

Prognostic signature of ovarian cancer based on 14 tumor microenvironment-related genes

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Abstract

Background: Ovarian cancer is one of the lethal gynecological diseases in women. However, using tumor microenvironment related genes to identify prognostic signature of ovarian cancer has not been discussed in detail.

Methods: The mRNA profiles of 386 ovarian cancer patients were retrieved from The Cancer Genome Atlas. Univariate Cox regression and LASSO Cox regression analyses were performed and 14 optimized prognostic genes related to tumor microenvironment were identified.

Results: The multivariate Cox hazards regression showed risk score was an independent prognostic signature for ovarian cancer. Nomogram model could reliably predict the patients' survival. Furthermore, M1 macrophages, M2 macrophages, and follicular helper T cells, differentially expressed between the high- and low-risk groups, were found to be associated with the risk score.

Conclusion: CTL-associated antigen 4 (CTLA4) and indoleamine 2,3-Dioxygenase 1 (IDO1), which were previously shown to be important immune checkpoints, probably contribute to the immunosuppressive microenvironment aberration. This study may shed light on the prognosis of ovarian cancer.

Abbreviations: GEO = Gene Expression Omnibus, TCGA = The Cancer Genome Atlas, TME = tumor microenvironment.

Keywords: nomogram, ovarian cancer, prognosis, tumor microenvironment

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Availability of data and material: Our data and related clinical information were retrieved from The Cancer Genome Atlas (TCGA, <https://tcga-data.nci.nih.gov/tcga/>) and the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>).

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1. Introduction

Ovarian cancer is the most lethal gynecological malignancy,^[1] followed by cervical cancer and uterine body cancer, and its morbidity and mortality rates are still increasing.^[2] Malignant ovarian lesions include primary lesions and secondary lesions. In the primary lesions, epithelial ovarian carcinoma is the most common malignant ovarian tumor (70% of all ovarian malignancies), besides, the stromal tumors of the ovary, germ-cell tumors, sex-cord stromal tumors, and other more rare types are also included.^[3] Unfortunately, early ovarian cancer is minimal, nonspecific, or even asymptomatic due to its anatomy features,^[4] therefore, most cases are diagnosed at an advanced stage.^[5,6] Epithelial ovarian carcinoma has considerable complexity and heterogeneity in biology, drug response, and survival time, representing a major obstacle for its precision medicine.

Recent studies have shown that the tumor microenvironment (TME), composed of a variety of immune cells and stromal cells, is crucial in the occurrence and development of cancer and cell response to chemotherapy.^[7] At the same time, the rise of immunotherapy, including immune checkpoint inhibitor, shows that the assessment of TME heterogeneity and the remodeling of the immune microenvironment have broad application and profound impact on cancer treatment.^[8] Recently, some studies have investigated the TME-related markers for tumor prognosis. The study of Zeng et al^[9] describes the comprehensive features of gastric cancer TME-related genes and provides new strategies for cancer treatment. Another study indicates that many elements of TME other than tumor epithelial cells could influence the progression of non-small cell lung cancer.^[10] Consequently, new treatment strategies and paradigms are of great need for these patients. Unfortunately, only a few immune therapies have been

Table 1
Clinicopathological characteristics of OV patients from TCGA database.

Characteristics	OV patients (N = 375)	
	No.	%
Age		
≤59 (median)	198	52.80%
>59 (median)	177	47.20%
Race		
White	326	86.93%
Black or African American	24	6.40%
Asian	11	2.93%
American Indian or Alaska	2	0.53%
Native Hawaiian or other Pacific islander	1	0.27%
Unknown	11	2.93%
Pathologic stage		
I	1	0.27%
ii	24	6.40%
iii	293	78.13%
iv	57	15.20%
Survival time		
Long (>5 years)	75	20.00%
Short (<5 years)	300	80.00%
OS status		
Dead	230	61.33%
Alive	145	38.67%

OS=overall survival; OV=ovarian serous cystadenocarcinoma; TCGA=The Cancer Genome Atlas.

approved for ovarian cancer treatment. Therefore, the mining of TME-related genes in the immune microenvironment significantly changes the treatment prospects of ovarian cancer. TME-related genes may be a potential solution to the overwhelmed drug resistance and improve the clinical outcome of ovarian cancer patients.^[11]

In this study, we analyzed the expression of 760 TME-related genes in ovarian cancer patients. Meanwhile, we constructed a risk score model by hub TME-related genes to predict the prognosis of ovarian cancer patients, and evaluated the efficiency of this model in multiple levels, which provides new ideas for improving the prognosis of ovarian cancer patients.

