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Development of a five-gene signature as a novel prognostic marker in ovarian cancer

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The prognosis of ovarian cancer (OC) remains poor. Thus, the present study aims to identify independent prognostic factor in OC patients. OC gene expression studies GSE26712 and TCGA-OV were included in this study. Prognosis-associated differentially expressed genes (DEGs) between normal ovarian tissue and OC were identified. LASSO Cox proportional hazards regression model was conducted and a prognostic signature was constructed based on these DEGs. The predictive ability of the signature was analyzed in the training set and test set. The prognosis performance of the signature was compared with CA-125 and HE4. Gene set enrichment analysis (GSEA) was conducted to identify relevant mechanism. 332 DEGs were identified, out of which 64 DEGs were significantly correlated with the overall survival (OS) of OC patients, and 5 DEGs (IGF2, PEG3, DCN, LYPD1 and RARRES1) were applied to build a 5-gene signature. Patients in the 5-gene signature low-risk group had significantly better OS compared to those in the 5-gene high-risk group (p=0.0004) in the training set. Similar results were found in the test set, and the signature was also an independent prognostic factor. The prognosis performance of the 5-gene signature was significantly better than that of CA-125 and HE4. GSEA suggested that OC samples in the 5-gene high-risk group were significantly enriched in WNT/β-catenin signaling and epithelial-mesenchymal transition. We developed and validated a 5-gene signature that might be used as an independent prognostic factor in patients with OS.

Key words: ovarian cancer; prognostic signature; overall survival

Ovarian cancer (OC) represents the most lethal type of gynecological malignance and is a clinically heterogeneous disease as demonstrated through associations with family history of cancer, genetic risk and histopathology of this disease [1, 2]. Epithelial cancer accounts for about 95% of the OC [2]. Owing to the fact that nearly 70% of OC patients are diagnosed at stages III and IV according to the International Federation of Gynecology and Obstetrics (FIGO) and that more than 30% of OC patients will develop acquired chemoresistance and eventually relapse, the 5-year overall survival remains poor [3, 4]. Thus, developing novel prognostic tools to stratify seemingly identical patients and redirect them to more precise therapies is of great importance. There have been many recent improvements in the sequencing technology. Subsequently, a variety of OC gene expression studies have been published [5, 6]. Therefore, in this study we developed and validated a five-gene based prognostic signature for patients with OC. It has been reported that these five genes (IGF2[7], PEG3[8], DCN[9], LYPD1[10] and RARRES1[11]) were associated with survival and cell growth of multiple human cancers.

Materials and methods

OC gene expression studies. OC gene expression study GSE26712 [5] and TCGA-OV [12] were included in this study. GSE26712, which included 195 ovary tissue samples (10 normal, 185 malignant) was used as a training set. TCGA-OV, which included 564 patients whose survival time was fully documented, was used as a test set.

Data processing and analysis. Raw data of GSE26712 was downloaded from gene expression omnibus (GEO)

database and preprocessed and normalized using R "affy" package [13], and then the DEGs between normal ovarian tissue and OC were calculated using R package "limma" [14]. Genes at $|\log_2 FC|>2$ and adjusted $p<0.05$ were treated as DEGs. Log-rank based survival analyses were conducted to identify DEGs that were significantly correlated with the overall survival (OS) of patients with OC. LASSO Cox regression model was applied to select prognostic DEGs to predict the OS by 10-fold cross-validation and the risk scores for each patient were calculated using R package "glmnet" [15]. Time-dependent receiver operating characteristic curve (ROC) analysis was conducted to find the optimal cut-off and stratify OC patients into low-risk group and high-risk group in the training set and test set [16]. Thus, we constructed a prognostic signature on the basis of LASSO Cox regression model. Logistic regression model and Cox proportional hazards regression model were performed to analyze the relation between the clinical features of OC patients and the 5-gene signature and to identify prognostic factors in OC. Odds ratios (ORs) or hazards ratios (HRs) and associated confidence intervals (CIs) were calculated using maximum likelihood estimates, along with Wald test

Table 1. Characteristics of OC patients in the test set.

