment.

Based on the TCGA database, the genes corresponding to methylation changes and mRNA expression in drug-resistant tissues of OC were screened via integration analysis.

Kaplan-Meier survival analysis was performed for prognostic analysis of the 55 genes, and survival curves had been drawn. Four genes related to prognosis of ovarian cancer PFS were screened, and 5 significantly associated with OS were selected. There were two genes that were related to both PFS and OS.

3. Results

3.1. Screening of methylated genes related to drug resistance in ovarian cancer (OC)

Genes were derived from the Coremine Medical database, where OC, ovarian carcinoma, methylation, resistant or resistance or chemoresistance were used as keywords. The advanced nested searches were performed to retrieve genes associated with OC,

gene methylation, and chemotherapeutic resistance in OC, resulting in a total of 1407 methylated genes significantly associated with OC and drug resistance in OC. The selected genes were put into cBioportal for examination, revealing that a total of 885 genes had data related to DNA methylation, mRNA expression and clinical data in OC tissues.

Microarray data of DNA methylation in 490 patients with OC from the TCGA database, related data on mRNA expression, and clinical prognoses were downloaded, including data from 90 platinum-resistant patients with OC and 197 platinum-sensitive patients with OC, which included 885 methylated genes screened. The t test was used to analyze the data, and a total of 55 differentially methylated genes markedly associated with drug resistance in OC were screened (Table 1), among which there were 27 genes with hypermethylation in drug-resistant tissues, and 28 with hypomethylation in drug-resistant tissues. Among these 55 genes, previous studies have reported that hyper-methylation changes of genes were clearly associated with drug resistance in OC, including death associated protein[7] and estrogen receptor 1 (ESR1),[8] midkine (MDK),[9] myelin and lymphocyte,[10] phosphatase and tensin homolog (PTEN),[11] Ras association domain family member 1 (RASSF1),[9] and homeo-box A11 (HOXA11);[9] hypomethylation changes of genes were clearly related to drug resistance in OC, including carbonic anhydrase 9,[12] cyclin dependent kinase 2 (CDK2),[13] and ETS proto-oncogene 1 (ETS1).[14]

3.2. Bioinformatics analysis of differentially methylated genes for drug resistance in OC

3.2.1. Pathway enrichment analysis of differentially methylated genes for drug resistance in OC.
DAVID online software was used to perform a biological process analysis of the above 55 differential DNA-methylated genes.[15] The results are shown in Table 2. In the cluster of 2 biological processes annotated to the highest score, cluster1 was mainly a biological process related to cell proliferation, covering 7 genes, while cluster2 was mainly related to the cell cycle process, covering a total of 3 genes. These results suggested that the above 55 differential DNA-methylated genes may be involved in the development, progression, and drug resistance in OC mainly via regulation of cell proliferation and cell cycle. There were 4 enriched pathways, ie, PI3K-Akt signaling pathway, p53 signaling pathway, FoxO signaling pathway, and Cell cycle, which were clearly related to drug resistance in OC. Previous studies have reported the relevance of PI3K-Akt signaling pathway,[9] p53 signaling pathway,[16,17] Cell cycle,[17] and FoxO signaling pathway in other cancers, as well as tumor resistance.[18,19]

3.2.2. Protein interaction analysis of the 55 differentially methylated genes.
In order to further analyze the association between differential DNA-methylated genes and drug resistance in OC, a comprehensive analysis of the interaction of 55 gene proteins by the String tool was performed as shown in Figure 1. In addition to the transmembrane protein 158, kinesin family member 1C, membrane bound transcription factor peptidase, site 1, DNA topoisomerase III beta, PDZ domain containing 1 (PDZK1), solute carrier family 19 member 1, CDC42 binding protein kinase beta (CDC42BP) and lysosomal protein trans-membrane 4 beta genes, the direct or indirect interactions were observed among the remaining 47 protein genes, which indicated that these genes might be involved in the regulation of drug resistance in OC. Among these protein genes, ESR1 interacted

Table 1

Screening of 55 significantly differentially methylated genes in drug resistance of ovarian cancer.

Low Methylation Gene[*]	Mean ± Std. Error Mean		P value	FDR
	Resistance	Sensitive		
ABCG4	0.62 ± 0.021	0.69 ± 0.011	.004	0.044
BGLAP	0.85 ± 0.003	0.86 ± 0.002	.001	0.001
BRD4	0.17 ± 0.018	0.22 ± 0.013	.028	0.053
CA9	0.37 ± 0.019	0.43 ± 0.013	.020	0.052
CDKN2B	0.78 ± 0.019	0.84 ± 0.007	.006	0.055
COL18A1	0.50 ± 0.016	0.55 ± 0.010	.006	0.049
CSF1R	0.66 ± 0.022	0.73 ± 0.010	.006	0.047
CSF2	0.81 ± 0.013	0.84 ± 0.004	.013	0.060
DYRK2	0.63 ± 0.017	0.69 ± 0.010	.002	0.073
ESR1	0.21 ± 0.010	0.24 ± 0.007	.020	0.055
F2	0.89 ± 0.008	0.90 ± 0.004	.047	0.049
GNA13	0.63 ± 0.022	0.68 ± 0.009	.043	0.050
HOXA11	0.29 ± 0.024	0.35 ± 0.017	.044	0.050
IL13	0.89 ± 0.005	0.91 ± 0.003	.026	0.048
LEP	0.66 ± 0.026	0.73 ± 0.015	.022	0.050
ORM2	0.80 ± 0.014	0.84 ± 0.006	.018	0.052
PAX8	0.36 ± 0.014	0.40 ± 0.012	.015	0.055
PDZK1	0.60 ± 0.020	0.65 ± 0.013	.041	0.050
PEA15	0.77 ± 0.011	0.80 ± 0.004	.029	0.048
PIK3C2B	0.86 ± 0.007	0.88 ± 0.002	.023	0.051
PTEN	0.06 ± 0.004	0.07 ± 0.004	.024	0.049
RASSF1	0.18 ± 0.025	0.27 ± 0.019	.004	0.055
REN	0.79 ± 0.015	0.83 ± 0.006	.006	0.041
RHAG	0.46 ± 0.022	0.52 ± 0.015	.025	0.047
SLC19A1	0.36 ± 0.019	0.40 ± 0.011	.023	0.046
SLC45A2	0.18 ± 0.010	0.21 ± 0.008	.037	0.050
TNFRSF10C	0.19 ± 0.029	0.27 ± 0.024	.029	0.047
TNFSF10	0.73 ± 0.015	0.79 ± 0.007	.001	0.001