2. Methods

2.1. Study population and TME-related genes

In the present study, 386 ovarian cancer patients' mRNA expression profiles and corresponding clinical information were retrieved from The Cancer Genome Atlas (TCGA, <https://tcga-data.nci.nih.gov/tcga/>). The patients with incomplete survival information were excluded. Among the TCGA cohort, 375 patients had complete survival information and were used in the following analysis. The detailed clinical information of these 375 patients was shown in Table 1. In addition, the datasets GSE26193 and GSE63885 were retrieved from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) database. GSE26193 contained the information of 107 ovarian cancer patients with complete survival information, and GSE63885 contained the information of 101 ovarian cancer patients, 78 of which had complete survival information. The mRNA levels of ovarian cancer samples in the 2 GEO datasets were quantified by the Affymetrix Human Genome U133 Plus 2.0 Array platform. The 760 TME-related genes were obtained based on previous studies^[12–16] (see Table, Supplemental Digital Content, <http://links.lww.com/MD/>

G248, which illustrates the information of the 760 genes related to tumor microenvironment).

2.2. Construction of prognostic model

Based on the expression levels of 760 TME-related genes, univariate Cox regression analysis was performed, and the genes with significant correlation with the prognosis of ovarian cancer were identified, with $P < .05$ considered as statistically significant. Then the LASSO Cox regression analysis was performed using glmnet package in R to further optimize the TME-related genes that were associated with the prognosis of ovarian cancer.^[17] The risk score of each sample was calculated with the optimized genes basing on the following formula:

$$\text{Risk score} = \sum_{i=1}^n \text{Coef}_i * X_i$$

In this formula, Coef_i is the risk coefficient of each factor calculated by the LASSO-Cox model, X_i is the expression value of each factor, and in this study it refers to the mRNA expression level of TME-related genes. Then, survival and survminer packages and 2-sided log-rank test were applied to determine the optimal cutoff value of risk score for stratifying the ovarian cancer patients. According to the cutoff value, patients were attributed into Low-Risk group and High-Risk group.

2.3. Survival analysis

Kaplan–Meier analysis was performed to estimate the overall survival rate of different groups using the survival package and survminer package. Log-rank test was performed to determine the difference in survival rate between distinct groups. Multivariate Cox regression model was used to analyze whether risk score could predict the survival of ovarian cancer patients independently of other factors.

2.4. Calculation of immune cell infiltration

We used CIBERSORT^[18] to calculate the relative proportion of 22 infiltrating immune cells in each sample. CIBERSORT characterizes the composition of infiltrating immune cells basing on deconvolution algorithm and 547 pre-set barcode genes according to the gene expression matrix. The sum of immune cell proportion in each sample was 1.

2.5. Establishment of nomogram model

Nomogram is widely used to predict the prognosis of cancer. In order to predict the 1-year, 3-year, and 5-year survival probabilities of ovarian cancer patients, the nomogram was established based on all the independent prognostic factors determined by the multivariate Cox regression by using the rms package in R. A calibration curve of the nomogram was drawn to compare the predicted overall survival probability of nomogram and the actual overall survival probability.

2.6. Statistical analysis

Kaplan–Meier method was used to estimate the overall survival rate of different groups. Log-rank test was used to analyze the significance of difference in survival rate between different groups. Wilcoxon signed-rank test was used to compare the difference in immune cell infiltration in different groups, with

