

Original Article



Ovarian cancer risk score predicts chemo-response and outcome in epithelial ovarian carcinoma patients

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OPEN ACCESS

Received: Jul 16, 2020

Revised: Nov 6, 2020

Accepted: Nov 8, 2020

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Conflict of Interest

No potential conflict of interest relevant to this
article was reported.

ABSTRACT

Objective: Cytoreductive surgery followed by adjuvant chemotherapy is a standard frontline treatment for epithelial ovarian cancer (EOC). We aimed to develop an ovarian cancer risk score (OVRs) based on the expression of 10 ovarian-cancer-related genes to predict the chemoresistance, and outcomes of EOC patients.

Methods: We designed a case-control study with total 149 EOC women including 75 chemosensitives and 74 chemoresistants. Gene expression was measured using the quantitative real-time polymerase chain reaction. We tested for correlation between the OVRs and chemosensitivity or chemoresistance, disease-free survival (DFS), and overall survival (OS), and validated the OVRs by analyzing patients from the TCGA database.

Results: The chemosensitive group had lower OVRs than the chemoresistant group (5 vs. 15, $p \leq 0.001$, Mann-Whitney U test). Patients with disease relapse (13 vs. 5, $p < 0.001$, Mann-Whitney U test) or disease-related death (13.5 vs. 6, $p < 0.001$) had higher OVRs than those without. OVRs ≥ 10 (hazard ratio=3.29; 95% confidence interval=1.94–5.58; $p < 0.001$) was the only predictor for chemoresistance in multivariate analysis. The median DFS (5 months vs. 24 months) and OS (39 months vs. >60 months) of patients with OVRs ≥ 10 were significantly shorter than those of patients with OVRs <9). The high OVRs group also had significantly shorter median OS than the low OVRs group in 255 patients in the TCGA database (39 vs. 49 months, $p = 0.046$).

Conclusions: Specific genes panel can be clinically applied in predicting the chemoresistance and outcome, and decision-making of epithelial ovarian cancer.

Keywords: Ovarian Cancer; Prognostic Factor; Gene Analysis; Drug Resistance; Risk Score

INTRODUCTION

Epithelial ovarian cancer (EOC) is the seventh most commonly diagnosed cancer among women in the world [1-3] and has received increasing attention in recent years because it is associated with the highest mortality rate among gynecologic malignancies [3,4]. The overall

Author Contributions

Conceptualization: C.W.F.; Data curation: L.H.Y., T.Y.J., C.Y.L., C.Y.C.; Formal analysis: T.Y.J.; Investigation: L.H.Y., C.W.F., C.W.F.; Methodology: L.H.Y., T.Y.J., C.Y.L., C.Y.C., H.H.C., C.W.F.; Project administration: C.W.F.; Supervision: C.W.F.; Writing - original draft: L.H.Y., C.W.F.; Writing - review & editing: T.Y.J., C.Y.L., C.Y.C., C.W.F.

5-year relative survival rate generally ranges between 30%–40% worldwide and has seen only very modest increases (2%–4%) since the 1990s [1]. More than 75% of affected patients are diagnosed at an advanced stage (stages III and IV) [1]. Early diagnosis of EOC is difficult due to the lack of obvious initial symptoms and accurate biomarkers: ovarian cancer patients are usually diagnosed at an advanced stage and have a poor prognosis [2]. The 5-year survival rate was around 80% for patients with early stage EOCs (stages I and II), but less than 30% for those with advanced stage (stages III and IV) disease [2].

The primary management of EOC is surgery, followed by adjuvant platinum-based chemotherapy. Maintenance strategies include anti-angiogenic agents or poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors. Patients with refractory disease or recurrence within 6 months of cessation of chemotherapy (chemotherapy resistance) had poor prognosis and short expected survival, usually <12 months [4]. Currently known factors affecting prognosis in ovarian cancer include cancer stage, histological type, tumor grade, residual tumor size after surgery, and chemosensitivity or chemoresistance. However, these factors present an incomplete picture of the tumor biology and are frequently interrelated [5]. All histologic subtypes display heterogeneity but are managed with the identical systemic regimens. There is an unmet need for new molecule(s) or biomarker(s) to predict individual disease course and chemoresistance.

So we selected the several genes as a panel, to test for correlation between their expression and the platinum sensitivity or resistance of ovarian cancers. We developed a scoring system named the ovarian cancer risk score (OVRS) with which to score each EOC patient to evaluate their chemoresistance, disease recurrence, and outcome. Finally, the OVRS was validated using the TCGA database of ovarian cancer patients.

MATERIALS AND METHODS

1. Patients and design of case-control study

The study protocol was approved by the Institutional Review Board of the hospital. We designed a case-control study to compare the expression of the genes in the panel among advanced stage (stages III/IV) EOC patients according to chemotherapy sensitivity (chemosensitive or chemoresistant). The two groups of patients were matched for age, FIGO stage, histologic type, tumor grade, and surgical status to avoid confounding. Patients were eligible if they met the following criteria: 1) had advanced stage EOC, 2) had received primary debulking surgery and adjuvant chemotherapy in our institute, and 3) had received platinum-based adjuvant chemotherapy for 6 to 8 cycles. Exclusion criteria included the following: 1) had received neoadjuvant chemotherapy before surgery (interval debulking), 2) had did not received staging or debulking surgery, 3) had received <6 or ≥9 cycles of adjuvant chemotherapy, 4) had received non-platinum-based chemotherapy, or 5) had received surgery or chemotherapy at another institute.