Variable	total number	Group		Logistic regression analysis			
		Low-risk	High-risk	OR	LCI	UCI	p-value
Age (year)							
<60	295	112	183	1.002	0.987	1.017	0.783
≥60	269	107	162				
Stage							
Early stage	46	26	20	0.872	0.778	0.974	0.016
Late stage	518	193	325				
Grade							
Grade 1	9	7	2				
Grade 2	69	28	41	0.719	0.481	1.068	0.103
Grade 3	476	178	298				
NA	10	6	4				

Abbreviations: OR, odds ratio; LCI, lower limit of confidence interval; UCI, upper limit of confidence.

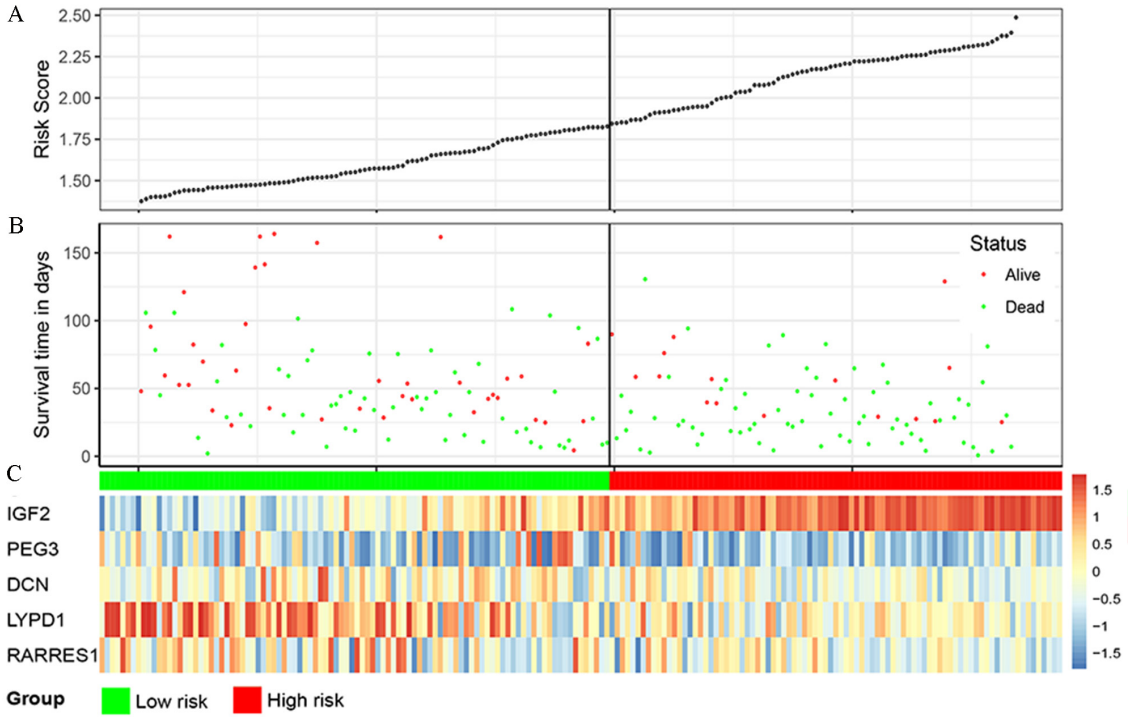

Figure 1. Characteristics of the 5-gene prognostic signature. A) the risk of each OC patients. B) the overall survival and survival status of each OC patients. C) heat-map of the 5 genes in the signature.

p-values. Thus, the prognostic role of the signature was investigated in the training set and test set. C-index, also known as concordance index, provides a global assessment of a fitted survival model. To evaluate the performance of the 5-gene signature, we compared the C-index of the 5-gene signature with other prognostic biomarkers (CA-125 and HE4) [17] using R package "survcomp [18]". Finally, to identify potentially relevant mechanisms that were associated with the OC patient survival, gene set enrichments analysis (GSEA) was conducted, and gene set at nominal p<0.05 and false discovery rate (FDR) <25% were treated as significantly enriched [19, 20].

Results

Characteristics of OC patients. A total of 185 high grade, advanced stage OC patients were included in the training set and the age of OC patients was not available. Meanwhile, a total of 564 OC patients were included in the TCGA-OV data set (the test set), of which 295 (52.3%) OC patients were younger than 60 years old and the remaining 269 OC patients were not younger than 60 years. Regarding the stage, 46 (8.2%) patients were early stage OC and 518 (91.8%) OC patients were advanced stage OC in the test set. As for the grade, 9 (1.6%) patients were grade 1 OC, 69 (12.2%) patients were grade 2 OC and 474 (84.4%) patients were grade 3 OC in the test set (Table 1).

