Identification of a Glycolysis-Related Gene Signature for Survival Prediction of Ovarian Cancer Patients

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Primary research

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Abstract

Background: Ovarian cancer (OV) is deemed as the most lethal gynecological cancer in women. The aim of this study was construct an effective gene prognostic model for OV patients.

Methods: The expression profiles of glycolysis-related genes (GRGs) and clinical data of patients with OV were extracted from The Cancer Genome Atlas (TCGA) database. Univariate, multivariate, and least absolute shrinkage and selection operator Cox regression analyses were conducted, and a prognostic signature based on GRGs was constructed. The predictive ability of the signature was analyzed in training and test sets.

Results: Based on nine GRGs (ISG20, CITED2, PYGB, IRS2, ANGPTL4, TGFBI, LHX9, PC, and DDIT4), a gene risk signature was identified to predict the outcome of patients with OV. The signature showed a good prognostic ability for OV, particularly high-grade OV, in the TCGA dataset, with areas under the curve of 0.709, 0.762, and 0.808 for 3-, 5- and 10-year survival, respectively. Similar results were found in the test sets, and the signature was also an independent prognostic factor. Moreover, a nomogram combining the prediction model and clinical factors was constructed.

Conclusion: Our study established a nine-GRG risk model and a nomogram to better perform on OV patients’ survival prediction. The risk model represents a promising and independent prognostic predictor for OV patients. Moreover, our study of GRGs could offer guidance for underlying mechanisms explorations in the future.

1. Introduction

Among gynecological cancers, ovarian cancer (OV) is considered the most fatal. And it is estimated that the respective numbers of new cases and new deaths were 22,530 and 13,980 in the United States in 2019.\[1\] That the main challenges in effective methods for screening and predicting prognosis are attributed to the significant heterogeneity at clinical, histopathological and molecular levels of this disease.\[2\] And clinical and pathologic factors are not sufficient to predict long-term survival.\[3\] More and more opportunities for exploring tumor prognostic markers emerged, and that attributed to the establishment and development of public biological databases, which provides available gene expression data and clinical data of cancers. Many biomarkers, including the EN2 and HE4 genes, which are associated with the prognosis and survival of OV, have been identified currently.\[4–7\] With the rapid development of high-throughput sequencing, a variety of patient genome databases have been constructed so that researches can get more systematic understanding of genomic changes. By mining the database, thousands of prognostic biomarkers have been identified. \[8, 9\] Additionally, studies found that the genetic model constructed by multiple genes has better prediction performance for cancer prognosis than the single gene.\[9, 10\] Gene model constructed based on tumor biopsy has practical significance for the guidance of targeted therapy. Currently, several studies have explored to do establish multigene signatures for assessing OV patients’ survival risk and predicting clinical outcomes.\[8–11\]
Glycolysis takes place in all cells of the body.[12] A previous study reported that genes involved in glycolysis are ubiquitously overexpressed in 24 cancer classes.[13] So far, the relationships between glycolysis and the processes of cancers’ oncogenesis, development, proliferation and invasion have been the focus of many studies.[14–16] The results from previous studies provide compelling evidence of new glycolysis-related biomarkers for the prediction of cancer patients’ survival. Poor prognosis occurs in pancreatic cancer patients with high expression of TCF7L2 compared to those with low expression levels, and the involved mechanism behind it was that the positive regulation of TCF7L2 on aerobic glycolysis through the axis of EGLN2/HIF-1α.[17] Four glycolysis-related genes (GRGs; AGRN, AKR1A1, DDIT4, and HMMR) were also identified in a previous study and are strongly associated with clinical outcome of lung adenocarcinoma patients with lung adenocarcinoma.[18] The combination model of nine GRGs were identified to effectively predict overall survival (OS) of endometrial cancer.[19] In addition, a glycolytic gene expression signature score, established on the basis of 10 glycolytic genes (HK2, HK3, LDHA, PKM2, GAPDH, ENO1, LDHB, PKLR, ALDOB, and GALM), predicts unfavorable clinical outcomes of patients with glioblastoma and is closely associated with the mesenchymal subtype.[14] However, no signature based on GRGs has been established to predict survival in patients with OV.

In this study, we aimed to investigate specific GRG markers that had close associations with the survival of patients with OV by using data from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) database and to evaluate the prognostic value of the biomarkers for the prediction of patient survival in OV. An effective 9-GRG risk predictive model was constructed for predicting outcomes of OV patients. Noteworthily, the GRG risk model enabled identification of patients with poor prognoses in the high-risk group. The results acquired from the multivariate Cox regression analyses implied that our risk model was a predictor independent of clinical factors, and did better in the prediction of OV patients’ survival.