Women diagnosed with advanced stage EOC at National Taiwan University Hospital between Jan 1st, 2013 and June 30th, 2016 were recruited. Clinico-pathologic data including age, disease stage, tumor grade, treatment history including primary and salvage therapy, recurrent status, and prognosis were collected and reviewed until July 30th, 2018 or their last visit, whichever came first. The disease stage and histologic types were defined according to the system of the International Federation of Gynecology and Obstetrics (FIGO) [6].

Recurrence was defined as abnormal results of imaging studies (including computerized tomography or magnetic resonance imaging), elevated CA125, CEA, or CA19-9 (more than 2 times the upper normal limit) in two consecutive tests at 2-week intervals, or biopsy-proven disease. Patients whose disease progressed or recurred 6 months or less after they completed adjuvant chemotherapy were defined as chemoresistant, while those without recurrence or recurrence more than 6 months after completing adjuvant chemotherapy were defined as chemosensitive. The time elapsed from completion of the primary treatment until the diagnosis of disease recurrence was defined as disease-free survival (DFS). The time elapsed from the diagnosis of disease until the date of death or last visit was defined as overall survival (OS). There were 149 matched patients with advanced-stage EOC (stage III or IV), including 75 chemosensitive patients and 74 chemoresistant patients, included for analysis.

2. Specimen collection and RNA extraction from cancerous tissues

Tissue specimens for experiments were collected in the surgery and stored at -80°C immediately until processing. Total tissue RNA was isolated using Trizol reagent (Invitrogen Corporation, Carlsbad, CA, USA), according to the manufacturer's instructions. The samples were subsequently passed over a Qiagen RNeasy column (Qiagen, Valencia, CA, USA) to remove small fragments of RNA that could affect the RT reaction and hybridization quality. After RNA recovery, cDNA was synthesized using a chimeric oligonucleotide containing oligo-dT and a T7 RNA polymerase promoter, at a concentration of 100 pmol/ μL .

3. Selection of the 10 candidate genes

Genes including those identified from our previous studies or reported to be related to the the progression, metastasis and outcome of human cancers, especially women cancers were selected to be candidate genes for the panel [7-13]. The candidate genes for further survey included glypican-1 (GPC1) [14], cyclophilin B (CYPB) [15]. Mesothelin (MSLN) [16], LIM domain kinase 2 (LIMK2) [17], dedicator of cytokinesis 4 (DOCK4) [18], serine/threonine kinase 31 (STK31) [19], insulin-like growth factor-1 (IGF1) [20], chitinase 3-like 1 (CHI3L1) [21], survivin [22-25], and transmembrane protein 102 (TMEM102) of common beta-chain-associated protein (CBAP) [26].

4. Quantitative real-time polymerase chain reaction (qRT-PCR)

QRT-PCR was performed using an Applied Biosystems (Foster City, CA, USA) Real-Time detection system. The relative abundance of cDNA was calculated by using the comparative method with G6PDH as the internal control. Detection of G6PDH and the respective target gene were performed using the primer (TaqMan Assays, Life Technologies Corporation, Carlsbad, CA, USA) with 40 cycles of 2 minutes at 50°C , 10 minutes at 95°C , 15 seconds at 95°C , and 1 minute at 60°C .

The comparative $2^{-\Delta\Delta\text{Ct}}$ method was used to calculate the expression of the target gene as described previously [27]. The number of cycles needed for amplification-generated fluorescence to reach a specific threshold of detection (the Ct value). For the relative quantification of gene expression, based on adding fixed amounts of RNA starting material to the reactions, the Ct values obtained for each real-time PCR were first transformed using the term $E^{-\text{Ct}}$, where E is reaction efficiency, divided by the corresponding value obtained for the same gene in the reference sample (normal ovarian tissue).

The following equation was used to calculate the expression level of the target gene in each sample: Relative expression level of the target gene = $2^{-\Delta\Delta\text{Ct}}$, where $\Delta\text{Ct} = \text{Ct}_{\text{target gene}} - \text{Ct}_{\text{housekeeping (G6PDH)}}$, and $\Delta\Delta\text{Ct} = \Delta\text{Ct}_{\text{sample (ovarian cancerous tissue)}} - \Delta\text{Ct}_{\text{calibrator (normal ovarian tissue)}}$ [27].

5. Development of the OVRS composed of 10 target genes

The OVRS was based on our previous publication [24]. The median expression levels of 10 target genes were used as cutoff values for analysis. The univariate Cox proportional-hazards model was performed to evaluate the impact of the expression of these 10 target genes on chemoresistance. The univariate analysis for chemoresistance by the Cox proportional-hazards model is shown in **Table 1**. Risk scores were calculated, reflecting the impact of the target genes on chemosensitivity or chemoresistance. First, we categorized the patients into low- and high-expressing groups according to their median expression levels of the 10 target genes. Patients with high expression of the target genes were more frequently resistant to chemotherapy.

