Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2022**; 49(12): 273
https://doi.org/10.31083/j.ceog4912273

Original Research

A Metabolic Gene Prognostic Risk Model for Cervical Cancer

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Academic Editor: Andrzej Semczuk
Submitted: 27 July 2022 Revised: 26 October 2022 Accepted: 1 November 2022 Published: 12 December 2022

Abstract

Background: Previous studies have identified hundreds of constantly changing metabolic genes in cervical cancer, however, their prognostic effect remains to be explored. **Methods**: In this paper, Cox univariate regression and Lasso regression models were used to identify metabolic genes associated with squamous cervical cancer prognosis, and developed a prognostic risk score. Next, on the basis of the median risk score, cervical squamous cancer patients were divided into two groups: high- and low-risk patients. Kaplan-Meier analysis and receiver operating characteristic (ROC) curves were used to evaluate the predictive efficacy of the metabolic gene prognostic risk model. In addition, we analysed the correlation between drug sensitivity, immune cell infiltration, and Gene set variation analysis (GSVA) and the metabolic gene prognostic risk model. **Results**: The results showed that the prognosis of patients in the high-risk group was worse. The metabolic gene prognostic model was correlated with immune cell infiltration. It is also correlated with sensitivity to common chemotherapeutic drugs. In addition, gene set enrichment analysis results revealed several significantly enriched pathways, which may help to explain the underlying mechanisms of cervical carcinogenesis. **Conclusions**: The proposed prediction model can be potentially used for prognosis prediction of cervical cancer.

Keywords: cervical cancer; metabolic gene; TCGA; prognostic risk model

1. Introduction

Cervical cancer is one of the most common malignant tumors in women. It is reported that there are approximately 530,000 new cases and 270,000 deaths per year worldwide [1–3]. Approximately 70% of cervical cancers are SCCs (squamous cell carcinomas), the most common type [4]. To treat the early-stage cervical cancer, the standard approach is through surgery, while concurrent chemoradiation are the main treatments for advanced cervical cancer. Even though the people with early stage or localized lesions can undergo surgery to improve their chances of long-term survival. However, treatment is limited due to drug resistance and recurrence [5], and the five-year survival rate for advanced cervical cancer (stages II–IV) is only 15%–69% [6], which seriously endangers the physical and mental health of women. Numerous studies have demonstrated the tight connection between metabolic disorders and an increased risk of cancer, and malignant tumors are capable of metabolic reprogramming [7]. With the deepening understanding of tumor biology and tumor metabolism, it is found that the metabolic abnormalities in tumor tissues are far more complex than previously recognized. In addition to abnormal energy metabolism, tumor tissues also exhibit defective carbon and amino acid metabolism [8,9]. Compared with normal tissues, different tumor cells and tumor tissues have metabolic heterogeneity. Local tumors also show a dependence on metabolic reprogramming during tumor metastasis [7]. The functions of metabolic-related mechanisms in cervical cancer patients, however, remain unclear. To build a more accurate prediction model, we used Cox univariate regression model and Lasso regression algorithm. Furthermore, the model's clinical predictive value was also studied using more bioinformatics analysis methods. In cervical squamous cell carcinoma (CESC) patients, the metabolic gene-associated model accurately predicts survival outcomes, which can lead to individualized treatment.

2. Materials and Methods

2.1 Data Preparation

From the TCGA database (https://portal.gdc.cancer.gov/), we downloaded the raw mRNA expression data of CESC, including the normal group (n = 3) and the CESC group (n = 306). To analyze differentially expressed genes, FPKM data of mRNA level 3 were integrated and normalized using the Limma package. The differential gene screening conditions were $|\text{LogFC}| > 1$ and p value < 0.05. Next, the Series Matrix File data of GSE44001 was downloaded from GEO public database, the annotation platform is GPL14951. The Series Matrix File data of GSE52903 were annotated with GPL6244. GeneCards (https://www.genecards.org/) database was used to extract 18,762 metabolism-related genes, and genes with Relevance scores >3 were analyzed as metabolic gene sets.