High methylation gene[†]	Mean ± Std. error mean		P value	FDR
	Resistance	Sensitive		
ANXA5	0.07 ± 0.007	0.05 ± 0.003	.027	0.048
CAPN5	0.06 ± 0.006	0.04 ± 0.003	.047	0.048
CD44	0.05 ± 0.002	0.04 ± 0.001	.038	0.050
CDC42BPB	0.05 ± 0.005	0.04 ± 0.002	.029	0.046
CDK2	0.11 ± 0.008	0.10 ± 0.004	.033	0.048
CDKN2C	0.08 ± 0.008	0.06 ± 0.004	.041	0.050
CTSL	0.12 ± 0.008	0.10 ± 0.004	.025	0.047
DAP	0.05 ± 0.004	0.04 ± 0.002	.044	0.049
ESRRA	0.26 ± 0.011	0.23 ± 0.006	.028	0.048
ETS1	0.07 ± 0.007	0.05 ± 0.004	.046	0.050
FUT4	0.06 ± 0.003	0.05 ± 0.001	.017	0.052
HIC1	0.06 ± 0.006	0.05 ± 0.003	.046	0.049
KIF1C	0.13 ± 0.014	0.09 ± 0.006	.010	0.050
KMT2A	0.06 ± 0.007	0.05 ± 0.003	.033	0.047
LAPTM4B	0.04 ± 0.002	0.04 ± 0.001	.016	0.049
MBTPS1	0.06 ± 0.006	0.05 ± 0.003	.042	0.050
MCL1	0.04 ± 0.002	0.04 ± 0.001	.021	0.053
MDK	0.05 ± 0.006	0.04 ± 0.003	.045	0.050
MDM2	0.07 ± 0.004	0.06 ± 0.002	.039	0.050
MIF	0.04 ± 0.004	0.03 ± 0.002	.016	0.052
MTA1	0.09 ± 0.009	0.07 ± 0.004	.014	0.050
PAX2	0.07 ± 0.006	0.05 ± 0.003	.019	0.052
PTK2	0.07 ± 0.005	0.06 ± 0.002	.014	0.055
SDC1	0.09 ± 0.010	0.07 ± 0.004	.014	0.050
TMEM158	0.08 ± 0.007	0.06 ± 0.003	.029	0.044
TOP3B	0.14 ± 0.014	0.10 ± 0.007	.008	0.044
YY1	0.08 ± 0.008	0.06 ± 0.005	.048	0.048

ABCG4 = ATP binding cassette subfamily G member 4, ANXA5 = annexin A5, BGLAP = bone gamma-carboxyglutamate protein, BRD4 = bromodomain containing 4, CA9 = carbonic anhydrase 9, CAPN5 = calpain 5, CD44 = cluster of differentiation 44, CDC42BPB = CDC42 binding protein kinase beta, CDK2 = cyclin dependent kinase 2, CDKN2B = cyclin dependent kinase inhibitor 2B, CDKN2C = cyclin dependent kinase inhibitor 2C, COL18A1 = collagen type XVIII alpha 1 chain, CSF1R = colony stimulating factor 1 receptor, CSF2 = colony stimulating factor 2, CTSL = cathepsin L, DAP = death associated protein, DYRK2 = dual specificity tyrosine phosphorylation regulated kinase 2, ESR1 = estrogen receptor 1, ESRRA = estrogen related receptor alpha, ETS1 = ETS proto-oncogene 1, F2 = coagulation factor II, thrombin, FUT4 = fucosyltransferase 4, GNA13 = G protein subunit alpha 13, HIC1 = HIC ZBTB transcriptional repressor 1, HOXA11 = homeobox A11, IL13 = interleukin 13, KIF1C = kinesin family member 1C, KMT2A = lysine methyltransferase 2A, LAPTM4B = lysosomal protein transmembrane 4 beta, LEP = leptin, MBTPS1 = membrane bound transcription factor peptidase, site 1, MCL1 = MCL1 apoptosis regulator, MDK = midkine, MDM2 = MDM2 proto-oncogene, MIF = macrophage migration inhibitory factor, MTA1 = metastasis associated 1, ORM2 = orosomucoid 2, PAX2 = paired box 2, PAX8 = paired box 8, PDZK1 = PDZ domain containing 1, PEA15 = proliferation and apoptosis adaptor protein 15, PIK3C2B = phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta, PTEN = phosphatase and tensin homolog, PTK2 = protein tyrosine kinase 2, RASSF1 = Ras association domain family member 1, REN = renin, RHAG = Rh associated glycoprotein, SDC1 = syndecan 1, SLC19A1 = solute carrier family 19 member 1, SLC45A2 = solute carrier family 45 member 2, TMEM158 = transmembrane protein 158, TNFRSF10C = TNF receptor superfamily member 10c, TNFSF10 = TNF superfamily member 10, TOP3B = DNA topoisomerase III beta, YY1 = YY1 transcription factor.

[*] Low methylation genes in ovarian in drug-resistance tissues of ovarian cancer.

[†] High methylation genes in ovarian in drug-resistance tissues of ovarian cancer.

Screening of 55 significantly differentially methylated genes in drug resistance of ovarian cancer.

Enriched biological processes	Enriched genes	*P* value	Benjamini
Negative regulation of cell proliferation	ETS1/COL18A1/CSF1R/CDKN2B/CDKN2C/KMT2A/PTEN	1.50E-03	1.10E-01
Regulation of cyclin-dependent protein serine/threonine kinase activity	CDKN2B/CDKN2C/PTEN	6.70E-03	2.70E-01
Immune response	ETS1/TNFRSF10C/CSF2/IL13/TNFSF10	4.40E-02	6.60E-01

Enriched pathway	Enriched genes	P value	Benjamini
PI3K-Akt signaling pathway	MCL1/MDM2/CSF1R/CDK2/PTEN/PTK2	2.30E-02	2.70E-01
p53 signaling pathway	MDM2/CDK2/PTEN	4.00E-02	3.30E-01
FoxO signaling pathway	MDM2/CDK2/CDKN2B/PTEN/TNFSF10	3.60E-03	8.30E-02
Cell cycle	ETS1/TNFRSF10C/CSF2/IL13/TNFSF10	4.40E-02	2.90E-01

ABCG4 = ATP binding cassette subfamily G member 4, ANXA5 = annexin A5, BGLAP = bone gamma-carboxyglutamate protein, BRD4 = bromodomain containing 4, CA9 = carbonic anhydrase 9, CAPN5 = calpain 5, CD44 = cluster of differentiation 44, CDC42BPB = CDC42 binding protein kinase beta, CDK2 = cyclin dependent kinase 2, CDKN2B = cyclin dependent kinase inhibitor 2B, CDKN2C = cyclin dependent kinase inhibitor 2C, COL18A1 = collagen type XVIII alpha 1 chain, CSF1R = colony stimulating factor 1 receptor, CSF2 = colony stimulating factor 2, CTSL = cathepsin L, DAP = death associated protein, DYRK2 = dual specificity tyrosine phosphorylation regulated kinase 2, ESR1 = estrogen receptor 1, ESRRA = estrogen related receptor alpha, ETS1 = ETS proto-oncogene 1, F2 = coagulation factor II, thrombin, FUT4 = fucosyltransferase 4, GNA13 = G protein subunit alpha 13, HIC1 = HIC ZBTB transcriptional repressor 1, HOXA11 = homeobox A11, IL13 = interleukin 13, KIF1C = kinesin family member 1C, KMT2A = lysine methyltransferase 2A, LAPTM4B = lysosomal protein transmembrane 4 beta, LEP = leptin, MBTPS1 = membrane bound transcription factor peptidase, site 1, MCL1 = MCL1 apoptosis regulator, MDK = midkine, MDM2 = MDM2 proto-oncogene, MIF = macrophage migration inhibitory factor, MTA1 = metastasis associated 1, ORM2 = orosomucoid 2, PAX2 = paired box 2, PAX8 = paired box 8, PDZK1 = PDZ domain containing 1, PEA15 = proliferation and apoptosis adaptor protein 15, PIK3C2B = phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta, PTEN = phosphatase and tensin homolog, PTK2 = protein tyrosine kinase 2, RASSF1 = Ras association domain family member 1, REN = renin, RHAG = Rh associated glycoprotein, SDC1 = syndecan 1, SLC19A1 = solute carrier family 19 member 1, SLC45A2 = solute carrier family 45 member 2, TMEM158 = transmembrane protein 158, TNFRSF10C = TNF receptor superfamily member 10c, TNFSF10 = TNF superfamily member 10, TOP3B = DNA topoisomerase III beta, YY1 = YY1 transcription factor.