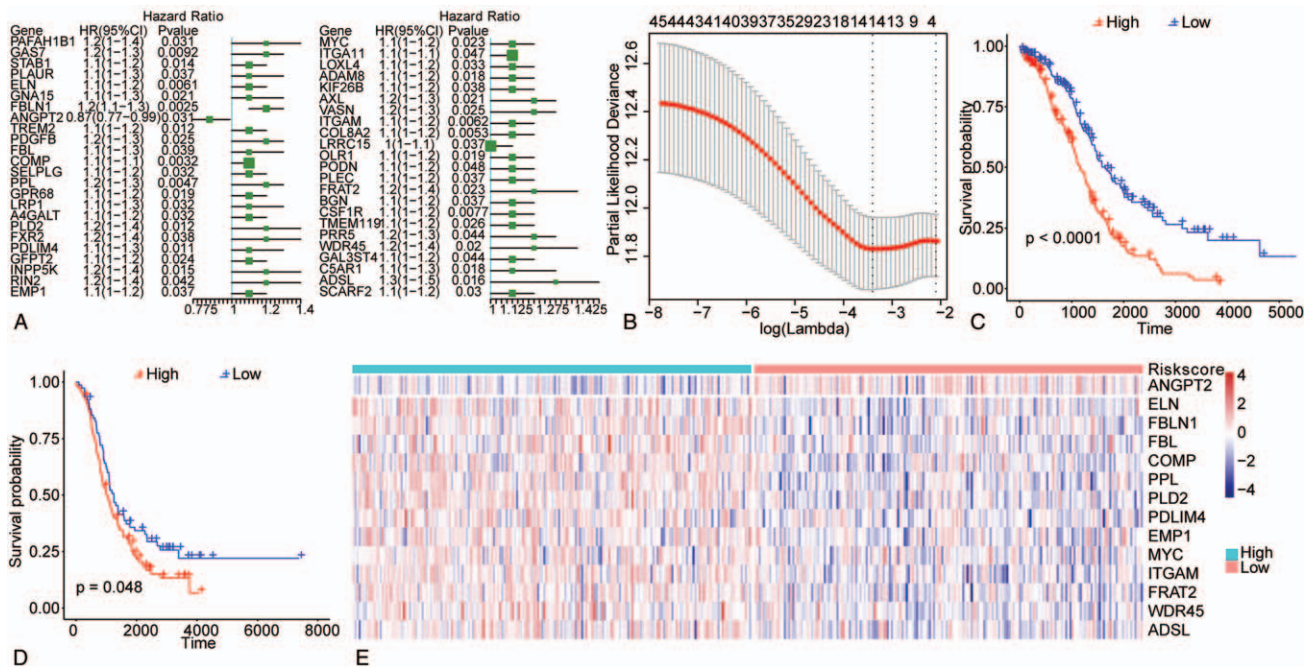


Figure 1. Construction of the prognostic model for ovarian cancer. (A) Univariate cox regression analysis of 48 tumor microenvironment (TME)-related genes that were significantly associated with the prognosis of ovarian cancer. (B) Determination of the tuning parameter lambda in the LASSO regression model. The horizontal axis was log (lambda), and the vertical axis was partial likelihood deviation. The lambda value corresponding to the smallest partial likelihood deviation value was the optimal, and the corresponding number on the top was the number of variables. (C) Kaplan–Meier survival curve in TCGA dataset. The horizontal axis was time and the vertical axis was survival rate. Different colors represented different groups. The P value was based on the log-rank test. (D) Kaplan–Meier survival curve in the meta-GEO dataset. (E) The heat map of the mRNA expression of the 14 selected genes in the high-risk and low-risk samples in the TCGA dataset. The horizontal axis was the sample, and the vertical axis was the gene. Red represented high expression, blue represented low expression, and the groups of the samples were marked with different colors on the heat map. 95% CI=95% confidence interval, HR=hazard ratio; GEO=Gene Expression Omnibus, TCGA=The Cancer Genome Atlas.

$P < .05$ as the threshold of statistical significance. R software (v3.5.2) was used for the statistical analysis of this study.

3. Results

3.1. Construction and verification of the prognostic model

Univariate Cox regression analysis was performed using the samples from TCGA database with the expression values of 760 TME-related genes as continuous variables, and the hazard ratio (HR) of each gene was calculated. A total of 48 genes were identified as prognostically relevant genes with P value $< .05$ as the threshold. The genes with HR value < 1 were protective genes that were conducive to the prognosis, and those with HR value > 1 were hazardous genes associated with unfavorable prognosis (Fig. 1A). LASSO Cox regression analysis was performed with the 48 identified genes. According to the lambda value which the gene number corresponded to in the LASSO Cox analysis, we selected 14 genes in the following analysis to achieve the smallest lambda value (Fig. 1B). The 14 genes were ELN, FBLN1, ANGPT2, FBL, COMP, PPL, PLD2, PDLIM4, EMP1, MYC, ITGAM, FRAT2, WDR45, and ADSL.

In order to obtain a unified cut-off value for the stratification of ovarian cancer patients, the expression values of these genes were normalized to the data with the average value of 0 and standard deviation (SD) value of 1. Then, the normalized expression value of each TME-related gene is weighted with the regression coefficient of LASSO Cox regression analysis to establish a risk score model

for predicting patients' prognosis basing the data of TCGA cohort. Risk Score = $(0.032648133 \times ELN) + (0.050148701 \times FBLN1) + (-0.182538141 \times ANGPT2) + (0.022643331 \times FBL) + (0.008810390 \times COMP) + (0.037391649 \times PPL) + (0.029721887 \times PLD2) + (0.055455835 \times PDLIM4) + (0.018301503 \times EMP1) + (0.008366183 \times MYC) + (0.039140799 \times ITGAM) + (0.018056371 \times FRAT2) + (0.032383440 \times WDR45) + (0.046671263 \times ADSL)$. Base on this formula, the risk score of each patient in the TCGA dataset and meta-GEO verification set (the combination of 2 GEO datasets) was calculated. And according to the optimal cut-off point (0.00515), these patients were divided into a high-risk group and a low-risk group. Survival analysis showed that in the TCGA and meta-GEO datasets, the high-risk ovarian cancer samples had poorer survival compared with the low-risk samples (Fig. 1C and D). In addition, significantly differential expression levels of these 14 genes between the high- and low-risk groups in TCGA cohort were observed (Fig. 1E). In conclusion, the risk score based on the 14 genes, ELN, FBLN1, ANGPT2, FBL, COMP, PPL, PLD2, PDLIM4, EMP1, MYC, ITGAM, FRAT2, WDR45, and ADSL, could predict the prognosis of patients with ovarian cancer.