We then developed the risk scores for the chemoresistance of EOC patient and their outcome by using the β coefficient of *MSLN*. Scores of “0” and “1” were defined as *MSLN* expression <76.01 and ≥ 76.01 (median), respectively. The risk scores of the other 9 target genes were calculated by dividing the respective β coefficients of the other 9 target genes by the β coefficient of *MSLN*, and then rounding to an integer value, as shown in **Table 1**. The risk scores of the target genes for chemo-response were summed for each patient to give their OVRS.

Table 1. Univariate Cox proportional hazard model of 10 target genes for chemosensitivity or chemoresistance of 149 epithelial ovarian carcinoma patients

Gene expression	HR (95% CI)*	β coefficient	p-value	Risk score [†]
<i>MSLN</i>				
<76.01	1			0
≥ 76.01	1.58 (0.98–2.53)	0.46	0.060	1
<i>GPCT</i>				
<0.44	1			0
≥ 0.44	2.16 (1.33–3.50)	0.77	0.002	2 (1.7)
<i>CYPB</i>				
<47.94	1			0
≥ 47.94	3.26 (1.97–5.40)	1.18	<0.001	3 (2.6)
<i>LIMK2</i>				
<4.15	1			0
≥ 4.15	2.50 (1.53–4.09)	0.92	<0.001	2
<i>DOCK4</i>				
<0.29	1			0
≥ 0.29	2.79 (1.69–4.61)	1.03	<0.001	2 (2.2)
<i>STK31</i>				
<0.41	1			0
≥ 0.41	2.57 (1.57–4.20)	0.94	<0.001	2
<i>IGF1</i>				
<4.17	1			0
≥ 4.17	3.11 (1.87–5.18)	1.14	<0.001	3 (2.5)
<i>CHI3L1</i>				
<5.90	1			0
≥ 5.90	1.93 (1.20–3.13)	0.66	0.007	1 (1.4)
<i>Survivin</i>				
<1.50	1			0
≥ 1.50	1.62 (1.00–2.61)	0.48	0.048	1
<i>CBAP</i>				
<1.41	1			0
≥ 1.41	3.71 (2.21–6.24)	1.31	<0.001	3 (2.8)

Expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

CI, confidence interval; HR, hazard ratio.

*Univariate Cox regression model; [†]Risk scores of each patient was calculated by the β coefficient of other 9 target genes divided by the β coefficient of *MSLN* and then rounded to an integer value.

6. TCGA database

The OVRS was applied to patients from the Cancer Genome Atlas (TCGA) database. We first obtained the EOC patient data from the GDC Data Portal of TCGA and used the R package (TCGA biolinks and data.table) to download patients' clinical and gene expression data (Project ID: TCGA-OV; disease type: ovarian serous cystadenocarcinoma; data type: gene expression quantification; file type: normalized results; platform: Illumina HiSeq). There were 255 patients available for external validation of our scoring system.

7. Statistical analysis

Statistical analysis was performed using SPSS software version 22.0 (IBM, Armonk, NY, USA). Patient characteristics and clinico-pathologic parameters were evaluated with the Chi Square test. The expression levels of the 10 target genes in patients with chemosensitivity and chemoresistance were analyzed using the Mann-Whitney U test with Bonferroni correction test. The comparisons of OVRS scores between chemo-sensitive and resistant, as well as between patients relapsing and those not relapsing, and between patients who died and those who did not, were also performed by Mann-Whitney U test. Univariate and multivariate Cox's regression model was used to analyze the risk score and risk factors for chemoresistance. Next, the performance of OVRS scores for chemoresistance, disease relapse, and disease-related death were estimated using a receiver operating characteristic (ROC) curve. We compared the DFS and OS between women with OVRS below the mean versus those with OVRS above the mean by Kaplan-Meier log rank test. A p-value less than 0.05 was considered to be statistically significant.

8. Ethics approval details

This study was approved by the Institutional Research Ethics Committee at the National Taiwan University Hospital (approval No. 201907088RIN). All of the patients' data were fully anonymized before we accessed them and the Research Ethics Committee waived the requirement for informed consent.

RESULTS

1. Clinicopathological characteristics of the EOC patients

A total of 149 ovarian carcinoma patients were enrolled in this study, including 75 chemosensitive patients and 74 chemoresistant patients. These 149 patients were matched by age, surgical status, histology type, FIGO stages, and tumor grades. The clinico-pathological characteristics of these 149 patients are displayed in **Table 2**. Except for the rates of recurrence and disease-related death, characteristics such as FIGO stages or residual tumor size after debulking surgery were not significantly different between chemosensitive and chemoresistant patients.