directly with other 21 genes, and PTEN interacted directly with other 17 genes, while MDM2 proto-oncogene (MDM2) and cluster of differentiation 44 (CD44) interacted directly with 14 genes. As a classical gene, PTEN has been widely studied in drug resistance in OC. Numerous studies have shown that PTEN, as a key link, is involved in the PI3K/AKT/PTEN drug-resistance pathway and participates in drug resistance in OC by affecting biological processes, such as DNA methylation, cell cycle, apoptosis, and cell
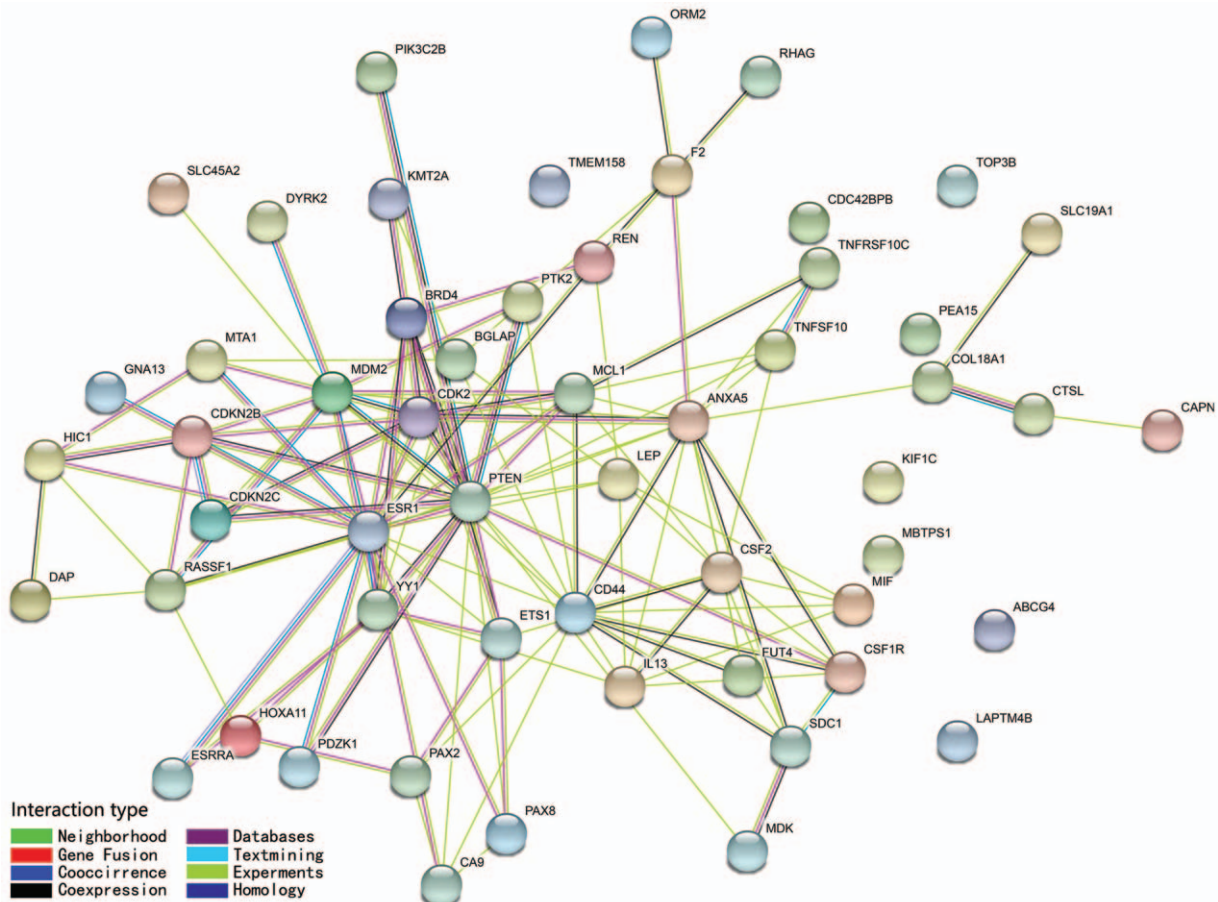

Figure 1. Protein interaction network analysis of 55 methylated genes related to drug resistance in ovarian cancer.

proliferation.[11,20–22] In addition, it had been reported that changes in gene methylation of ESR1[23,24] are involved in the development and drug resistance in OC and are associated with prognosis. With reference to MDM2 and CD44, although no previous studies have shown that changes in methylation are associated with OC and drug resistance in OC, numerous studies have indicated that both MDM2 and CD44 genes are involved in drug resistance in OC, and are related to the prognosis of OC. Based on these and our findings, it can be concluded that MDM2 and CD44 genes may be involved in the development of OC and drug resistance in OC through changes in gene methylation.

3.3. Analysis of the negative correlation between methylation level of differentially methylated genes for drug resistance in OC and mRNA expression

Based on the TCGA database, 10 genes corresponding to the methylation degree of differential methylated genes and mRNA expression from the above 55 genes were further screened. Among these, 5 were hypermethylated in drug-resistant tissues, including calpain 5, cathepsin L (CTSL), ETS1, membrane bound

transcription factor peptidase, and syndecan 1. The mRNA expressions of these 5 genes were decreased in drug-resistant tissues; coagulation factor II, PDZK1, Rh associated glycoprotein (RHAG), TNF receptor superfamily member 10c (TNFRSF10C), and orosomucoid 2 showed hypomethylation in drug-resistant tissues, and mRNA expressions of these 5 genes were up-regulated in drug-resistant tissues, as shown in Figure 2. In addition, CTSL has been previously reported to be clearly associated with drug resistance in OC.[25]

3.4. Association between methylation level of differentially methylated genes for drug resistance in OC and prognosis of OC