2.2 GO and KEGG Functional Annotations

ClusterProfiler (R3.6, R Core Team, Vienna, Austria) was used to annotate the functions of differential genes and explore their functional relevance. Relevant functional categories were identified using GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes). We considered a statistical difference if p value and q value all less than 0.05.

2.3 Model Construction and Prognosis

The prognostic correlation models were constructed using Lasso regression once differential metabolism genes were selected. For each patient, a risk score formula was constructed by incorporating the expression values of each specific gene and weighting them with their regression coefficients in Lasso regression analysis. The risk score formula is shown below.

$$\text{Risk score} = \sum_i \text{regression coefficient of metabolic gene } i \times \text{expression of metabolic gene } i.$$

Where Metabolic gene i is the identifier of the i-th selected core metabolic gene. According to this formula, median risk score was used to distinguish low-risk and high-risk patients. A Kaplan-Meier survival curve was computed and then compared using log-rank statistics. Using Lasso regression and hierarchical multiple regression, we examined the role of risk score in predicting patient prognosis. We evaluated the model prediction performance using ROC curves.

2.4 Drug Sensitivity Analysis

We predicted each tumor sample's chemotherapy susceptibility using the "pRRophetic" function in R software (R3.6, R Core Team, Vienna, Austria) based on the GDSC Cancer Drug Susceptibility Genomics Database. Based on the GDSC dataset, 10 cross-validations were conducted to determine the accuracy of the IC50 estimates for each specific chemotherapy drug treatment. We set all the parameter to default settings, including the "combat", which removes batch effects, and the average of duplicate gene expression.

2.5 Analyses of Immune Cell Infiltration

RNA-seq data from different subgroups of CESC patients was analyzed using the CIBERSORT algorithm to determine the relative proportions of immune infiltrating cells. Pearson correlation analysis is performed for gene expression and immune cell content, and statistical significance is defined as $p < 0.05$.

2.6 Gene Set Difference Analysis

By thoroughly scoring the gene sets of interest, Gene set variation analysis (GSVA) turns changes at the gene level into changes at the pathway level and establishes the biological function of the samples. In this study, molecular signatures database (v7.0) gene sets were downloaded, and GSVA scores were used to assess possible biological functional changes between samples.

2.7 Statistical Analysis

R software (R3.6, R Core Team, Vienna, Austria) and SPSS 19.0 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses. Kaplan-Meier survival curves were generated and compared using log-rank. Multivariate analysis is performed using Cox proportional risk models. All statistical tests were two-sided, and we consider is as statistically significant if $p < 0.05$.

3. Results

3.1 Expression of Metabolism-Related Genes in the CESC Cohort

We extracted a collection of 2235 metabolism-related regulatory factors from the downloaded mRNA expression data (FPKM). The differential expression between cervical cancer patients and control patients are investigated using the Wilcox test. According to the results, 378 metabolism-related genes, including 177 up-regulated and 201 down-regulated genes, were differentially expressed (logFC >1, logFC <-1, and $p < 0.05$). The changes in differential gene expression values (log2(expression value+1)) are shown by heat map in Fig. 1A, while the differential ploidy and p value of these differential genes are presented using a volcano plot in Fig. 1B.

3.2 Functional Enrichment of DEGs and Co-Expression Network Construction

As a result of GO and KEGG pathway enrichment analysis, a large number of pathways were significantly enriched for differentially expressed genes, such as coenzyme metabolic process, cytoplasmic vesicle lumen, coenzyme binding, etc. (Fig. 2A). In the KEGG enrichment process, there are central carbon metabolism in cancer, biosynthesis of amino acids and other metabolism-related pathways as illustrated in Fig. 2B. At the same time, through the Metascape database, we further analyzed candidate gene pathways. According to the results, these candidates were mainly enriched in hormone, organic hydroxy compound metabolic, and small molecule biosynthetic pathways (Fig. 2C). Fig. 2D shows the co-expression network analysis result of genes in the differential gene set.