2. Expression levels of the 10 target genes between chemosensitive and chemoresistant patients

The expression levels of the 10 target genes (*GPC1*, *CYPB*, *MSLN*, *LIMK2*, *DOCK4*, *STK31*, *IGF1*, *CHI3L1*, *Survivin*, and *CBAP*) were analyzed by qRT-PCR. Representative qRT-PCR figures for the 10 target genes are shown in **Supplementary Fig. 1**. The median expression levels of the genes were significantly lower in the chemosensitive group than in the chemoresistant group (*MSLN* 41.95 vs. 152.53, $p=0.041$; *GPC1* 0.19 vs. 0.80, $p=0.012$; *CYPB* 23.28 vs. 75.77, $p\leq 0.01$; *LIMK2* 2.01 vs. 8.74, $p<0.01$; *DOCK4* 0.13 vs. 0.98, $p\leq 0.01$; *STK31* (0.17 vs. 1.09, $p<0.01$); *IGF1* 1.44 vs.

Table 2. Clinicopathological characteristics of 149 epithelial ovarian carcinoma patients

Characteristics	Chemotherapy		p-value*
	Sensitive (n=75)	Resistant (n=74)	
Age (years)			0.63
<50	11 (14.7)	13 (17.6)	
≥50	64 (85.3)	61 (82.4)	
Residual tumor size after debulking surgery			0.16
<1 cm	43 (57.3)	34 (45.6)	
>1 cm	32 (42.7)	40 (54.1)	
Histology			0.27
Serous	57 (76.0)	54 (73.0)	
Endometrioid	9 (12.0)	5 (6.8)	
Clear cell	9 (12.0)	13 (17.6)	
Mixed type [†]	0 (0.0)	2 (2.7)	
FIGO stage			0.27
III	61 (81.3)	65 (87.8)	
IV	14 (18.7)	9 (12.2)	
Tumor grade			0.21
I	5 (6.7)	1 (1.4)	
II	2 (2.7)	1 (1.4)	
III	68 (90.7)	72 (97.3)	
CA-125 (U/mL)			0.28
Median (range)	877.5 (5.3–9,892)	982.4 (5.3–36,908.3)	
Disease recurrence			<0.001
Yes	35 (46.7)	74 (100.0)	
No	40 (53.3)	0 (0.0)	
Disease-related death			<0.001
Yes	14 (18.7)	52 (70.3)	
No	61 (81.3)	22 (29.7)	

Data are presented as number (%).

*Chi-square test; [†]Mixed type: mixed endometrioid and serous carcinoma.

9.39, $p < 0.01$; *CH13L1* 3.02 vs. 17.80, $p = 0.013$; *Survivin* 0.94 vs. 3.12, $p = 0.036$; *CBAP* 0.63 vs. 2.93, $p < 0.01$) (all by Mann-Whitney U test by Bonferroni correction) (**Supplementary Table 1**).

3. OVRS was correlated with chemo-resistance, disease relapse, and disease-related death

The individual risk scores for the 149 patients were calculated by summing the respective scores of the 10 target genes. The OVRS ranged from 0 to 20 (**Fig. 1A**). The median OVRS was significantly lower in the chemosensitive than in the chemoresistant group (5 vs. 15, $p < 0.001$, Mann-Whitney U test).

The distributions of OVRS for disease relapse and disease-related death are shown in **Fig. 1B and C**. The patients with disease relapse (13 vs. 5, $p < 0.001$, Mann-Whitney U test) or disease-related death (13.5 vs. 6, $p < 0.001$, Mann-Whitney U test) had higher median OVRS than those without.

4. ROC curves for determining prediction performance of OVRS

We then calculated the area under the ROC (AUROC) curve to assess the discrimination of OVRS in chemoresistance, disease relapse, and disease-related death. The maximal value of the Youden index was determined as the optimal cutoff point. The AUROC was defined as providing outstanding, excellent, or acceptable discrimination if it was higher than 0.8, between 0.6 and 0.8, or between 0.4 and 0.6, respectively. The AUROC of the OVRS was 0.803 for chemoresistance (Youden index 0.531 and cut-off value of OVRS 8.5), 0.75 for