Univariate Kaplan-Meier analysis was performed for the association between the changes in the above 55 gene methylation and clinical prognosis of ovarian epithelioma (including disease-free survival [DFS] and overall survival [OS]). The results are shown in Table 3 and Figures 3–5. The methylation of PTEN and TNFRSF10C genes had an effect on the prognostic DFS in OC (P < .05), and the 3 genes,
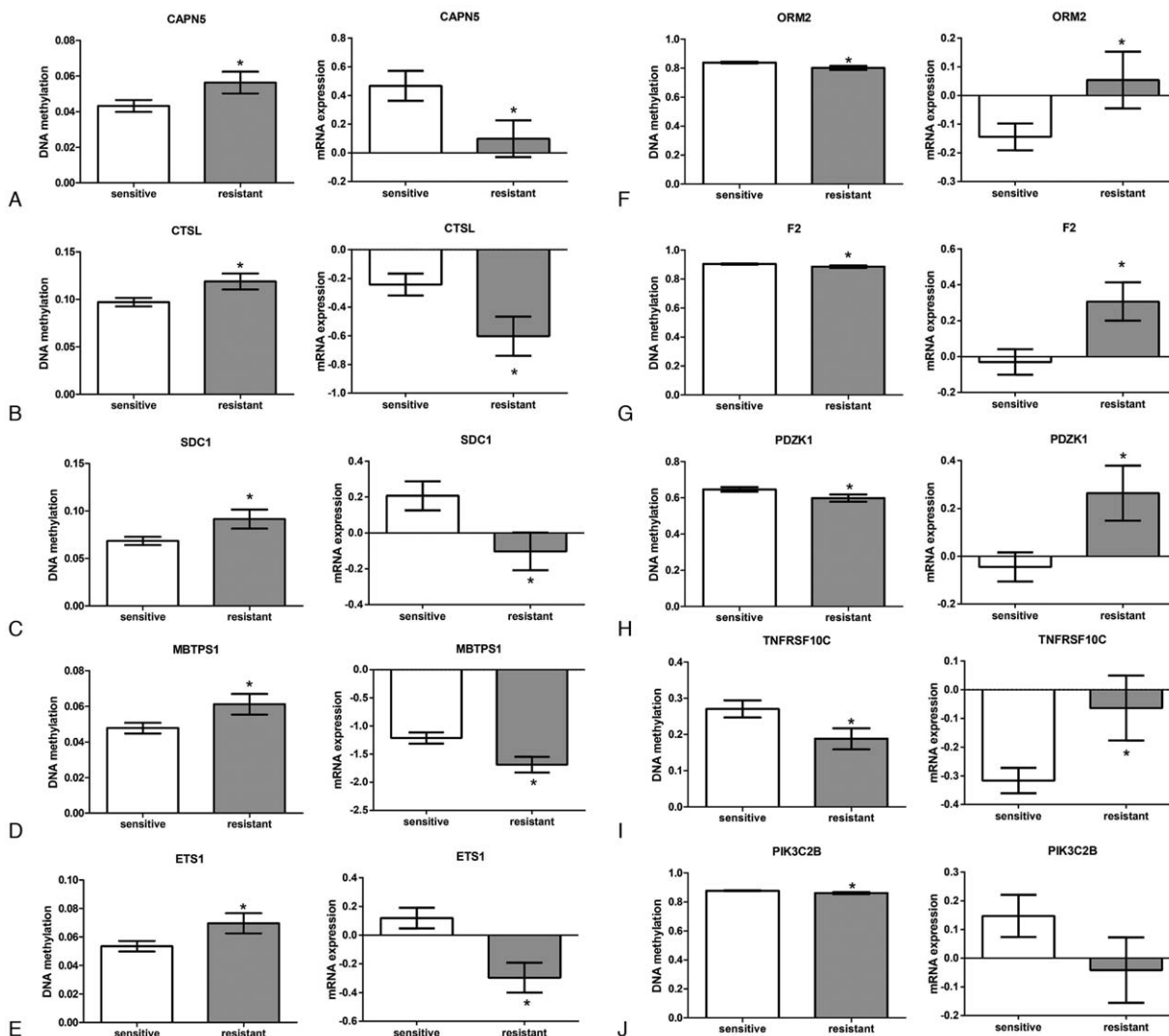

Figure 2. Analysis of gene methylation level and mRNA expression in drug-sensitive and resistant tissues of ovarian cancer.

Table 3

Methylated genes associated with the prognosis of ovarian cancer.

		Disease-free survival					Overall survival				
				95% Confidence Interval					95% Confidence Interval		
		Estimate	Std. Error	Lower	Upper	p value	Estimate	Std. Error	Lower	Upper	p value
DYRK2	H	–	–	–	–	–	48.03	3.136772	41.88193	54.17807	.037
	L	–	–	–	–	–	38.04	2.070551	33.98172	42.09828	
	Overall	–	–	–	–	–	43.79	2.117305	39.64008	47.93992	
PIK3C2B	H	–	–	–	–	–	47.51	1.981098	43.62705	51.39295	.017
	L	–	–	–	–	–	38.41	1.882595	34.72011	42.09989	
	Overall	–	–	–	–	–	43.79	2.117305	39.64008	47.93992	
RHAG	H	–	–	–	–	–	48.29	2.598983	43.19599	53.38401	.005
	L	–	–	–	–	–	38.34	2.187783	34.05195	42.62805	
	Overall	–	–	–	–	–	38.34	2.187783	34.05195	42.62805	
BRD4	H	17.97	1.223946	15.57107	20.36893	0.038	48.29	4.75322	38.97369	57.60631	.006
	L	15.41	1.137698	13.18011	17.63989		41.36	2.157245	37.1318	45.5882	
	Overall	16.85	0.742849	15.39402	18.30598		43.79	2.117305	39.64008	47.93992	
PDZK1	H	19.51	1.007644	17.53502	21.48498	0.01	47.67	1.677993	44.38113	50.95887	.028
	L	14.72	0.811729	13.12901	16.31099		39.06	2.671631	33.8236	44.2964	
	Overall	16.85	0.742849	15.39402	18.30598		43.79	2.117305	39.64008	47.93992	
PTEN	H	18.86	1.055861	16.79051	20.92949	0.011	–	–	–	–	–
	L	15.38	0.725749	13.95753	16.80247		–	–	–	–	–
	Overall	16.85	0.742849	15.39402	18.30598		–	–	–	–	–
TNFRSF10C	H	17.97	1.123973	15.76701	20.17299	0.041	–	–	–	–	–
	L	14.72	1.068611	12.62552	16.81448		–	–	·	–	–
	Overall	16.85	0.742849	15.39402	18.30598		–	–	–	–	–
DYRK2	H	48.03	3.136772	41.88193	54.17807	0.037	–	–	–	–	–
	L	38.04	2.070551	33.98172	42.09828		–	–	–	–	–
	Overall	43.79	2.117305	39.64008	47.93992		–	–	–	–	–
PIK3C2B	H	47.51	1.981098	43.62705	51.39295	0.017	–	–	–	–	–
	L	38.41	1.882595	34.72011	42.09989		–	–	–	–	–
	Overall	43.79	2.117305	39.64008	47.93992		–	–	–	–	–
RHAG	H	48.29	2.598983	43.19599	53.38401	0.005	–	–	–	–	–
	L	38.34	2.187783	34.05195	42.62805		–	–	–	–	–
	Overall	38.34	2.187783	34.05195	42.62805		–	–	–	–	–

BRD4 = bromodomain containing 4, DYRK2 = dual specificity tyrosine phosphorylation regulated kinase 2, PDZK1 = PDZ domain containing 1, PIK3C2B = phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta, PTEN = phosphatase and tensin homolog, RHAG = Rh associated glycoprotein, TNFRSF10C = TNF receptor superfamily member 10c.

phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta (PIK3C2B), RHAG, and dual specificity tyrosine phosphorylation regulated kinase 2 (DYRK2), had effects on the prognostic OS for OC ($P < .05$). The methylation of bromodomain containing 4 (BRD4) and PDZK1 genes had effects on the prognostic DFS and OS of OC ($P < .05$). Among them, the hypomethylation or demethylation of 4 genes, BRD4, PDZK1, PTEN, and TNFRSF10C, which had effects on the prognostic DFS of ovarian cancer, shortened the median survival time of DFS by 3 to 5 months in OC. The hypomethylation or demethylation of the 5 genes, BRD4, PDZK1, PIK3C2B, RHAG, and DYRK2, which had effects on prognostic OS of OC, resulted in a 7 to 10 month reduction in the median survival time of OS in ovarian cancer. The methylation changes of these 7 genes in the methylation degree and mRNA expression in drug-resistant and sensitive tissues of OC are shown in Figure 6. Among them, methylation of PDZK1, RHAG, and TNFRSF10C corresponded to mRNA expression, and these 3 genes showed hypomethylation and high mRNA expression in drug-resistant tissues of OC.

4. Discussion

At present, OC has high fatality rates, and the predominant cause of chemotherapy failure is the formation of multidrug resistance (MDR). DNA methylation is 1 of the epigenetic mechanisms that

has an important regulatory role in the development, progression, and drug resistance of OC. Numerous studies have reported on the association between DNA methylation and OC and drug resistance in OC are available, showing that drug resistance in OC may occur through cell cycle, cell proliferation, and apoptosis. In the current study, we used TCGA database to systematically integrate the association of the 55 methylated genes with OC, MDR, and clinical prognosis. This study can be used as sort of a guide for the clinical selection of chemotherapy drugs, early detection, and prognosis. In addition, we performed bioinformatics analysis on these 55 genes, including annotation of biological processes and protein interaction, thus providing a basis for the overall study of DNA-methylated genes involved in the OC and drug resistance.