3.3 Prognosis Gene Retrieval and Prediction Model Construction

In order to identify the key metabolic genes, we collected clinical information from CESC patients. According to Fig. 3A–C, we used Lasso regression and Cox univariate regression algorithms to identify cervical cancer signature genes. By using Cox univariate regression, 59 prognosis-related genes were identified: *ISCU, TCN2,*

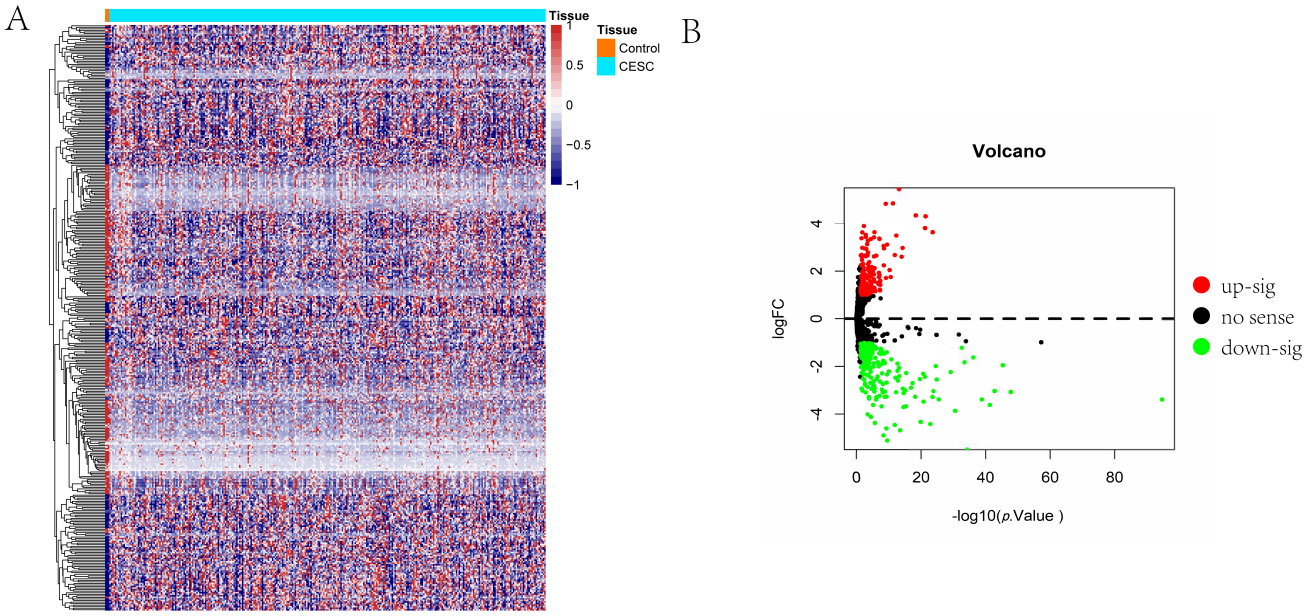

Fig. 1. The identification of metabolism-related genes with differential expression. (A) Heat map of the differentially expressed genes (DEGs). (B) Volcano plot of the DEGs.