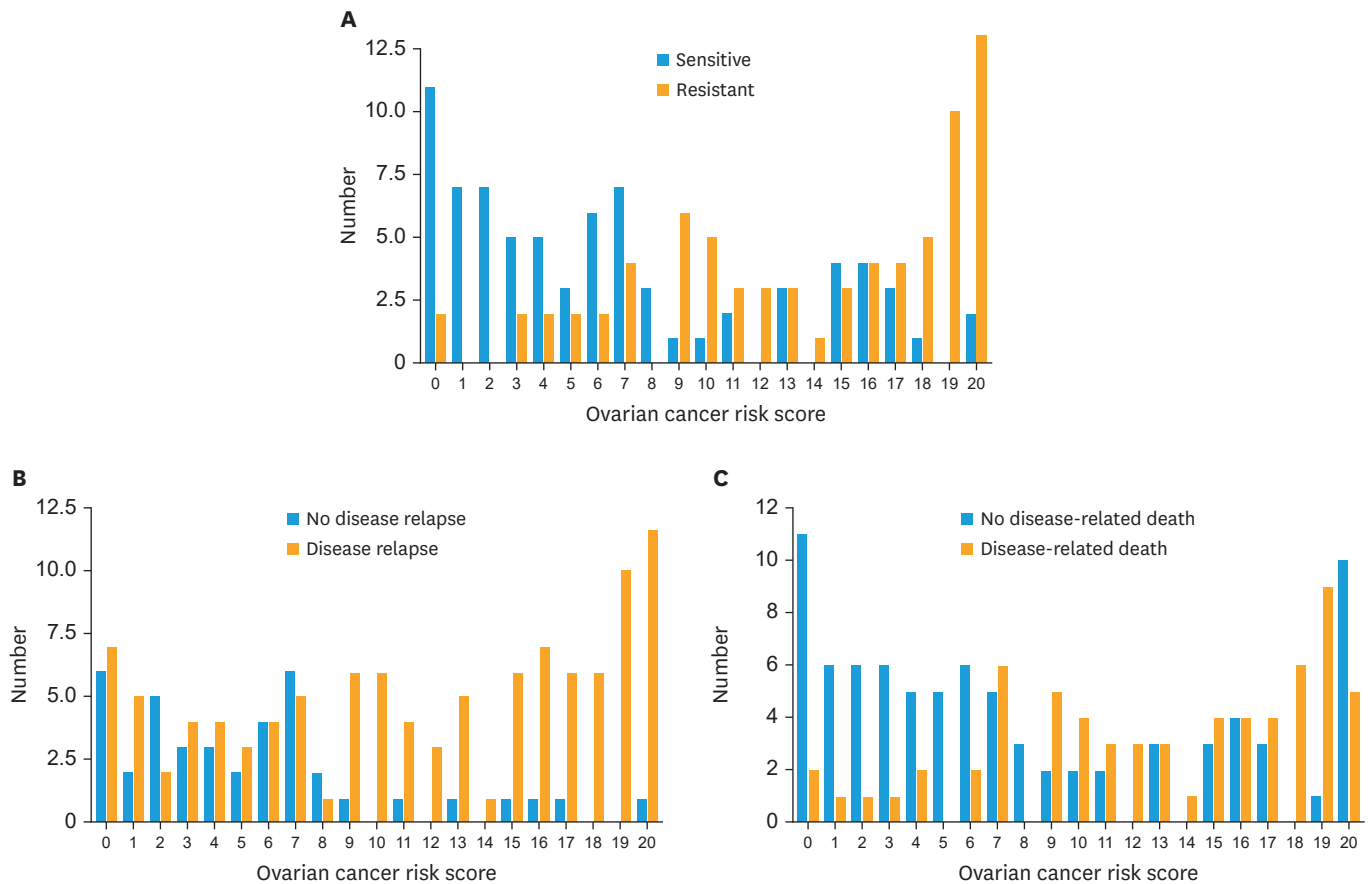


Fig. 1. (A) Distribution of OVRS of 149 epithelial ovarian carcinoma patients in different conditions. (A) Chemosensitivity and chemoresistance. (B) Disease relapse. (C) Disease-related death. OVRS, ovarian cancer risk score.

disease relapse (Youden index 0.504 and cut-off value of OVRS 8.5), and 0.71 for disease-related death (Youden index 0.411 and cut-off value of OVRS 8.5) (**Fig. 2A-C**).

5. OVRS was the only poor prognostic factor of chemoresistance and correlated with EOC patient outcome

We further evaluated the prognostic factors of chemoresistance by the various clinicopathologic parameters and the OVRS score. The prognostic factors of chemoresistance were evaluated in univariate and multivariate analyses as shown in **Table 3**. OVRS ≥ 10 (hazard ratio=3.29; 95% confidence interval=1.94–5.58; $p < 0.001$) was the only predictor for chemoresistance in multivariate analysis.

The Kaplan-Meier log rank method was used to investigate whether OVRS predicted survival in EOC patients. Because the median OVRS score of the 149 patients was also 10, so we divided the patients into two groups (above or below the mean score 10). The DFS and OS of 149 patients according to their OVRS are shown in **Figure 3**. The median DFS and OS were significantly reduced in EOC patients with a high risk score (OVRS ≥ 10) compared with those with a low risk score (DFS: 5 vs. 24 months, $p < 0.001$; OS: 39 vs. >60 months, $p < 0.001$, by log rank test).

Patients who had residual tumor size >1 cm after debulking surgery had worse survival with OVRS ≥ 10 (DFS: 5 vs. 29 months, $p < 0.001$; OS: 35 vs. >60 months, $p = 0.001$, log rank test)