Based on the TCGA database, 55 differentially methylated genes in drug-resistant and sensitive tissues of OC were screened. Among the 55 genes, BRD4,[26]CD44,[27]CTSL,[25]DYRK2,[28] MCL1 apoptosis regulator,[29]MDM2.[30] proliferation and apoptosis adaptor protein,[31]PTEN,[22]RASSF1A,[32]TNFRSF10C,[33]MDK,[9]HOXA11,[34] and lysosomal protein transmembrane 4 beta[35] have been identified as clearly associated with drug resistance in OC. Furthermore, previous studies have reported that hypermethylation changes of death associated protein, ESR1, MDK, myelin and lymphocyte, PTEN, RASSF1, and HOXA11 genes are clearly related to drug resistance in OC, while hypomethylation
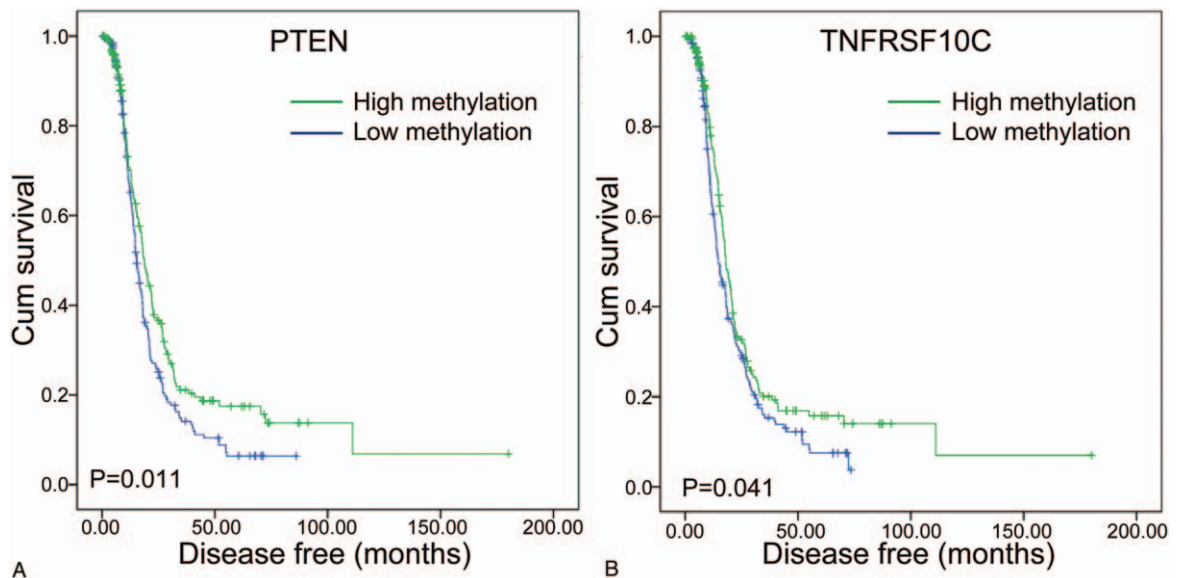
Figure 3. DNA-methylated genes related to the disease free survival of ovarian cancer.

changes of carbonic anhydrase 9, *CDK2*, and *ETS1* are clearly related to drug resistance in ovarian cancer.

Bioinformatics analysis was performed for 55 genes that were significantly methylated in OC tissues. DAVID analysis revealed that the biological processes in which 55 genes had the highest score were cell proliferation and cell cycle processes (Table 2). The pathways with the highest score were PI3K-Akt signaling pathway and p53 signaling pathway (Table 2). These results suggested that these methylated genes may be involved in the regulation of drug resistance in OC mainly via regulation of cell proliferation and cell cycle processes as well as via PI3K-Akt and p53 signaling pathways. In addition, protein interaction analysis revealed that the PTEN gene directly interacted with other 17 genes, ESR1 gene directly interacted with other 21 genes, and that most of the 55 genes had direct or indirect interactions with PTEN or ESR1. It also suggested that many genes were involved in the drug resistance of OC via interaction with PTEN and ESR1 gene. These results were consistent with the existing studies.

As shown in Table 2, at least seven genes including *ETS1* and *PTEN* were involved in the regulation of drug resistance in OC through the cell proliferation pathway, and at least 3 genes participated in the regulation of drug resistance in OC through the cell cycle pathway. Existing studies have shown that many genes are involved in the regulation of drug resistance in OC through cell proliferation and cell cycle processes, as well as via PI3K-Akt and p53 signaling pathways. For example, the proto-oncogene *ETS1* methylation participates in the drug resistance in ovarian cancer through multiple physiological and pathological processes, such as phylogeny, stimulus response, vascular endothelial growth factor production, morphogenesis, cell proliferation, cell adhesion, and signal transduction.[36]*MDM2*, as a suppressor gene of P53 can lead to drug resistance by inhibiting the P53 pathway. The use of drugs to inhibit the binding of MDM2 to P53 can effectively activate P53, which leads to increased cell cycle and apoptosis, thereby reversing drug resistance.[30] In addition, upregulation of PTEN increases P53
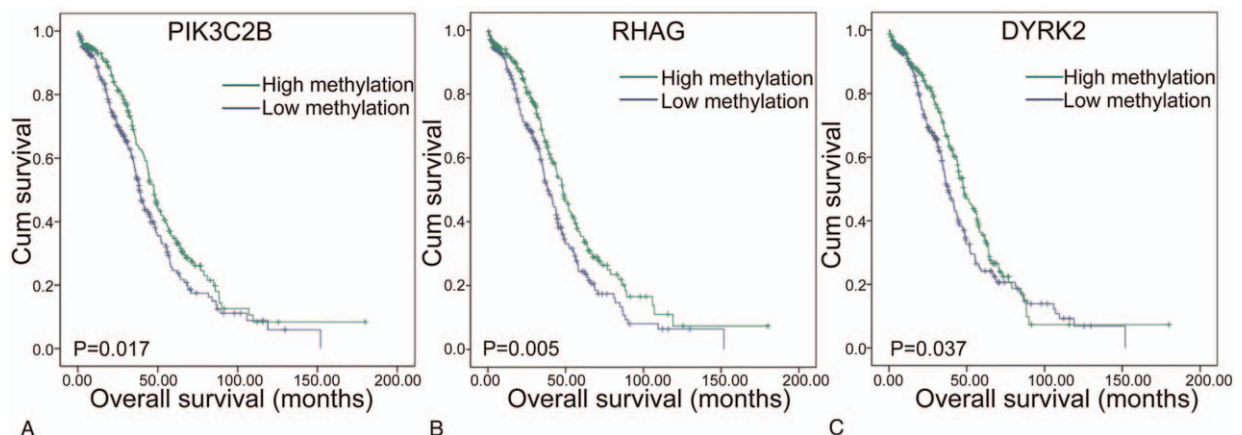