FOXP3, PGK1, UCP2, SPINT2, FASN, MOCS1, TFRC, ADH1B, TNF, MIR200A, GAPDH, MMP1, PFKFB4, GCH1, CDIPT, ACOT4, LDHA, LEPR, BCL2, SPP1, TNFRSF1B, TGFA, CPE, CA9, NPL, SPTBN1, GALNT3, DHCR7, FGFR2, PFKM, NME4, SDS, SQLE, SLC25A42, JUN, NT5E, TRPV4, HSPG2, PLA2G7, SLC25A10, CKB, APOC1, GART, SLC2A3, SCD, VDAC1, ENO1, APOD, SELP, HMGCS1, NR1D2, TREM2, PLIN2, FGFR3, PIP4K2B, COX5A and *HK2*. After randomly dividing TCGA patients into training and validation sets in an equal ratio, Lasso regression analysis was used to determine the best risk score. The metabolic gene prognostic risk score model was constructed as the formula below: Risk Score = *FGFR2* × (–0.4067) + *FOXP3* × (–0.2731) + *NPL* × (–0.2704) + *CKB* × (–0.1992) + *APOD* × (–0.1027) + *TCN2* × (–0.0673) + *TNFRSF1B* × (–0.0378) + *FGFR3* × (–0.0154) + *HSPG2* × 0.0067 + *TFRC* × 0.0089 + *NT5E* × 0.0220 + *CA9* × 0.0313 + *CPE* × 0.0438 + *FASN* × 0.0442 + *NME4* × 0.0828 + *PIP4K2B* × 0.0977 + *SPP1* × 0.0989 + *MMP1* × 0.1013 + *JUN* × 0.1311 + *SPINT2* × 0.1474 + *GAPDH* × 0.1894 + *MIR200A* × 0.1902 + *TNF* × 0.1972 + *SQLE* × 0.2018 + *ADH1B* × 0.3305. Based on the median risk score, patients were divided into high and low risk groups. In both the training and test sets, the OS of the high-risk group was significantly lower ($p < 0.001$) than that of the low-risk group, as shown in Fig. 3D,E. Furthermore, the ROC curve results showed a C-index of 0.85 and 0.79 in the training set and test set, both indicating good validation efficacy, as shown in Fig. 4A,B. Baseline characteristics of the patients is presented in **Supplementary Table 1**.

3.4 Multi-Omics Study to Explore the Model's Clinical Predictive Value

The relationship between the tumor immune infiltration and the risk score was further investigated. The results showed that risk score was significantly and positively correlated with Macrophages M0 ($p < 0.01$), Dendritic cells activated ($p < 0.05$), Mast cells activated ($p < 0.01$) content, and significantly negatively correlated with Mast cells resting ($p < 0.01$), T cells CD8 ($p < 0.01$), B cells naive ($p < 0.01$), as shown in Fig. 5A. The interaction between immune cells is shown in Fig. 5D. A combination of surgery and chemotherapy is effective in treating cervical cancer at an early stage. We use the R package's "pRRophetic" function to determine chemotherapy sensitivity of tumor samples from the GDSC database. A significant relationship was found between the risk score and the sensitivity to Bortezomib, Docetaxel, Erlotinib, Metformin, Mitomycin.C, and Paclitaxel (Fig. 5B). Additionally, we examined the mutation profiles of high-risk and low-risk patients. High-risk individuals had a significantly higher proportion of mutations of *KMT2C* and *TTN* than low-risk individuals, shown in Fig. 5C. At the same time, we also found that there were significant differences in microsatellite instability (MSI) and tumor mutation burden (TMB) between the two groups, and the expression of TMB and MSI was increased in the high-risk group (Fig. 5E,F).