Prognostic signature construction. As shown in Table S1, a total of 332 DEGs were identified between normal ovarian tissue and OC in the training set (Table S1). Then, 64 genes were significantly correlated with the OS of the OC patients using univariate Cox proportional hazards regression analysis (Table S2). We then constructed a 5-gene based prognostic signature using L1-penalized Cox proportional hazards regression on the training set (Figure 1, Table S3).

The prognostic role of the 5-gene signature in OC. We divided the OC patients into the 5-gene signature low-risk group and high-risk group on the basis of the cutoff (1.575) calculated using the time-dependent ROC analysis (Figure 2A). As shown in Figure 2B, patients in the 5-gene signature low-risk group had significantly better OS compared to those in the 5-gene high-risk group (HR= 0.5391, 95% CI: 0.3801–0.7646, p=0.0004).

Validation of the prognostic role of the 5-gene signature in the test set. To validate the predictive role of the 5-gene signature, we first performed logistic regression analysis. As shown in Table 1, the 5-gene signature was significantly correlated with the stage of OC patients (OR=0.872, 95% CI: 0.778–0.974, p=0.016, Table 1). The results of KM survival analysis suggest that the OS favors patients in 5-gene signature low-risk group over those in high-risk group (HR= 0.6186, 95% CI: 0.4849–0.7891, p=0.0001, Figure 3A) in the test set. Furthermore, although the 5-gene signature did not play a prognostic role in patients with early stage OC (HR= 0.4689, 95% CI: 0.1196–1.839, p=0.3, Figure 3B), a lower risk of signature was related with significantly better prognosis of patients with advanced stage OC (HR=0.6274, 95% CI: 0.4892–0.8047, p=0.0002, Figure 3C) in the set. Univariate and multivariable hazards regression analysis suggest that the 5-gene signature is an independent prognostic factor for OC (Table 2). Meanwhile, the results of Kaplan-Meier survival analysis suggest that lower expression of IGF2, DCN, LYPD1 and RARRES1 is associated with better OS in the training set and test set (Figure S1 and Figure S2).

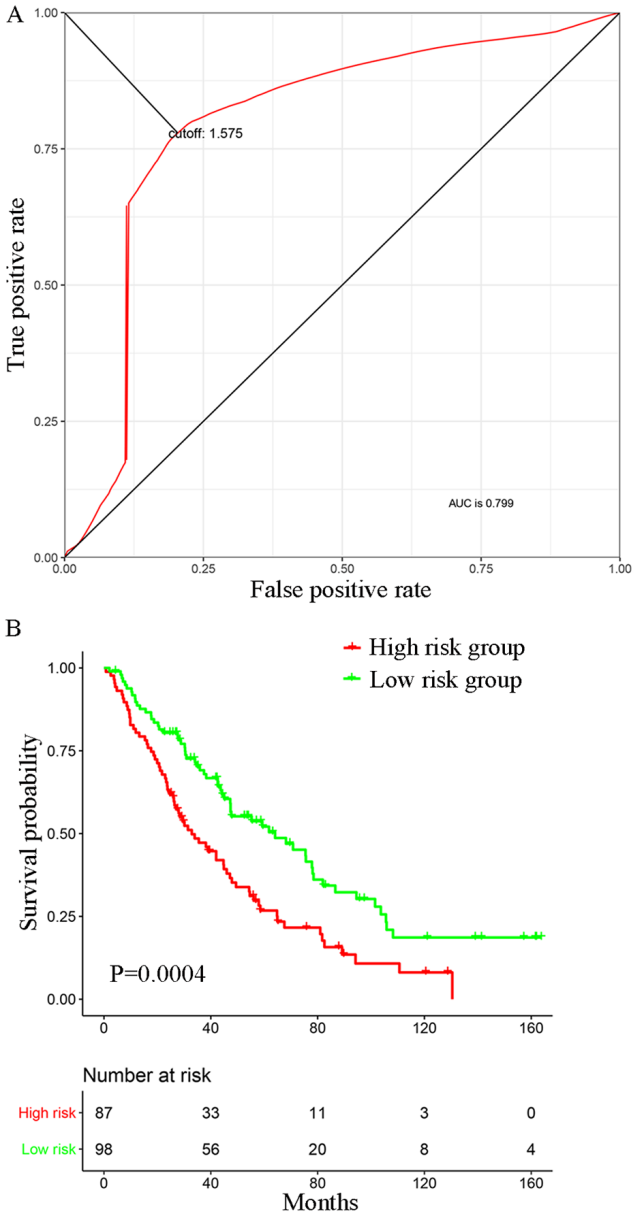

Figure 2. The prognostic role of the 5-gene signature in the training set. A) time-dependent survival ROC analysis. B) the overall survival of patients in low-risk group and high-risk group.