Figure 4. DNA-methylated genes related to the overall survival of ovarian cancer.
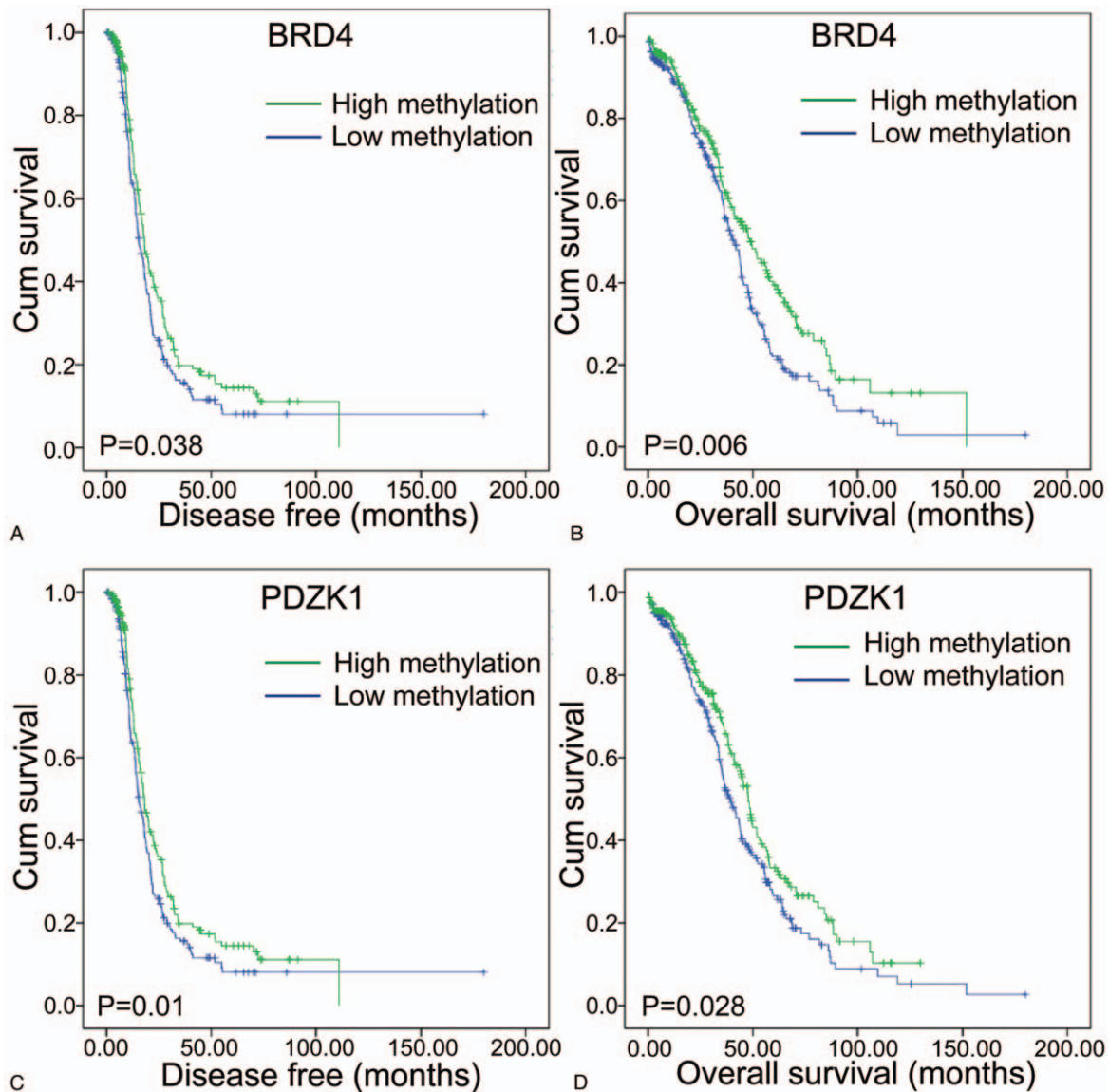
Figure 5. DNA-methylated genes related to the disease free survival and disease free survival of ovarian cancer.

levels by inhibiting the AKT pathway and MDM2, leading to cell cycle G(2)/M arrest and apoptosis.[37] Further evidence comes from the gene proliferation and apoptosis adaptor protein, which participates in the development and progression of OC by regulating cell proliferation, autophagy, apoptosis and glucose metabolism. Its overexpression can destroy the microtubule instable protein function, thereby promoting paclitaxel-induced mitosis and apoptosis and increasing the sensitivity of tumor cells to paclitaxel.[31] Similarly, *CDK2* is considered to have an important role in regulating cell cycle, particularly when changing from G1 to S, and it is known to be involved in cancer progression and cancer cell proliferation. In OC tissues, *CDK2* methylation leads to significant decrease in the mRNA expression, thereby participating in the development and progression of OC via cell cycle pathway.[13] The above findings indicate that cell proliferation and cell cycle processes might be important mechanisms through which DNA-methylated genes regulate the drug resistance in OC. Accordingly, an in-depth study of the regulating effect of DNA-methylated genes on cell

proliferation and cell cycle could provide more possibilities to address MDR in ovarian cancer. After adjustment by the Benjamini method, P values were above 0.05 for the identified genes (Table 2). Since the actual P value represents an important parameter in the DAVID's statistical model, these genes might still have a certain biological significance in drug resistance. Studies have been initiated by our team to confirm these findings.

Previous studies have reported the importance of DNA-methylated genes in prognosis of OC. In the current study, survival analysis of 55 differential DNA-methylated genes revealed that the methylation of *PTEN* and *TNFRSF10C* genes affected the prognostic DFS of ovarian cancer; *PIK3C2B*, *RHAG*, and *DYRK2* genes had effect on the prognostic OS of OC. The methylation of 2 genes, *BRD4* and *PDZK1*, had effects on the prognostic DFS and OS in ovarian cancer. Previous studies have shown that among these seven genes, *DYRK2* has low expression in cisplatin-resistant tissues of OC and is associated with low postoperative overall survival rate in patients with OC.[28] Other genes have also been studied in OC tissues. For
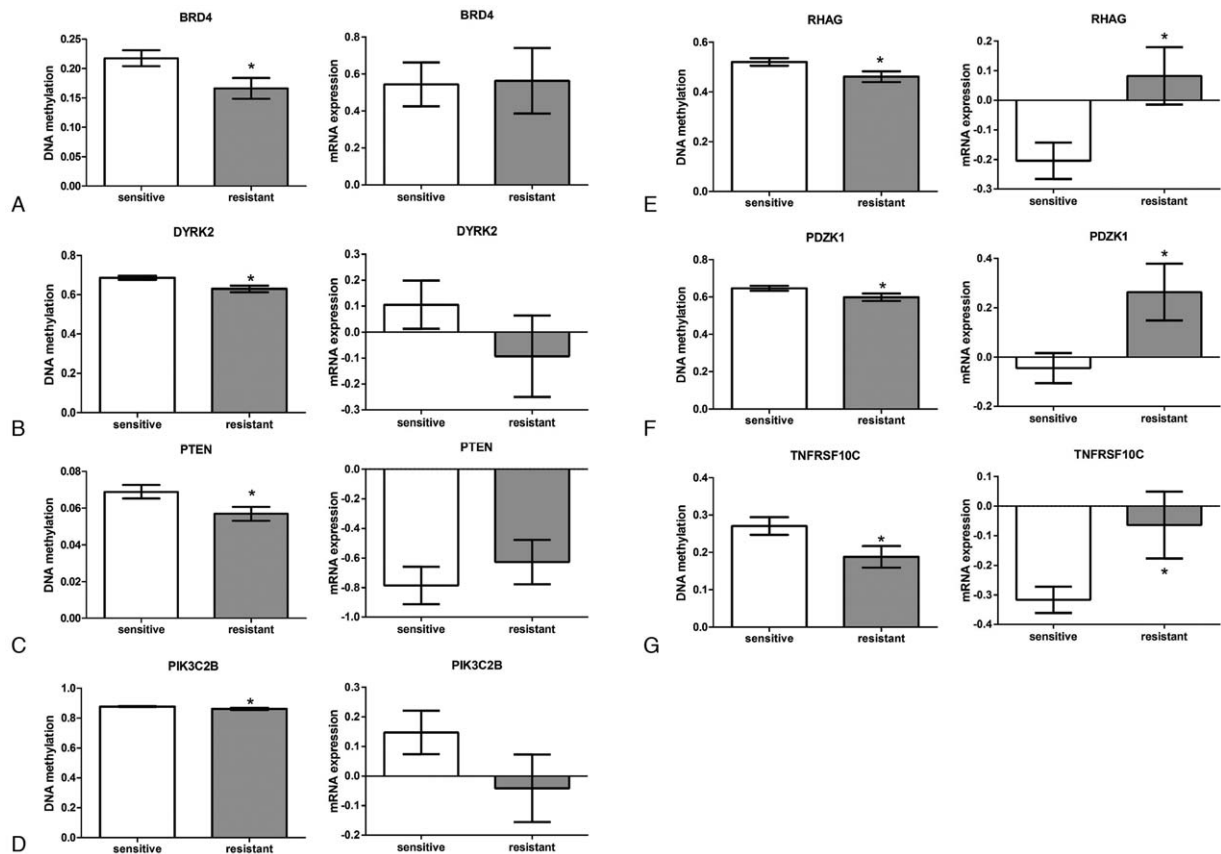
Figure 6. Analysis of gene methylation level and mRNA expression related to the prognosis of ovarian cancer.