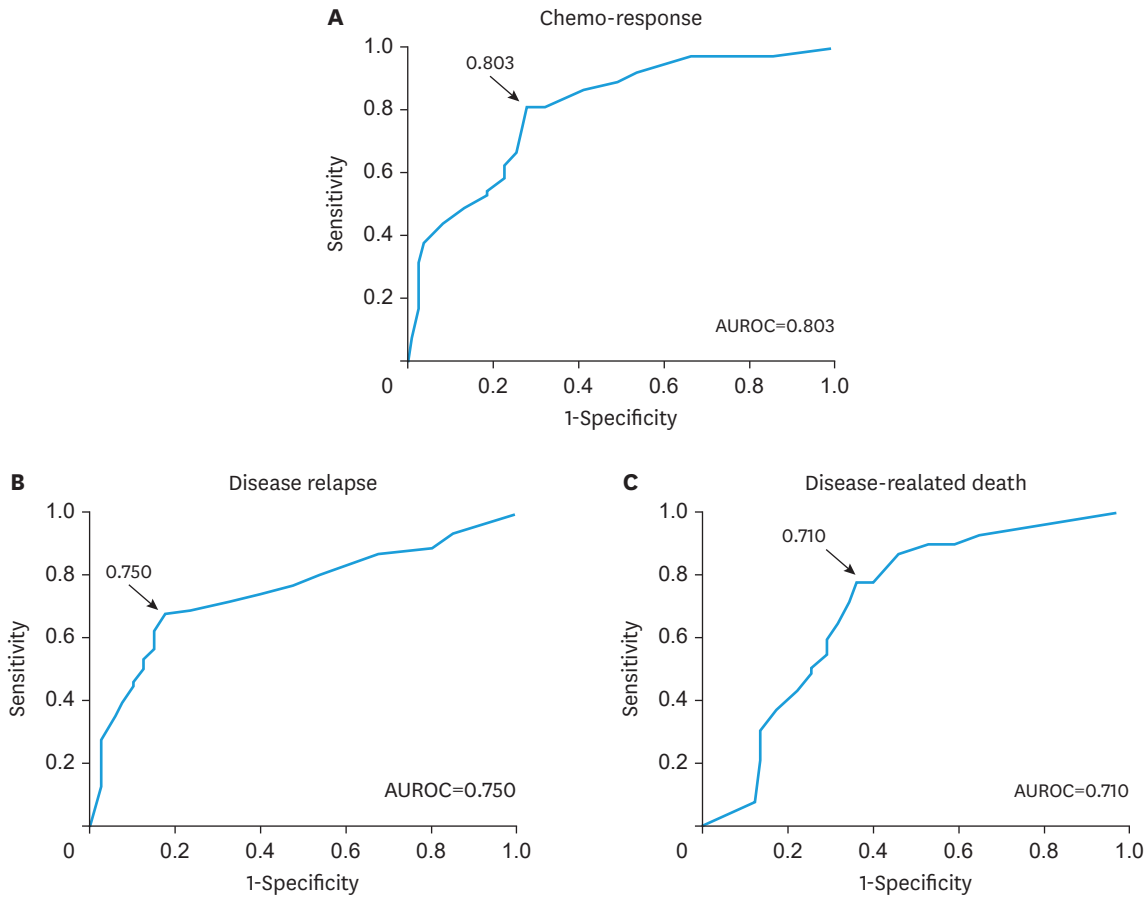


Fig. 2. The receiver operating characteristic curves of 149 epithelial ovarian carcinoma patients using ovarian cancer risk score=8.5 in different condition. (A) Chemoresistance. (B) Disease relapse. (C) Disease-related death. AUROC, area under the receiver operating characteristic curve.

Table 3. Cox proportional hazards model of risk factors for chemoresistance of 149 epithelial ovarian carcinoma patients

Characteristics	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (yr)				
<50	1		1	
≥50	0.81 (0.44–1.47)	0.49	0.81 (0.44–1.50)	0.50
Residual tumor size after debulking surgery				
≤1 cm	1		1	
>1 cm	1.36 (0.86–2.15)	0.20	1.36 (0.81–2.27)	0.24
Histology				
Serous	1		1	
Non-serous	1.19 (0.7–2.03)	0.51	1.72 (0.93–3.19)	0.08
Tumor grade				
Grade I	1		1	
Grades II/III	7.13 (0.99–51.4)	0.051	3.39 (0.79–14.6)	0.10
OVRs				
<10	1		1	
≥10	3.75 (2.23–6.29)	<0.001	3.29 (1.94–5.58)	<0.001

CI, confidence interval; HR, hazard ratio; OVRS, ovarian cancer risk score.

(**Supplementary Fig. 2A and B**). Patients with OVRS ≥10 had dismal outcomes even with tumors that were thoroughly and optimally debulked (DFS: 5 vs. 14 months, p=0.001; OS: 41 vs. >60 months, p=0.001, log rank test) (**Supplementary Fig. 3A and B**).