3.2. Risk score is an independent prognostic marker for ovarian cancer

Age, TNM stage, race, and risk score were adopted for multivariate Cox regression analysis to determine whether the risk score was an independent prognostic indicator (Fig. 2A). It was found that risk score and stage were still significantly

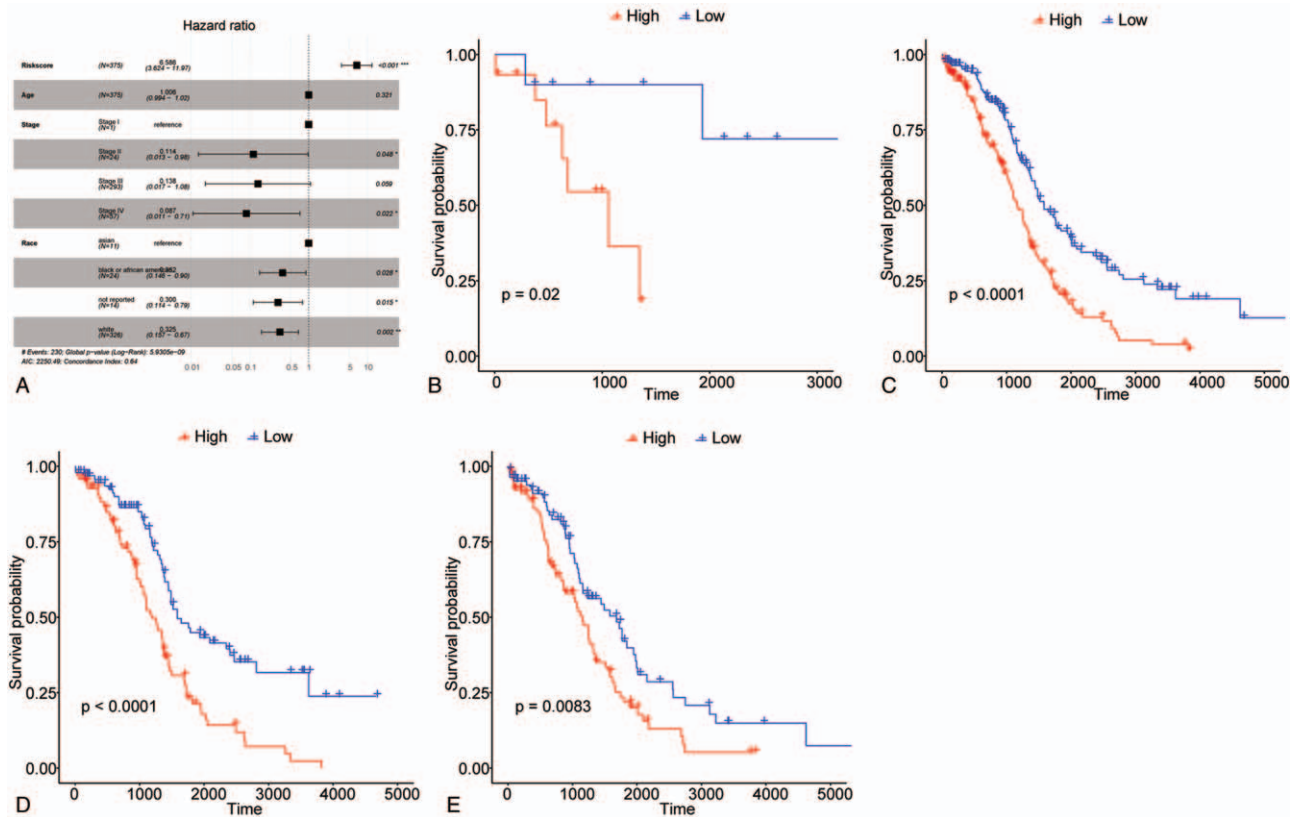


Figure 2. Risk score was an independent prognostic marker of ovarian cancer. (A) Forest map of multivariate Cox regression analysis. Compared with the reference sample, samples with a hazard ratio >1 had a higher risk of death, and samples with a hazard ratio <1 had a lower risk of death. (B and C) Kaplan–Meier survival curves of ovarian cancer samples with different stages. (D and E) Kaplan–Meier survival curves of ovarian cancer samples of different age groups.

correlated with the overall survival of ovarian cancer patients. Samples with a high risk score were at greater risk of death (HR = 6.33, 95% CI: 3.50–11.45, $P < .001$).

To further explore the prognostic value of risk score in ovarian cancer samples with different clinicopathological factors (including age and TNM stage), we regrouped the ovarian cancer patients according to these factors and conducted Kaplan–Meier survival analysis. It was found that, the overall survival rate of samples in the high-risk group was significantly lower than that in the low-risk group in early stage (Stage I + Stage II) and advanced stage (Stage III + Stage IV) samples (Fig. 2B and C); also, in samples ≤ 59 years old and samples >59 years old (Fig. 2D and E). These results indicated that the risk score could be used as an independent indicator to predict the prognosis of ovarian cancer patients.