2. Materials And Methods

2.1. Data collection

We extracted clinical and RNA sequencing data of OV patients from TCGA (https://portal.gdc.cancer.gov/). The exclusion criteria were set as follows: (1) confirmed non-OV pathological diagnosis; and (2) OV patients with incomplete information regarding clinical characteristics (age, tumor stage, and histological grade, survival time and status). Finally, the covered clinical information of 583 patients was complete integrated. Other datasets (GSE63885, GSE26193, and GSE30161) from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) were selected as independent validation sets for validation of the robustness of the eight-DRG prognostic model. The GRG sets were obtained from the Molecular Signatures Database (MSigDB).[20] The gene list was obtained by synthesizing the genes of five related gene sets after excluding duplicate genes.

2.2. Construction and evaluation of the nine-GRG prediction model

We applied log2 transformation to standardize the expression of each gene. We used p value of < 0.05 as the screening criterion, and performed univariate Cox analysis, the least absolute shrinkage and selection
operator (LASSO) method lasso, [21, 22] as well as multivariate Cox regression analysis in order. Follow the criterion of Akaike information, the final selected GRGs related to prognosis are used to construct gene prediction models. We calculated the risk score using the following formula: Risk score 
\[ \text{Risk score} = \sum_{i=1}^{n} \text{coef} \times \text{id} \] [23] The Kaplan-Meier survival curve constructed using the R package “survival” [24, 25] demonstrated the OS of the high- and low-risk groups, which were stratified according to risk signature. The predictive performance and validity of the model was assessed by a time-dependent receiver-operating characteristic (ROC) curve, which was conducted by using the R package “survivalROC.” [24] Univariate and multivariate Cox regression analyses were performed to determine the prognostic values of the signature and some clinic-pathological features.

2.3. Statistical analyses

We compared the distribution of the clinical features using the chi-square tests, which included age, tumor stage, and histological grade, between the different subgroups. The alteration in selected genes is obtained from the cBioPortal website (http://www.cbioportal.org/). We made use of the R version 3.6.2 software to conduct the statistical analyses. When p value is less than 0.05, the statistical differences is deemed significant.

3. Results

3.1. Patient characteristics and collection of GRGs

From TCGA database, we downloaded the complete data of 583 OV patients which contained clinical information and the expression profiles of RNA sequencing. We manually searched for glycolysis-related gene sets from MSigDB version 6.2 and referenced relevant literature. Five related gene sets (REACTOME_GLYCOLYSIS, HALLMARK_GLYCOLYSIS, GO_GLYCOLYTIC_PROCESS, KEGG_GLYCOLYSIS_GLUCONEOGENESIS, and BIOCARTA_GLYCOLYSIS_PATHWAY) were downloaded, and 443 genes were obtained. After excluding duplicate genes, the corresponding 386 genes remained for the subsequent analysis. The integrated clinical data and list of the GRGs are shown in Tables 1 and S1, respectively.
Table 1
Clinic pathological characteristics of extracted patients with ovarian cancer.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th>No. of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>≤ 65</td>
<td>403(69.13)</td>
</tr>
<tr>
<td></td>
<td>&gt; 65</td>
<td>180(30.87)</td>
</tr>
<tr>
<td>TNM stage</td>
<td>Stage I</td>
<td>33(5.66)</td>
</tr>
<tr>
<td></td>
<td>Stage II</td>
<td>41(7.03)</td>
</tr>
<tr>
<td></td>
<td>Stage III</td>
<td>389(66.7)</td>
</tr>
<tr>
<td></td>
<td>Stage IV</td>
<td>102(17.50)</td>
</tr>
<tr>
<td></td>
<td>Stage X</td>
<td>18(3.08)</td>
</tr>
<tr>
<td>Histologic grade</td>
<td>G1</td>
<td>11(1.89)</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>101(17.32)</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>456(78.21)</td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>1(0.17)</td>
</tr>
<tr>
<td></td>
<td>GX</td>
<td>14(2.40)</td>
</tr>
<tr>
<td>Vital status</td>
<td>Alive</td>
<td>241(41.34)</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td>342(58.66)</td>
</tr>
</tbody>
</table>

Abbreviation: Stage X: unknown pathological stage; GX: unknown histological grade.