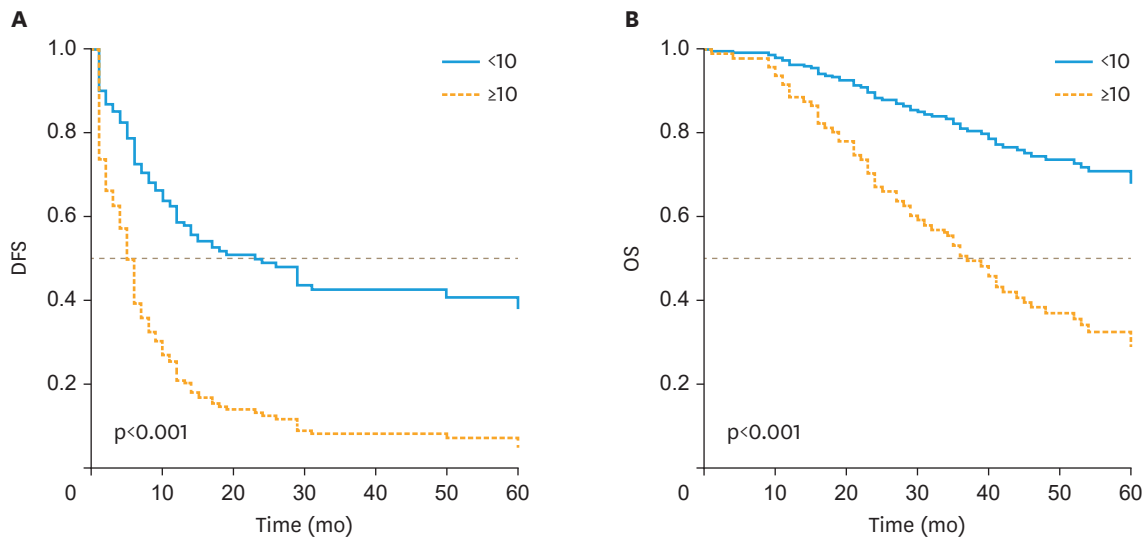


Fig. 3. (A) The DFS curves of 149 EOC patients with low and high OVRs. (B) The OS curves of 149 EOC patients with low and high OVRs groups. (The cutoff score of OVRs was 10). AUROC, area under the receiver operating characteristic curve; DFS, disease-free survival; EOC, epithelial ovarian cancer; OS, overall survival; OVRs, ovarian cancer risk score

6. Validation of OVRs in the TCGA Cohort

The OVRs prediction model was subsequently validated in the TCGA cohort. The TCGA cohort consisted of 255 EOC patients, including 245 chemosensitive and 10 chemoresistant patients for whom all 10 target genes were represented by RNA-Seq data in TCGA. The distributions of OVRs in the 245 chemosensitive and 10 chemoresistant patients are shown in **Supplementary Fig. 4A**. The median OVRs score of the 245 patients was 10. The OVRs did not differ significantly between the chemosensitive (median score=11, range 7–13) and chemoresistant (median score=10, range=2–19) groups ($p=0.73$, Mann-Whitney U test).

We also divided the patients into two groups (above or below the median score, ≥ 10 vs. < 10). Patients with a high risk score (OVRs ≥ 10) had significantly shorter median OS compared to those with a low risk score (OVRs < 10) (39 vs. 49 months, $p=0.046$, log rank test) (**Supplementary Fig. 4B**).

DISCUSSION

We selected 10 target genes involved in cell growth, regulation, apoptosis, and/or cancer progression as a panel to test for correlation with the chemosensitivity and chemoresistance of ovarian cancer patients. We then developed a scoring system we named the OVRs and calculated the score of each EOC patient to evaluate their chemoresistance, disease recurrence, and outcome. Finally, the OVRs was validated using the TCGA database of ovarian cancer patients. Our results found that patients with high OVRs were chemoresistant, and worse prognosis. Therefore, the OVRs can be a useful biomarker to predict chemoresistance and outcomes of epithelial ovarian carcinoma patients.

Currently, CA125 expression is used to monitor the treatment response of ovarian cancer patients [28]. However, its specificity is low and some histological types, such as a mucinous or clear cell carcinoma, do not express CA125 [28]. There is also evidence that mesothelin

expression is correlated with chemotherapeutic response and promotes chemoresistance through inhibiting paclitaxel-induced cell death [10,11]. The presence of BRCA 1/2 mutations was associated with a better chemotherapy response [29]. Cancer/testis antigen 45 is another protein linked to DNA-damage signaling and was identified as a platinum-sensitive regulator [30]. All these molecules have been investigated in the past decade in the hope of developing effective biomarkers and/or targeted therapies besides standard treatment algorithms. Immunotherapy of ovarian cancer is another promising treatment strategy, as ovarian cancer cells express immunogenic tumor-associated antigens [31]. Ovarian cancer is a heterogeneous disease and encompasses distinct histologic subtypes, molecular features, and genetic events [32]. Biomarkers have been variably associated with clinico-pathological factors and disease prognosis.

Some of clinico-pathological, molecular and genetic factors could also determine the prognosis of ovarian cancer including BRCA gene, hepatoceliac or mesenteric lymph node involvement, and so on [33-35]. An individual biomarker that best predicts chemo-sensitivity has not been found. Thinking the combination of several biomarkers could be more informative, we developed the OVRS, which comprises the 10 genes identified from our prior work. Analysis of the clinico-pathologic parameters and survival data for 255 tumor samples in the TCGA ovarian serous cystadenocarcinoma dataset served as external validation of the OVRS. A high OVRS uniformly predicted poor survival of patients in the TCGA database.

The current standard treatment of ovarian cancer is cytoreductive surgery followed by platinum-based chemotherapy and maintenance therapy. Maintenance therapy with PARP inhibitors extended survival substantially in newly diagnosed and recurrent ovarian patients exhibiting alterations in the homologous recombination repair pathway [36]. Treatment with anti-angiogenic agents such as bevacizumab in the first-line and maintenance treatment of ovarian cancer has also been evaluated, and it lengthened the disease-free (chemotherapy-free) interval [37]. However, molecular markers, including BRCA1/2, homologous recombination repair pathway mutations, and the endothelial cell protein CD31 could not be a good biomarker to predict the response to anti-angiogenic drugs. The OVRS has the potential to predict bevacizumab efficacy in our future studies. Antibodies targeting the products of the 10 OVRS genes are of potential interest in the future treatment of epithelial ovarian carcinoma patients.