3.3. Nomogram model could reliably predict the long-term survival of ovarian cancer patients

The 2 independent prognostic factors including stage and risk score, were used to construct the nomogram model (Fig. 3A). Three lines were drawn upwards to determine the points obtained from each factor in the nomogram for each patient. The sum of these points was located on the “Total Points” axis, and a line from the Total Points axis was drawn to determine the survival probability of ovarian cancer patients at 1, 3, and 5 years. The calibration curve was close to the ideal curve (a 45° line that

passed through the origin of the coordinate axis with a slope of 1), indicating that the predicted overall survival probability agreed well with the actual result (Fig. 3B, C and D).

3.4. Immune landscape of the ovarian cancer patients

We used the CIBERSORT software combined with the LM22 feature matrix to estimate the difference in infiltration of 22 immune cell types between high-risk and low-risk ovarian cancer patients. The immune cell infiltration in 375 ovarian cancer patients from TCGA were summarized in Fig. 4A. The change in the proportion of tumor infiltrating immune cells in different patients might represent the inherent characteristics of individuals. There were significant differences in the relative proportion of infiltrating immune cell types such as M1 macrophages between the high-risk and low-risk groups (Fig. 4B), and these 3 immune cell types were correlated with the risk score (Fig. 4C). Through principle component analysis (PCA) analysis, it was found that based on these 3 types of immune cells, samples within the low-risk and high-risk groups could be well separated (Fig. 4D).

The expression of immune checkpoints has become a biomarker for ovarian cancer patients to choose personalized immunotherapy.^[19] The correlation between the patient’s risk score and the key immune checkpoints [CTL-associated antigen 4 (CTLA4), PD1, indoleamine 2,3-Dioxygenase 1 (IDO1), TDO2, LAG3, TIGIT] was analyzed and it was found that the risk score was significantly correlated with them (Fig. 5A). Meanwhile, the