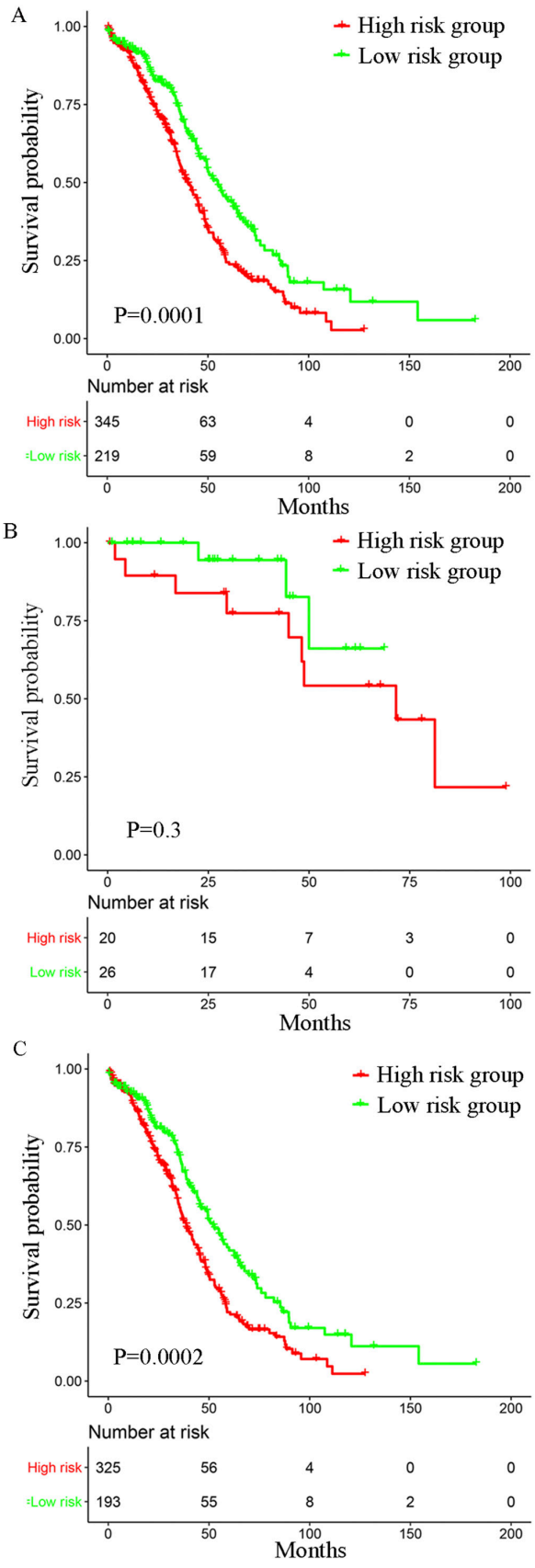

Figure 3. Validation of the prognostic role in the test set. A) the overall survival of patients in the whole population. B) the overall survival of patients with early stage OC. C) the overall survival of patients with advanced stage OC.

Comparison of the prognostic performance between the 5-gene signature and CA-125 and HE4. CA 125, also known as mucin 16 (MUC16), is a large membrane glycoprotein belonging to the wide mucin family and widely used as a tumor marker of OC [21]. Human epididymis protein 4 (HE4) is the FDC2 (HE4) gene product that has been treated as a new biomarker in OC[22]. Thus, we compared the prognosis performance of the 5-gene signature with CA-125 and HE4 in the TCGA ovarian cancer cohort (n=564). As shown in Figure 4, the C-index for the 5-gene signature is significantly higher compared to that for CA-125 (0.686 vs 0.539, p<0.001) and HE4 (0.686 vs 0.576, p<0.001) (Figure 4).