There are two major limitations in this study that could be addressed in future research. First, the design of our study is retrospective with a matched cohort to examine the difference in expression of 10 target genes. Pathways incorporating these genes have not been thoroughly studied and the genes may be functionally interrelated. Second, the optimal OVRS cut-off point identified in the TCGA dataset was different from that in our study cohort. This may be due to the two cohorts having distinct clinico-pathological characteristics, such as the rate of residual tumor size ≤ 1 cm after debulking surgery (NTUH 51.7% vs. TCGA 74.1%), proportion of chemoresistant patients (NTUH 49.7% vs. TCGA 3.9%), percentage of patients with recurrent disease (NTUH 73.2% vs. TCGA 58%), and the distribution of overall scores. Besides, our cohort recruited patients with different histologic types including serous, endometrioid, and clear cell histologies. The TCGA cohort only recruited patients with serous histology. We analyzed the 111 women with serous histology alone in our cohort. As shown in **Supplementary Fig. 5**, 54 chemo-resistant women of serous histology had higher median OVRS than those 57 chemo-sensitive women (5 vs. 16, $p < 0.001$, Kruskal Wallis test). Besides, Women of serous histology with OVRS ≥ 10 had significantly shorter DFS (6 vs. 18 months,

p<0.001, **Supplementary Fig. 5B**) and OS (41 vs. >60 months, p=0.002, **Supplementary Fig. 5C**) than those with OVRS <10. Despite these variables, the results from the TCGA dataset tended in the same direction like ours. Our study revealed that the OVRS developed from this study could have potential to apply in different histologies of ovarian cancer.

The ROC of the OVRS alone in this survey was 0.803 for chemotherapy response. Our previous study revealed that the ROC curves for disease recurrence or disease-related death of ovarian cancer were 0.84 and 0.76, when using IL-17 and IL-21 [10]. Zhao et al investigated N-glycosylation patterns in ovarian cancer patients with different drug response and a panel of three increased glycans combined with CA125 could achieve an AUC value of 0.88 [38]. Another study which utilized a multiparametric biomarker panel by analyzing serum CA125, kallikreins, B7-H4, Spondin-2 could provide predictive accuracy with AUC of 0.82 [39]. The ROC curve could be improved from 0.75 to 0.91 when combining clinical variables such as stage and debulking [40]. It showed that the OVRS alone in this survey and the other studies had similar ROC. It will be worth to improve the ROC of OVRS when combined with the other clinical variables.

In conclusion, the OVRS has excellent correlation with chemoresistance and outcome for both our studied cohort and the patients in the TCGA database. Therefore, the OVRS can serve as a useful biomarker in clinically predicting the chemoresistance and outcomes of EOC patients.

ACKNOWLEDGEMENTS

This work was supported in part by the 7th Core Laboratory Facility of the Department of Medical Research of National Taiwan University Hospital. We would like to thank the cancer registries of National Taiwan University Hospital, And Tzu-Pin Lu for TCGA data analysis. We sincerely thank Prof. San-Lin You (Big Data Research Centre, Fu-Jen Catholic University) to provide the consultation of statistical methology and analysis.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Median expression levels* of 10 target genes between 75 chemo-sensitive and 74 chemo-resistant epithelial ovarian carcinoma patients

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Supplementary Fig. 1

Representative quantitative polymerase chain reaction figures for 10 target genes and a housekeeping gene. (A) *GAPDH*, (B) *MSLN*, (C) *GPC1*, (D) *CYPB*, (E) *LIMK2*, (F) *DOCK4*, (G) *STK31*, (H) *IGF1*, (I) *CH13L1*, (J) *Survivin*, (K) *CBAP*.

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Supplementary Fig. 2

- (A) Disease-free survival curves of patients with residual tumor size >1 cm after debulking surgery.
 (B) Overall survival curves of patients with residual tumor size >1 cm after debulking surgery.

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Supplementary Fig. 3

- (A) Disease-free survival curves of patients with residual tumor size ≤1 cm after debulking surgery.
 (B) Overall survival curves of patients with residual tumor size ≤1 cm after debulking surgery.

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Supplementary Fig. 4

- (A) Distribution of the OVRS in 255 chemosensitive and chemoresistant ovarian carcinoma patients in the TCGA database. (B) Overall survival curves of 255 ovarian carcinoma patients in the TCGA database in the low- and high-OVRS groups (The cutoff score of OVRS was 10).

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Supplementary Fig. 5

- (A) Distribution of OVRS of 111 serous ovarian carcinoma patients. Fifty four chemo-resistant women of serous histology had higher median OVRS than those 57 chemo-sensitive women (5 vs. 16, $p < 0.001$, Kruskal Wallis test). (B) The disease-free survival curves of 111 EOC serous patients with low and high OVRS. (C) The overall survival curves of 111 EOC patients with low and high OVRS groups (The cutoff score of OVRS was 10).

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