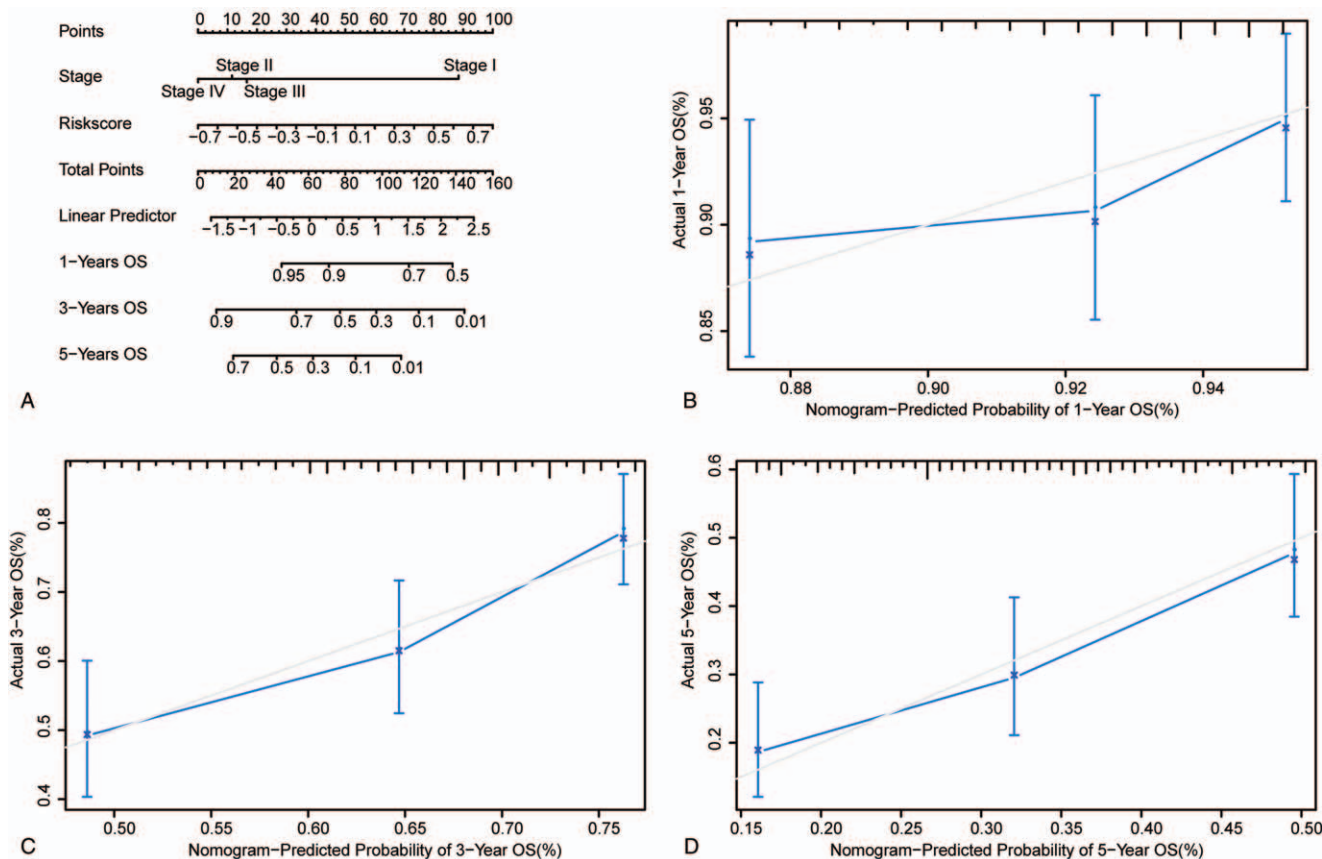


Figure 3. Establishment of nomogram to predict the survival of ovarian cancer patients. (A) Nomogram predicting the survival probability at 1, 3, and 5 years in ovarian cancer patients. (B–D) The calibration curve for the nomogram predicting the survival probability at 1, 3, and 5 years in ovarian cancer patients. The X axis represented the predicted survival rate of the nomogram, and the Y axis represented the actual survival rate.

expressions of CTLA4 and IDO1 among these 6 immune checkpoints were significantly different between the ovarian cancer patients within high-risk and low-risk groups (Fig. 5B). The result implicated that the poor prognosis of patients with high-risk ovarian cancer might be associated with the immune cell infiltration and immune checkpoints, however, further investigation is still needed.

4. Discussion

Ovarian cancer has the highest mortality rate among all gynecological malignancies. Nearly 80% of the patients are diagnosed after the occurrence of symptoms, and the disease has already progressed into a late stage.^[20] The focus of cancer treatment, especially cancer immunotherapeutic strategy, has shifted from killing the cancer cell by conventional cytotoxic chemotherapy towards re-building the immune system to eliminate cancer cells and prevent the recurrence and relapse of cancer.^[21] Here is a strong call for the methods to identify and develop clinically valuable gene signatures for ovarian cancer prognosis, especially the analyses basing on comprehensive and unbiased whole-genome data.

In this study, we first identified 48 TME-related genes associated with the prognosis of ovarian cancer in TCGA cohort. By using the LASSO Cox analysis, we finally selected 14 genes (ELN, FBLN1, ANGPT2, FBL, COMP, PPL, PLD2,

PDLIM4, EMP1, MYC, ITGAM, FRAT2, WDR45, and ADSL) to construct the risk score model for prognosis, and the scoring system showed strong discriminative power to separate patients with good or poor survival. Some of these 14 genes are already reported to contribute to ovarian cancers' pathophysiological features. For example, EMP1, belongs to the EMP family and encodes the glycoprotein with 4 conserved domains.^[22,23] EMP1 promotes the proliferation and invasion of ovarian cancer cells by activating the MAPK pathway.^[24] C-myc, a common over-activated oncogene by amplification, has been found in >70% of the advanced ovarian cancer patients.^[25,26] ITGAM is a potentially significant gene which plays a key role in the metastasis of high-grade serous ovarian cancer.^[27] In the ITGAM knockout mice, the tumor growth and immunosuppressive cytokine mRNA levels are enhanced.^[28,29] ANGPT2 is highly expressed in ovarian cancer tissues and cells, which promotes the intraperitoneal growth of ovarian cancer, contributing to the poor prognosis of mice infected by ovarian cancer cells.^[30] COMP encodes the extracellular matrix glycoprotein, and its increased expression is implicated in the development of epithelial ovarian cancer involving in the proliferation, migration, invasion, and apoptosis of ovarian cancer cells regulated by SNHG25.^[31,32] PDLIM4, a gene which is frequently suppressed in various cancers, is considered as a tumor suppressor.^[33] It has been shown that PDLIM4 expression is significantly decreased in ovarian cancer, which is related to the aggressive characteristics