3.2. Construction of the glycolysis-related risk signature

To examine the prognostic value of the 386 GRGs, 11 genes were obtained by preliminary screening, which was performed by univariate Cox regression analysis with the criterion of adjusted p < 0.05. Finally, nine genes (ISG20, CITED2, PYGB, IRS2, LHX9, PC, ANGPTL4, TGFBI, and DDIT4) significantly correlated with OS (adjusted p < 0.05) after filtration using LASSO and multivariable Cox regression analyses (Fig. 1A, B and C, Table 2). And then a predictive signature based on GRGs was constructed and the formula to assess the risk of every patient’s survival was showed as follows: risk score = (− 0.25414) × ISG20 expression level + 0.07897 × CITED2 expression level + 0.11769 × PYGB expression level + 0.09112 × IRS2 expression level + 0.06399 × ANGPTL4 expression level + 0.04811 × TGFBI expression level + 0.03555 × LHX9 expression level + 0.05593 × PC expression level + 0.05907 × DDIT4 expression level.

Additionally, Fig. 1D showed the mutation of the nine selected GRGs of 583 OV patients in the cBioPortal database. And we used the gene model calculated every patient’s risk score in the training set on the basis of the nine-GRG expression levels). The results suggested that patients with high risk scores were associated with a worse prognosis, when compare to patients with low risk scores (P < 0.0001, log-rank test; Fig. 2A and 2B). The areas under the curve (AUC) for 3-, 5-, and 10-year OS were 0.709, 0.762, and
The heatmap revealed the expression patterns of the 9 GRGs between the two different prognostic patient groups (Fig. 2D).

Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ensemble ID</th>
<th>Coefficient</th>
<th>HR (95%CIs)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISG20</td>
<td>ENSG00000172183</td>
<td>-0.25414</td>
<td>0.78 (0.70–0.87)</td>
<td>5.22E-06</td>
</tr>
<tr>
<td>CITED2</td>
<td>ENSG00000164442</td>
<td>0.078975</td>
<td>1.08 (0.92–1.28)</td>
<td>0.356061</td>
</tr>
<tr>
<td>PYGB</td>
<td>ENSG00000100994</td>
<td>0.117691</td>
<td>1.12 (0.94–1.34)</td>
<td>0.186993</td>
</tr>
<tr>
<td>IRS2</td>
<td>ENSG00000185950</td>
<td>0.091117</td>
<td>1.10 (0.97–1.23)</td>
<td>0.133015</td>
</tr>
<tr>
<td>ANGPTL4</td>
<td>ENSG00000167772</td>
<td>0.063993</td>
<td>1.07 (0.96–1.18)</td>
<td>0.218495</td>
</tr>
<tr>
<td>TGFBI</td>
<td>ENSG00000120708</td>
<td>0.048112</td>
<td>1.05 (0.94–1.17)</td>
<td>0.393959</td>
</tr>
<tr>
<td>LHX9</td>
<td>ENSG00000143355</td>
<td>0.035546</td>
<td>1.04 (0.97–1.11)</td>
<td>0.326248</td>
</tr>
<tr>
<td>PC</td>
<td>ENSG00000173599</td>
<td>0.055931</td>
<td>1.05 (0.91–1.23)</td>
<td>0.47917</td>
</tr>
<tr>
<td>DDIT4</td>
<td>ENSG00000168209</td>
<td>0.059074</td>
<td>1.06 (0.94–1.19)</td>
<td>0.328672</td>
</tr>
</tbody>
</table>

Abbreviation: HR: hazard ratio.

3.3. Evaluation of the predictive capability of the nine-GRG risk signature

After constructing the GRG predictive model, we selected three datasets to verify its prediction performance. The sets for validation including GSE63885, GSE26193, and GSE30161 datasets with 101, 107, and 58 patients with OV, respectively (Fig. 3). The AUCs of 3-, 5-, and 10-year OS were respectively 0.716, 0.767, and 0.819 in the GSE26193 dataset; 0.808, 0.8, and 0.853 in the GSE30161 dataset; and 0.636, 0.722, and 0.815 in the GSE63885 dataset. Survival analysis revealed that our risk signature performed well in the validation sets. And the survival differences between the satisfied high-risk and low-risk groups were all statistically significant (P value of < 0.05). The distribution of risk scores and survival statuses of the patients with OV in the three sets are also displayed in Fig. 3.