example, as the transcriptional regulator of epigenetics, BRD4 is involved in the development and progression of ovarian epithelial cancer and drug resistance.[26] The protein encoded by *BRD4* has a *BET* structure, and administration of a *BET* inhibitor can effectively inhibit the proliferation of ovarian epithelial cancer cells and reverse the drug resistance by blocking the PIK3-AKT and ERK pathways. Previous studies have also shown that the low expression of *TNFRSF10C* is significantly related to the chemotherapy resistance of serous OC. In addition, there are also reports on the gene expression and prognosis of ovarian cancer for other genes. For example, the degree of methylation of *ESR1* in normal ovarian tissues is higher than that in OC tissues,[8] and patients with hypermethylation of ESR1 in ovarian cancer tissues have better OS,[23] which is consistent with our results. The association between the degree of *ESR1* methylation and the prognosis of OC is more significant in serous and mucinous tissues.[38] However, some studies have also shown that in low-grade serous ovarian cancer, the degree of methylation of the *ESR1* promoter is negatively related to survival rate.[24] Another example is that *ETS1* is a key regulator of the *PARP1* gene in *BRCA1* mutant ovarian cancer, and its degree of methylation is significantly associated with the prognosis of OC.[14] In addition, CD44-positive cells of OC are associated with poor prognosis of OC.[39] Some studies have shown that CD44+ phenotypic stem cells of OC are more invasive and have higher tendency to be resistant to paclitaxel and platinum.[27] At the same time, the CD44+ phenotypic stem cells of ovarian cancer have higher autophagy, and when platinum drugs are used for anti-tumor,

they can inhibit autophagy of cancer stem cells, improve the efficacy of chemotherapy, and effectively prevent drug resistance.[40] Similarly, *MLH1, RASSF1A, HOXA10*, and *HOXA11* are hypermethylated in OC tissues, and after the use of demethylating drug decitabine, PFS in patients with ovarian cancer is prolonged, and their prognosis is improved, while the sensitivity of tumor tissue to carboplatin chemotherapy is restored.[32,34]

Among the seven genes related to the prognosis of ovarian cancer, *PDZK1, RHAG,* and *TNFRSF10C* showed significant hypomethylation in drug-resistant tissues of OC, indicating that these 3 genes may be involved in the drug resistance of OC through changes in gene methylation. Some scholars have previously confirmed that the low expression of *TNFRSF10C* is associated with chemotherapy resistance of OC.[33] Yet, there are only few reports on the association between the other 2 genes with ovarian cancer and drug resistance in OC. One such study has reported that for the *TNFRSF10C* in melanoma cells and tissues, hypermethylation of the promoter is involved in tumorigenesis and progression.[41] Another example is *PDZK1*, a scaffold protein that binds to plasma membrane proteins and regulatory elements, regulating its surface expression in the apical region of epithelial cells, which is considered an oncogene of breast cancer.[42] Overexpression of *PDZK1* is associated with resistance to paclitaxel, 5-fluorouracil, and etoposide in breast cancer.[43] In pheochromocytoma and paraganglioma, methylation of *PDZK1* is involved in tumor formation through apoptosis and cell invasion pathways.[44] Low expression of

PDZK1 in renal clear cell carcinoma is related to recurrence, metastasis, and poor prognosis of patients.[45] *PDZK1* expression is significantly down-regulated in pancreatic cancer tissues. Furthermore, low expression of *PDZK1* is significantly associated with poor prognosis in pancreatic cancer. *In vitro* studies have shown the overexpression of *PDZK1* in combination with *PTEN* can significantly reduce phosphorylation of *PTEN* and *AKT*, thus effectively reversing the proliferation and migration of pancreatic cancer cells.[46] In addition, the protein expressed by RHAG is an erythrocyte-associated glycoprotein, and the silencing expression of RHAG has been previously identified in esophageal cancer tissues.[47]

5. Conclusions

PDZK1, RHAG, and *TNFRSF10C* genes are likely to be involved in the drug resistance in OC through changes in gene methylation, and may become markers for predicting the prognosis of OC.

Author contributions

Hua Bai contributed to the conception of the study; Bingbing Yan performed the data analyses and wrote the manuscript; Chunqiu Xiong contributed significantly to analysis and manuscript preparation; Feifeng Huang helped perform the analysis with constructive discussions; Mingming Zhang contributed significantly to Literature research; Yan Mo contributed to the date acquisition.

References

[1] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–386.

[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7–30.

[3] Wang Z, Zhang J, Zhang Y, et al. Expression and mutations of BRCA in breast cancer and ovarian cancer: evidence from bioinformatics analyses. Int J Mol Med 2018;42:3542–50.

[4] Weinstein JN, Collisson EA, et al. Cancer Genome Atlas Research N.The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 2013;45:1113–20.

[5] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:l1.

[6] Finak G, Mayer B, Fulp W, et al. DataPackageR: reproducible data preprocessing, standardization and sharing using R/Bioconductor for collaborative data analysis. Gates Open Res 2018;2:31.

[7] Collins Y, Dicioccio R, Keitz B, et al. Methylation of death-associated protein kinase in ovarian carcinomas. Int J Gynecol Cancer 2006;16 (Suppl 1):195–9.

[8] Chmelarova M, Krepinska E, Spacek J, et al. Methylation analysis of tumour suppressor genes in ovarian cancer using MS-MLPA. Folia Biol (Praha) 2012;58:246–50.

[9] Zeller C, Dai W, Steele NL, et al. Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling. Oncogene 2012;31:4567–76.

[10] Lee PS, Teaberry VS, Bland AE, et al. Elevated MAL expression is accompanied by promoter hypomethylation and platinum resistance in epithelial ovarian cancer. Int J Cancer 2010;126:1378–89.

[11] Cheaib B, Auguste A, Leary A. The PI3K/Akt/mTOR pathway in ovarian cancer: therapeutic opportunities and challenges. Chin J Cancer 2015;34: 4–16.

[12] Sung HY, Ju W, Ahn JH. DNA hypomethylation-mediated over-expression of carbonic anhydrase 9 induces an aggressive phenotype in ovarian cancer cells. Yonsei Med J 2014;55:1656–63.