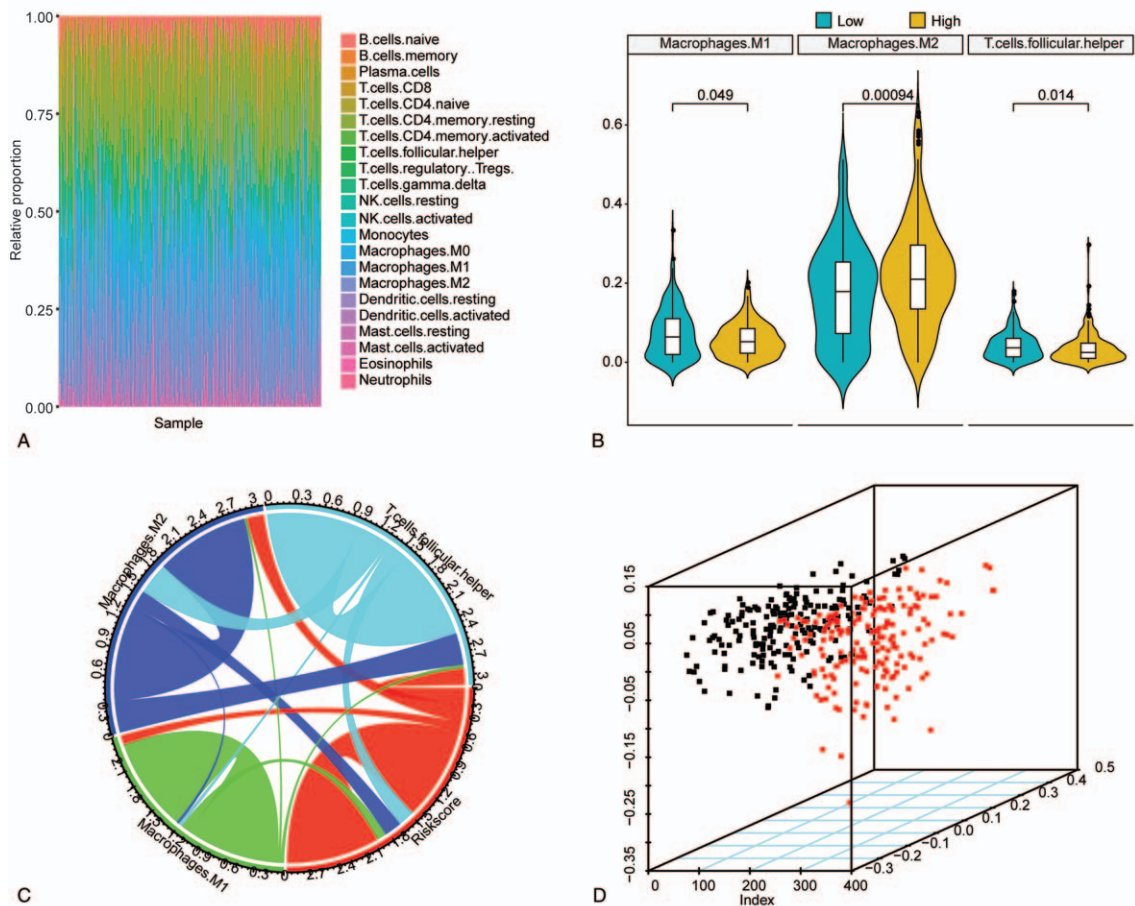


Figure 4. Immune infiltration in high-risk and low-risk ovarian cancer patients. (A) The relative proportion of infiltrating immune cells in all patients. (B) Immune cells with significant difference between the high-risk and low-risk groups. The horizontal axis was the high-risk and low-risk groups, and the vertical axis was the relative infiltration rate of immune cells. The *P* value was calculated by the Wilcoxon method. (C) The Chord diagram showing the correlation between the risk score and the three significantly different immune cell types between the high-risk and low-risk groups, the thicker link between them represented the stronger correlation. (D) The PCA analysis of the samples based on the 3 significantly different immune cell types between the high-risk and low-risk groups. The dots with different colors represented different types of samples.

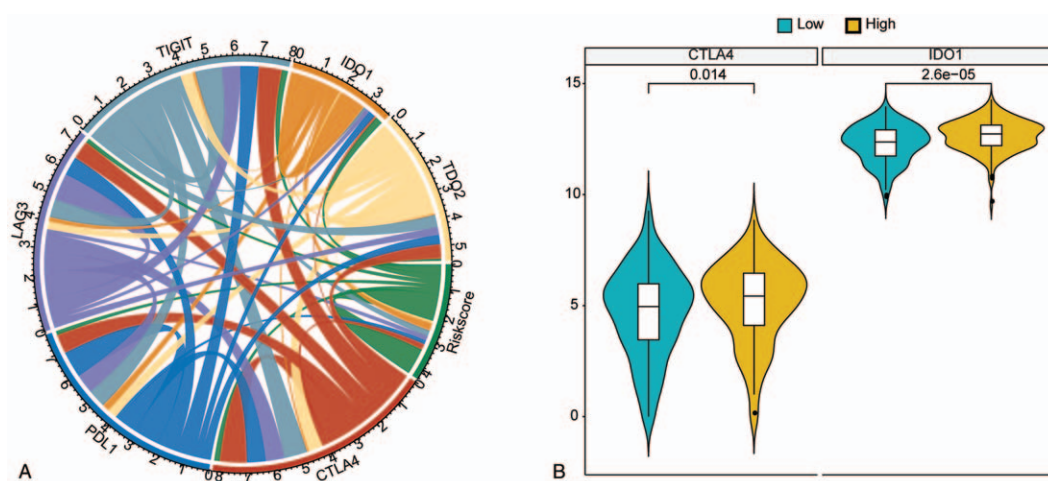


Figure 5. The relationship between important immune checkpoints and risk score. (A) The Chord diagram showing the correlation between the risk score and the expression of immune checkpoints. The thicker connection between them indicated the stronger correlation. (B) Violin charts of immune checkpoints with significantly different expression levels between the high-risk and low-risk groups. Different colors represented the high-risk and low-risk groups. The vertical axis was the expression level. The *P*-value was calculated using the Wilcoxon method.