3.4. Risk score generated from the nine-gene signature as an independent prognostic indicator

The exploration of independent predictive factors is through univariate analysis of clinical factors and risk models combined with multivariate regression analysis. Table 3 showed that, in addition to age and tumor stage, our GRG risk model could independently predict the OS according to the results from univariate analysis (HR [95% CI], 2.334 [1.817 – 2.997]; P < 0.001) and multivariate analysis (HR [95% CI],
2.361 [1.830 – 3.047]; P < 0.001), referring to the statistical standard of adjusted P value of < 0.05. Furthermore, the AUC of the nine-gene signature was higher than that of any single clinicopathological variable (Fig. 4A). The findings in the present study suggest that the gene model had independently and effectively predictive ability in the survival prediction for OV patients. To develop a quantitative method that can predict the OS of patients with OV, a nomogram was constructed. The predictors included risk score and age (Fig. 4B).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Age (≤ 65 / &gt;65)</td>
<td>1.023(1.010–1.036)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TNM stage ( I/II / III/IV )</td>
<td>1.643(1.028–1.752)</td>
<td>&lt; 0.039</td>
</tr>
<tr>
<td>Histologic grade(G₁/₂ / G₃/₄)</td>
<td>1.213(0.816–1.801)</td>
<td>0.340</td>
</tr>
<tr>
<td>Risk score (H / L)</td>
<td>2.334(1.817–2.997)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviation: GRGs, glycolysis-related genes; H, High; L, low.

### 3.5. Validation of the nine-GRG signature in predicting survival using Kaplan-Meier curves

The clinical features of age, histological grade, and tumor stage represent predictive prognostic factors of OV after the performance of univariate Cox regression analysis of OS. Kaplan-Meier curves revealed that clinical features that showed consistent results, namely patient age of > 65 years and disease stages III and IV, were associated with poor prognosis (Figs. 4C–4D).

For the purpose of testing whether our nine-GRG signature can play a role in different TNM stages, histological grades, and ages, a subgroup analysis was performed for each clinical feature. After the analyses of Kaplan-Meier survival, the risk signature was demonstrated to be stably prognostic power and applicable to the OV patients when they were stratified in to different age and TNM stage groups (Figs. 5A and 5B). However, when the patients were stratified into high-grade (grades 3 and 4) and low-grade (grades 1 and 2) subgroups, the risk score of the eight-gene signature remained an independent prognostic indicator in the high-grade subgroup (P < 0.001), but not in the low-grade subgroup (P = 0.067; Fig. 5C). The risk model showed more effective prediction in the patients groups of high-grade OV.

### 4. Discussion

More and more attentions to the globe burden ovarian cancer have been paid. Despite current advances in surgery and chemotherapy, its poor prognosis remains big challenges.[3] Because of its heterogeneity
and the lack of convenient and accurate biomarkers, the current prognostic tools of OV patients has limited clinically predictive abilities.[2, 26] Subtype identification, risk stratification, and characterization of the underlying mechanism are critical for improvement of the existing treatment methods, development of more precise and personalized therapies, and prolongation of survival time. Thus, a predictive model with a broad scope of application is needed for accurate prediction of OS in patients with OV and for guiding clinicians in targeted treatment and better prognosis. With the popular application of large databases, more and more prognostic markers are recognized.[8, 9, 27] Many studies have explored the biological function of GRGs in cancers.[14–16] In addition, considering that an increasing number of studies have discovered prognostic markers of glycolysis-related genes, the establishment of GRG-based risk signature to predict the survival of OV patients is necessary.

In our predictive model, this study consisted of a training set and 3 validation cohorts, which included 813 patients with OV. Nine genes with prognostic value for patients with OV were identified using univariate, multivariate, and LASSO Cox regression analyses. The study results indicate that the nine-GRG signature developed herein significantly correlates with poor prognosis in OV. In addition, this risk signature was still an independent prognostic factor in the multivariate Cox analyses. Results of survival analysis suggested that patients with higher risk score tend to have worse clinical outcomes. The 8-gene model showed better predictive ability than any single gene and clinicopathological factors. Nevertheless, due to the lack of relevant data in TCGA database, one limitation is that our model cannot predict recurrence and distant metastasis of OV patients. The model established in present study de well in the OS prediction. And a novel nomogram was established in our study by combing the prediction model and clinical characteristics. The nomogram used the complementary values of clinical characteristics and the prediction model and provided superior estimation of OS. Moreover, the gene signature could further assessed the survival risk when the patients have different clinical features (age, TNM stage, and histological grade). The risk model have effective prediction power for patients with diverse clinical characteristics, but its predictive power showed limited when patients with low grade which should be explored in depth in the future. This result implies that the clinical application of genetic models is far-reaching and the means for patients to predict prognosis in clinic will become more diverse, which can guide clinicians to treat more accurately.