[13] Kanwal A, Kaur M, Singh A, et al. Hypo/unmethylated promoter status of Cdk2 gene correlates with its over-expression in ovarian cancer in north Indian population. Cell Mol Biol (Noisy-le-grand) 2016;62:67–72.

[14] Li D, Bi FF, Cao JM, et al. Poly (ADP-ribose) polymerase 1 transcriptional regulation: a novel crosstalk between histone modification H3K9ac and ETS1 motif hypomethylation in BRCA1-mutated ovarian cancer. Oncotarget 2014;5:291–7.

[15] Zhang B, Kirov S, Snoddy J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res 2005;33:W741–8.

[16] Xu Y, Wang S, Chan HF, et al. dihydromyricetin induces apoptosis and reverses drug resistance in ovarian cancer cells by p53-mediated downregulation of survivin. Sci Rep 2017;7:46060.

[17] Li B, Gao Y, Rankin GO, et al. Chaetoglobosin K induces apoptosis and G2 cell cycle arrest through p53-dependent pathway in cisplatin-resistant ovarian cancer cells. Cancer Lett 2015;356:418–33.

[18] Zhao F, Lam EW. Role of the forkhead transcription factor FOXO-FOXM1 axis in cancer and drug resistance. Front Med 2012;6:376–80.

[19] Zhang Y, Gan B, Liu D, et al. FoxO family members in cancer. Cancer Biol Ther 2011;12:253–9.

[20] Singh M, Chaudhry P, Fabi F, et al. Cisplatin-induced caspase activation mediates PTEN cleavage in ovarian cancer cells: a potential mechanism of chemoresistance. BMC Cancer 2013;13:233.

[21] Yan B, Yin F, Wang QI, et al. Integration and bioinformatics analysis of DNA-methylated genes associated with drug resistance in ovarian cancer. Oncol Lett 2016;12:157–66.

[22] Yu X, Chen Y, Tian R, et al. miRNA-21 enhances chemoresistance to cisplatin in epithelial ovarian cancer by negatively regulating PTEN. Oncol Lett 2017;14:1807–10.

[23] Giannopoulou L, Mastoraki S, Buderath P, et al. ESR1 methylation in primary tumors and paired circulating tumor DNA of patients with high-grade serous ovarian cancer. Gynecol Oncol 2018;150:355–60.

[24] Kirn V, Shi R, Heublein S, et al. Estrogen receptor promoter methylation predicts survival in low-grade ovarian carcinoma patients. J Cancer Res Clin Oncol 2014;140:1681–7.

[25] Zhang H, Zhang L, Wei L, et al. Knockdown of cathepsin L sensitizes ovarian cancer cells to chemotherapy. Oncol Lett 2016;11:4235–9.

[26] Baratta MG, Schinzel AC, Zwang Y, et al. An in-tumor genetic screen reveals that the BET bromodomain protein, BRD4, is a potential therapeutic target in ovarian carcinoma. Proc Natl Acad Sci U S A 2015;112:232–7.

[27] Akhter MZ, Sharawat SK, Kumar V, et al. Aggressive serous epithelial ovarian cancer is potentially propagated by EpCAM(+)CD45(+) phenotype. Oncogene 2018;37:2089–103.

[28] Yamaguchi N, Mimoto R, Yanaihara N, et al. DYRK2 regulates epithelial-mesenchymal-transition and chemosensitivity through Snail degradation in ovarian serous adenocarcinoma. Tumour Biol 2015;36: 5913–23.

[29] Yasui K, Mihara S, Zhao C, et al. Alteration in copy numbers of genes as a mechanism for acquired drug resistance. Cancer Res 2004;64: 1403–10.

[30] Zanjirband M, Edmondson RJ, Lunec J. Pre-clinical efficacy and synergistic potential of the MDM2-p53 antagonists, Nutlin-3 and RG7388, as single agents and in combined treatment with cisplatin in ovarian cancer. Oncotarget 2016;7:40115–34.

[31] Xie X, Bartholomeusz C, Ahmed AA, et al. Bisphosphorylated PEA-15 sensitizes ovarian cancer cells to paclitaxel by impairing the microtubule-destabilizing effect of SCLIP. Mol Cancer Ther 2013;12:1099–111.

[32] Matei D, Fang F, Shen C, et al. Epigenetic resensitization to platinum in ovarian cancer. Cancer Res 2012;72:2197–205.

[33] Kang KW, Lee MJ, Song JA, et al. Overexpression of goosecoid homeobox is associated with chemoresistance and poor prognosis in ovarian carcinoma. Oncol Rep 2014;32:189–98.

[34] Fiegl H, Windbichler G, Mueller-Holzner E, et al. HOXA11 DNA methylation–a novel prognostic biomarker in ovarian cancer. Int J Cancer 2008;123:725–9.

[35] Yin M, Li J, Li X, et al. Over-expression of LAPTM4B is associated with poor prognosis and chemotherapy resistance in stages III and IV epithelial ovarian cancer. J Surg Oncol 2011;104:29–36.

[36] Wan SM, Peng P, Guan T. Ets-1 regulates its target genes mainly by DNA methylation in human ovarian cancer. J Obstet Gynaecol 2013;33: 877–81.

[37] Selvendiran K, Tong L, Vishwanath S, et al. EF24 induces G2/M arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by increasing PTEN expression. J Biol Chem 2007;282:28609–18.

[38] Losi L, Fonda S, Saponaro S, et al. Distinct DNA methylation profiles in ovarian tumors: opportunities for novel biomarkers. Int J Mol Sci 2018;19:1559–74.

[39] Zhu LC, Gao J, Hu ZH, et al. Membranous expressions of Lewis y and CAM-DR-related markers are independent factors of chemotherapy resistance and poor prognosis in epithelial ovarian cancer. Am J Cancer Res 2015;5:830–43.

[40] Pagotto A, Pilotto G, Mazzoldi EL, et al. Autophagy inhibition reduces chemoresistance and tumorigenic potential of human ovarian cancer stem cells. Cell Death Dis 2017;8:e2943.

[41] Liu S, Ren S, Howell P, et al. Identification of novel epigenetically modified genes in human melanoma via promoter methylation gene profiling. Pigment Cell Melanoma Res 2008;21:545–58.

[42] Kim H, Abd Elmageed ZY, Ju J, et al. PDZK1 is a novel factor in breast cancer that is indirectly regulated by estrogen through IGF-1R and promotes estrogen-mediated growth. Mol Med 2013;19:253–62.

[43] Kim H, Abd Elmageed ZY, Davis C, et al. Correlation between PDZK1, Cdc37, Akt and breast cancer malignancy: the role of PDZK1 in cell growth through Akt stabilization by increasing and interacting with Cdc37. Mol Med 2014;20:270–9.

[44] de Cubas AA, Korpershoek E, Inglada-Perez L, et al. DNA methylation profiling in pheochromocytoma and paraganglioma reveals diagnostic and prognostic markers. Clin Cancer Res 2015;21:3020–30.

[45] Zheng J, Wang L, Peng Z, et al. Low level of PDZ domain containing 1 (PDZK1) predicts poor clinical outcome in patients with clear cell renal cell carcinoma. EBioMedicine 2017;15:62–72.

[46] Ma Q, Wu X, Wu J, et al. PDZ-containing 1 acts as a suppressor of pancreatic cancer by regulating PTEN phosphorylation. Oncotarget 2017;8:72893–909.

[47] Chen BS, Xu ZX, Xu X, et al. RhCG is downregulated in oesophageal squamous cell carcinomas, but expressed in multiple squamous epithelia. Eur J Cancer 2002;38:1927–36.