GSEA of OC samples. Finally, we conducted GSEA to find associated mechanisms confirming that the 5-gene signature affected the prognosis of patients with OC. As shown in Figure 5, OC samples in the 5-gene high-risk group were significantly enriched in WNT/β-catenin signaling (enrichment score: 0.514782, P: 0.024, FDR: 18.83%) and epithelial-mesenchymal transition (EMT) (enrichment score: 0.706814, p=0.0397, FDR: 5.07%).

Discussion

In this study, we identified DEGs between normal ovarian tissue and OC cells, identified prognostic DEGs correlated with the OS of OC patients, and a 5-gene signature was constructed after these prognostic DEGs were included into a Cox proportional hazards regression model combined with the least absolute shrinkage and selection operator. The prognostic role of the 5-gene signature was analyzed and validated in the training set and test set. Finally, GSEA was conducted to investigate potentially relevant mechanism.

Five genes in the prognostic signature were IGF2, PEG3, DCN, LYPD1 and RARRES1. In fact, there were several studies that have reported the 5 genes in the cancer pathogenesis and progression. Xu et al. suggested that the expression levels of IGF2 and CD133 were positively correlated with each other in primary ESCC [23] and that concurrent upregulation of IGF2 and CD133 expression was significantly related with poor patient prognosis. They were also found to be involved in colorectal cancer, liver cancer, adrenocortical carcinomas, etc. [7, 24, 25]. Meanwhile, Jiang et al. demonstrated that down-regulation of PEG3 stimulated beta-catenin pathway and promoted glioma cell growth, which was similar to the results of our GSEA showing that OC patients in the 5-gene high-risk group were significantly enriched in WNT/beta-catenin signaling pathway [26]. Li et al. demonstrated that DCN, accompanied by HSPD1, could be considered as a biomarker for colon cancer [27]. Xu Y et al. demonstrated that decreased expression of DCN promoted proliferation

Table 2. Univariate and multivariable Cox proportional hazards regression analysis on the overall survival of OC patients.

Variable	Univariate Cox proportional hazards regression analysis				Multivariable Cox proportional hazards regression analysis			
	HR	LCI	UCI	p-value	HR	LCI	UCI	p-value
Age	1.021	1.01	1.032	<0.001	1.021	1.011	1.032	<0.001
Stage	1.173	1.054	1.305	0.003	1.155	1.037	1.288	0.009
Grade	1.337	0.999	1.787	0.05	1.275	0.951	1.711	0.104
5-gene signature	3.484	1.187	10.23	0.023	3.842	1.289	11.459	0.016

Abbreviations: HR, hazards ratio; LCI, lower limit of confidence interval; UCI, upper limit of confidence interval.