The 9 GRGs we identified include ISG20, CITED2, PYGB, IRS2, ANGPTL4, TGFBI, LHX9, PC, and DDIT4. Among the 9 biomarker genes identified in the present study, DDIT4 (DNA damage-inducible transcript 4) with high expression level actively responded to hypoxia-inducible factor 1 and act together to regulated the generation of cell reactive oxygen species.[28] As an oncogene, [28, 29] its overexpression correlates with tumor progression and worse outcomes in several human cancers, including OV.[18, 30–32] PYGB (brain-type glycogen phosphorylase) could regulate multiple biological characteristics of cancer cells, such as proliferation, invasion, and apoptosis and metastatic phenotypes of several cancers.[33–38] PYGB regulates the Wnt/β-catenin signaling pathway to achieve cancer-promoting effects in OV.[39], non-small cell lung cancer (NSCLC),[40] and gastric cancer.[41] IRS2 (insulin receptor substrates 2) mediates mitogenic and antiapoptotic signaling of insulin-like growth factor 1 receptor (IGF-IR), insulin receptor (IR), and other oncoproteins[42, 43] and is essential for cancer cell motility and metastasis.[44–46] IRS2
acted as an oncogene in OV and was involved in cell proliferation and ascites migration effect in OV progression.\cite{47, 48} **ANGPTL4** (angiopoietin-like 4) has been reported to be involved in ferroptotic cell death and chemoresistance of epithelial OV.\cite{49} Moreover, large amounts of it has been detected in malignant ascites of serous OV patients.\cite{50} High **ANGPTL4** levels predict shorter relapse-free survival in serous OV.\cite{50, 51} Studies have found that high promoter hypermethylation of **TGFBI** (transforming growth factor-beta-inducible gene) is involved in chemotherapy resistance of paclitaxel in OV.\cite{52, 53} A study showed that **TGFBI** and periostin, predict poor prognosis in serous epithelial OV.\cite{54} **PC** (pyruvate carboxylase) is a biotin-containing enzyme that converts pyruvate to oxaloacetate and has been implicated in cancer progression. **PC** is strongly involved in tumorigenesis in several cancers, such as breast cancer, NSCLC, glioblastoma, renal carcinoma, and gallbladder cancer.\cite{55–58} Moreover, **PC** may mediate the regulation of TNKS (tankyrase) in aerobic glycolysis and may be involved in the TNKS-regulated development of OV, as its oncogenic activity is induced by TNKS activating Wnt/β-catenin/snail signaling.\cite{59} Not much evidence has been accumulated on the following genes from OV basic research: **ISG20** (interferon-stimulated gene 20), an RNA exonuclease,\cite{60} stimulates tumor progression in hepatocellular carcinoma, clear cell renal cell carcinoma, and glioma.\cite{61–63} The high expression level of **ISG20** has association with the poor clinical outcome of OV patients.\cite{63} **CITED2** (Cbp/p300-interacting transactivator 2), a pleiotropic protein, has been reported to participate in several biological functions of cells, which included transcription and differentiation. High **CITED2** expression level is correlated with poor patient survival in breast \cite{64} and prostate cancers.\cite{65} **CITED2** participated in the regulation of the cell cycle, promoted cell proliferation and then played active role in progression of lung cancer.\cite{66, 67} and supports gastric cancer cell colony formation and proliferation.\cite{68} **CITED2** was involved in resistance to platinum-based chemotherapy in OV.\cite{69} **LHX9** (LIM homeobox 9) is a developmentally expressed transcription factor\cite{70} and is strongly expressed in the ovarian surface epithelium.\cite{71} Previous research has the development of childhood malignant gliomas involved **LHX9** abnormal methylation and silencing \cite{72} The relationship of **ISG20**, **CITED2**, and **LHX9** with OV and its molecular mechanism must be examined in depth in future studies. We integrated the 9 GRGs into a panel and established a novel multigene signature for predicting prognosis in OV. This signature showed a strong predictive ability and acted as an independent prognostic molecular factor in patients with OV.