of this disease such as the proliferation, migration, and invasion abilities of cancer cells, leading to an inferior prognosis.^[34] ELN is significantly differentially expressed between primary ovarian cancer and peritoneal metastatic ovarian cancers, indicating that ELN may be associated with the metastasis of ovarian cancer.^[35] PLD2 overexpression in ovarian cancer cells could increase the production of lysophosphatidic acid, a lipid mediator which enhances the motility and growth of ovarian cancer cells.^[36] Therefore, PLD2 may involve in ovarian cancer by regulating the generation of lysophosphatidic acid. For the remaining genes, although there are no direct researches on their association with ovarian cancer, most of them are reported to be associated with other cancers. FBLN1, located on chromosome 22 (22q13), is reported to be closely associated with the migrative, adhesive, and invasive features of tumor cells, and is adopted as a signature in multiple cancers.^[37] For example, the FBLN1 in serum is considered as a noninvasive biomarker in identifying colorectal cancer.^[38] FRAT2 is located on human chromosome 10q24.1 and could regulate the WNT signaling pathway.^[39] Increased expression of FRAT2 is observed in multiple cancers, such as the gastric cancer and lung cancer, which is probably associated with the regulation of WNT signaling pathway.^[40,41] WDR45 encodes the beta-propeller protein, and its mutation occurs in cancers including endometrial carcinoma and clear cell renal carcinoma.^[42,43] ADSL plays a key role in the de novo purine synthesis pathway, and is an oncogenic driver in several cancers, thus being considered as an essential therapeutic target in cancer.^[44] FBL encodes the nucleolar protein and enhances the proliferation of cancer cells through modulating mRNA translation and rRNAs methylation, which is considered as a potential oncogene in some cancers such as breast cancer and prostate gland cancer.^[45] These results also confirm the potential reliability of our study indirectly.

We analyzed the fractions of infiltrating immune cells in ovarian cancer patients stratified by the risk score. It was found that the M1 macrophages, M2 macrophages, and follicular helper T cells were associated with the risk score. Moreover, compared with the low-risk group, the high-risk group showed decreased infiltration proportions of M1 macrophages and follicular helper T cells, and increased infiltration proportion of M2 macrophages. Liu et al^[46] indicates obesity could promote the metastasis of ovarian cancer by down-regulating the infiltration of M1 macrophages, which may result in the poor survival outcome of the ovarian cancer patients. Lan et al^[47] has demonstrated the M2 macrophages infiltration and the activation effects on the transformation from macrophages to M2 macrophages probably lead to the inferior prognosis of ovarian cancer. Additionally, the infiltration of follicular helper T cells are proved to be correlated with superior prognosis in breast cancer patients.^[48] These researches are consistent with our result on the association between infiltrations of M1 macrophages, follicular helper T cells, M2 macrophages, and ovarian cancer prognosis.

As the immune checkpoints are instructive for personalized immunotherapy, also analyzed the key immune checkpoints in the ovarian cancer patients. It was found that the risk score was correlated to all key immune checkpoint including CTLA4, PD1, IDO1, TDO2, LAG3, and TIGIT. Additionally, CTLA4 and IDO1 were differentially expressed between the high-risk and low-risk ovarian cancer patients. The patients with high risk score had increased expression levels of CTLA4 and IDO1, indicating that the poor prognosis of high-risk ovarian cancer patients may be associated with the immune checkpoints, and immunotherapy targeting CTLA4 and IDO1 is a potential alternative for ovarian cancer treatment. Though the early study

showed CTLA4 blockade had minimal activity in ovarian cancer models,^[49] recent studies reported CTLA4 blockade could boost the expansion of tumor-reactive CD8+ tumor-infiltrating lymphocytes in ovarian cancer.^[50] In the present data, tailor targeted therapy against IDO1 may enhance the effectiveness of treatment. IDO1 inhibitors have been designed, screened, and tested in preclinical models of disease, but further verification in clinical trials is still required.

5. Conclusions

In the present work, we are concerned with the genetic characteristics of the microenvironment. Our systematic analysis and assessment demonstrate that 14 TME-related genes probably play a pivotal role in the prognosis of ovarian cancer patients, and the risk score based on these 14 genes may help to outline the prognosis of patients with ovarian cancer. However, although our results obtained from bioinformatics analysis exhibit promising performance, experimental validation is needed to further validate our results.

Author contributions

Conceptualization: Xiazi Nie, Bo Qu.

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Formal analysis: Xiazi Nie, Lina Song, Xiaohua Li, Yirong Wang, Bo Qu.

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Writing – original draft: Xiazi Nie, Bo Qu.

Writing – review & editing: Bo Qu.

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