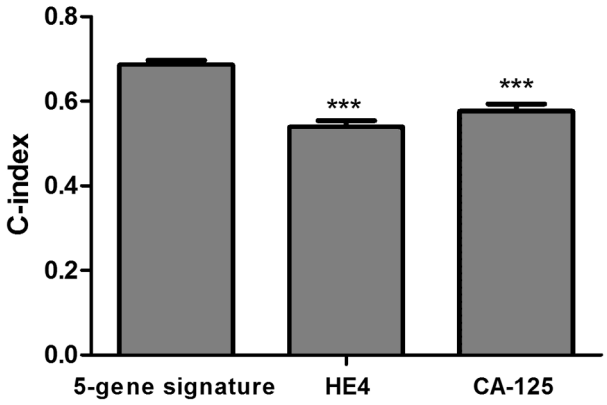

Figure 4. The C-index for the 5-gene signature, CA-125, and HE4. ***p<0.001

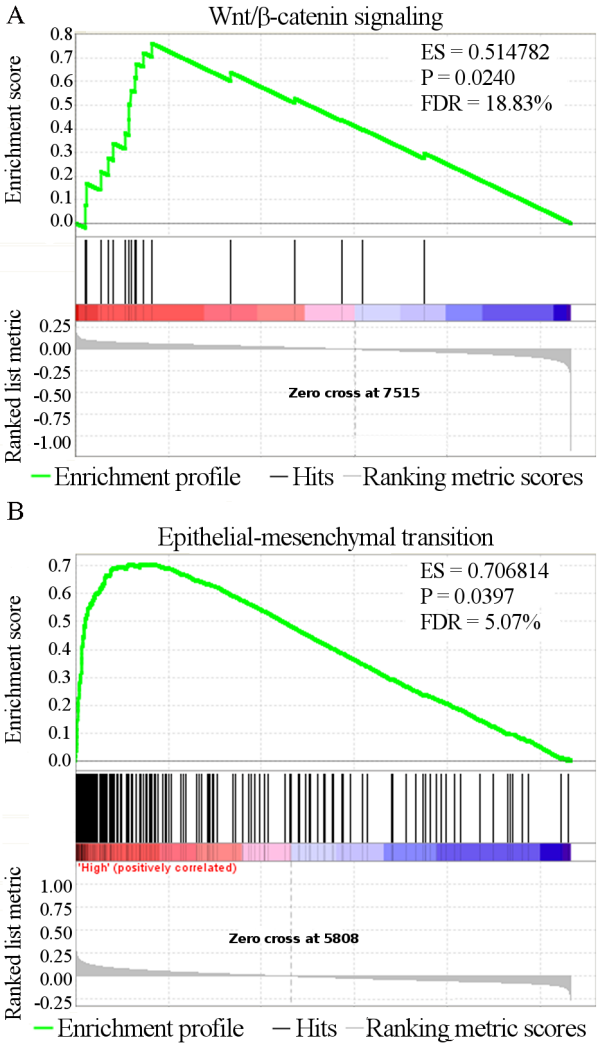

Figure 5. Gene set enrichment analysis of OC samples in the 5-gene signature low-risk group and high-risk group.

and metastasis of renal cell carcinoma cells [9]. Burnett et al. demonstrated that LYPD1 was up-regulated in breast cancer cells and was associated with the metastasis of the disease [28]. Oldridge et al. demonstrated that retinoic acid inhibited proliferation and invasion through inducting RARRES1 and LXN [29]. Wu et al. demonstrated that the expression of RARRES1 was significantly associated with tumor differentiation and staging in colorectal adenocarcinoma [11]. The above studies show that our signature might play an important role in the pathogenesis and progression of OC.

The result of GSEA suggest that the 5-gene signature might affect progression of the OC through WNT/β-catenin signaling and epithelial-mesenchymal transition. Wnt signaling was activated in epithelial OC and niclosamide inhibited the OC growth through suppressing WNT signaling. The Wnt signaling pathway plays a critical role in embryogenesis and oncogenesis. In the canonical Wnt signaling pathway, dysregulation of the Wnt/β-catenin signaling pathway has been identified in OC [30]. Mutations in the β-catenin (CTNNB1) gene leading to alteration of the Wnt/β-catenin signaling pathway have been found in the endometrioid subtype of OC [31, 32]. Aberrant accumulation of β-catenin is associated with increasing OC grade and poor survival [33, 34]. In contrast to canonical Wnt signaling, non-canonical Wnt signaling pathways may have transcriptional and non-transcriptional effects [34]. In the non-canonical Wnt/Ca2+ signaling pathway, Wnt ligands binding to Fzd receptors initiate activation of the phospholipase C via G protein-couple receptor signaling, causing an increase in intracellular Ca2+ and resulting in activation of Ca2+/calmodulin-dependent kinase II (CaMKII) and protein kinase C [35]. Meanwhile, previous studies have identified that deregulation of the Wnt/Ca2+ signaling pathway mediates cytoskeleton rearrangements, cellular

proliferation, cellular motility and epithelial-mesenchymal transition in cancer development and progression [36, 37].

Meanwhile, EMT has been found in multiple human cancers, especially in the metastasis process, where epithelial cells acquire increased motility and invasive properties to become mesenchymal like cells [38]. In OC, EMT promoted migration and invasion ability of the OC cells, contributed to chemoresistance and thus participated in the progression of the disease [39]. This could also explain the clinical role of the 5-gene signature in patients with OC to some extent.

Survival analysis on the 5-gene suggest that it could classify OC patients into high-risk group and low-risk group. Patients in low-risk group were associated with better clinical outcome compared with those in high-risk group. Although the conclusion was validated in an independent cohort, for the sake of caution we propose to conduct multicenter, large-scale clinical studies to validate our conclusions in the future.

In conclusion, we developed a 5-gene signature that might be used as an independent prognostic factor in patients with OC.

Supplementary information is available in the online version of the paper.

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