To our knowledge, our study firstly identified the GRG risk predictive signature using the data from TCGA public database. The nine-GRG risk model showed a promising survival prediction ability for the prognosis of OV. We also analyzed mutations in the 9 selected genes in the cBioPortal database. Despite these promising results, questions remain. First, our study was not a prospective study, and all the patients with OV were identified from public databases. Second, large-scale multicenter cohorts are necessary to verify our findings, and further studies are need to further explored the functional roles of the GRGs involved in the initiation and development of OV. Moreover, the gene signature performed more effectively in high-grade OV patients than in low-grade patients, and the mechanism of this observation is should be fully investigated in the future. To further validate the utility of this risk model, we the works of clinical data and specimens collection have been undertaken.
Conclusion

We constructed a valid, innovative, reliable nine-GRG prognostic model (ISG20, CITED2, PYGB, IRS2, ANGPTL4, TGFBI, LHX9, PC, and DDIT4) for predicting patient outcomes in OV. Moreover, our signature is an independent and important risk factor of OV. The predictive capability of this model in OV requires further testing to improve prognostic stratification and treatment management.

Abbreviations

ROC curve: receiver operating characteristic curve; AUC: areas under the curve; OV: ovarian cancer; OS: overall survival; GRG: glycolysis-related gene; HR: hazard ratio; TCGA: The Cancer Genome Atlas database; GEO: the Gene Expression Omnibus database; LASSO: the least absolute shrinkage and selection operator cox; GX: unknown histological grade; Stage X: unknown pathological stage.

Declarations

Acknowledgments

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Authors’ contributions

Dai Zhang, Si Yang and Yiche Li: Data curation, Writing - Original Draft, Methodology, Software. Meng Wang, Jia Yao and Yi Zheng: Conceptualization, Methodology, Software, Validation. Yujiao Deng and Na Li: Visualization, Investigation. Zhen Zhai, Ying Wu and Bajin Wei: Software, Validation. ZJD and HFK: Supervision, Project administration. All the authors read and approved the final manuscript.

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Ethics approval and consent to participate

This study has been proved by the Institutional Review Board of the First Affiliated Hospital of Zhejiang University in Zhejiang Province (Hangzhou, China).

Availability of data and materials

The datasets generated and analyzed during the current study are available in the TCGA (http://cancergenome.nih.gov/abouttcga), GEO (https://www.ncbi.nlm.nih.gov/geo/) and cBioPortal (http://www.cbioportal.org) databases. ZJD and HFK had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Conflicts of interest

The authors declare that there are no conflicts of interest.

Consent for publication

Written informed consent for publication was obtained from all participants.

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**Figures**

**Figure 1**

Figure 2

KM survival analysis, risk score assessment by the GRG risk signature and time-dependent ROC curve in the training set. A, KM survival analysis of high- and low- risk samples in the TCGA data set. B, Relationship between the survival status / risk score rank and survival time (years)/risk score rank. C, Time-dependent ROC curve for OS of the TCGA data set. The AUC was assessed at 3, 5 and 10 y. D: Nine
GRGs expression patterns for patients in high- and low-risk groups by the nine-GRG signature. Abbreviation: GRGs, glycolysis-related genes; OS: overall survival

Figure 3

KM survival analysis, risk score assessment by the GRG-related gene signature and time-dependent ROC curves in the GEO validation data sets. A, GSE26193, B, GSE30161, C, GSE63885. a. KM survival analysis of high- and low-risk samples. b. Relationship between the survival status / risk score rank and survival time (years) / risk score rank. c. ROC curve for overall survival of the validation data sets. The AUC was assessed at 3, 5 and 10 years.
Figure 4

ROC curve with respect to clinical features and risk model, nomogram and Kaplan–Meier survival analysis for OV patients with clinical features: A, time-dependent ROC curve with respect to single clinical features and risk model. B. The nomogram for predicting probabilities of OV patients overall survival. Kaplan–Meier survival analysis for OV patients with different clinical features that can predict patient survival (C, Age, D, Stage, E, Grade). Abbreviation: OV: ovarian cancer.
Figure 5

KM survival subgroup analysis of all patients with OV according to the GRG-related gene signature stratified by clinical characteristics. A, Age ≤ 65 y, Age > 65 y. B, Early stage (stage I-II), Late stage (stage III-IV). C, Low grade 1-2, High grade 3-4. Abbreviation: GRGs, glycolysis-related genes; OV: ovarian cancer.

Supplementary Files
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- TableS1.docx