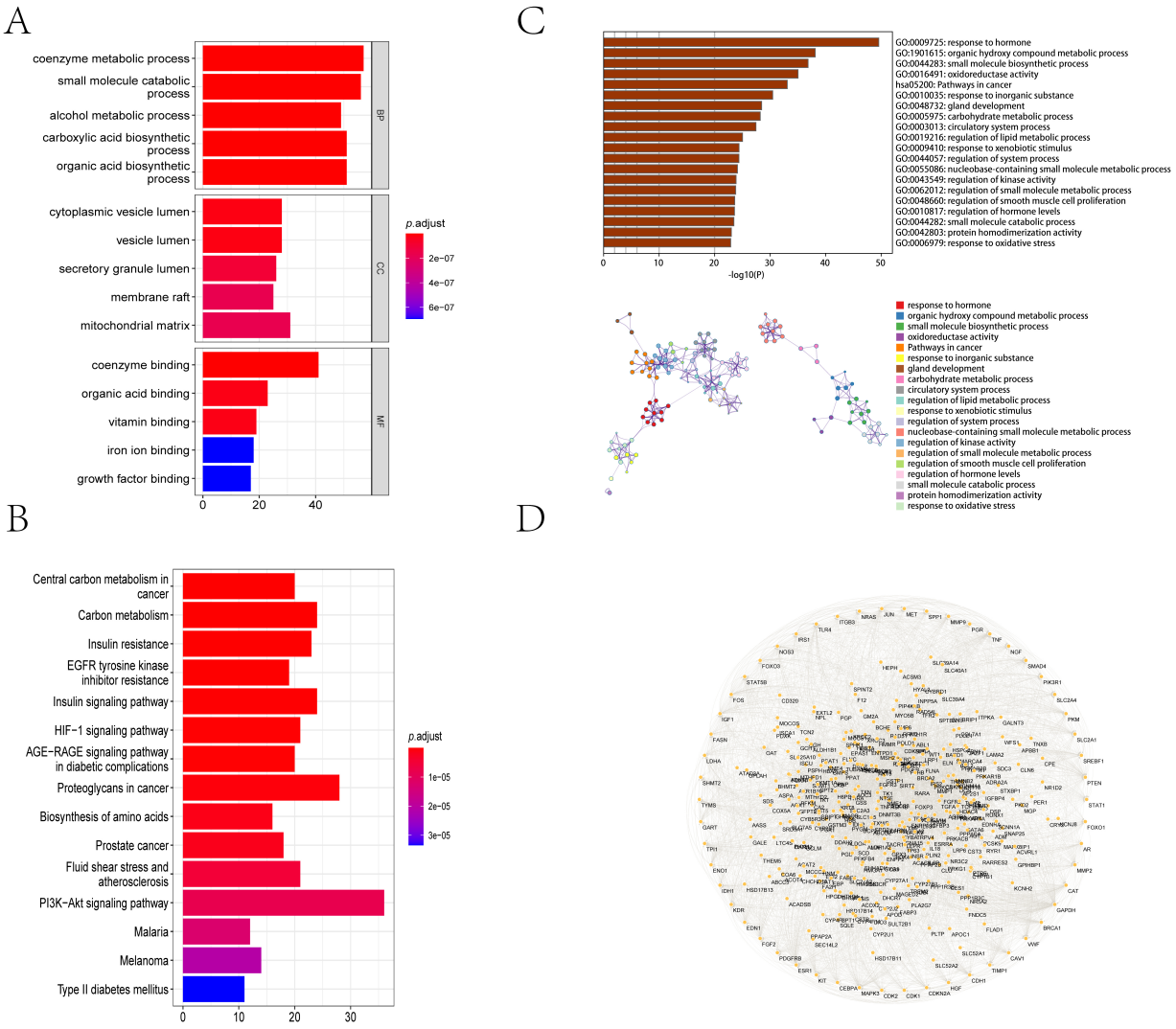

Fig. 2. Functional analysis of metabolism-related genes. (A) DEGs enriched by GO. (B) KEGG enrichment analysis of DEGs. (C) Related pathways of DEGs. (D) Protein network analysis of DEGs.

3.5 Signaling Mechanisms for Prognostic Model

We then studied two groups' specific signaling pathways. Results of the GSVA revealed that ADIPOGENESIS, TNFA_SIGNALING_VIA_NFKB, ANDROGEN_RESPONSE, MYOGENESIS, and KRAS_SIGNALING_DN were the most enriched pathways for the two groups of patients, as shown in Fig. 6. It has been shown that altering these signaling pathways can affect the prognosis of cervical cancer patients.

3.6 Prognostic Model Robustness.

Data on processed CESC patients with survival information was downloaded from GEO (GSE44001, GSE52903). And Kaplan-Meier survival analysis was used to assess survival differences. Compared to the low-risk group, OS was significantly lower in the high-risk group (Fig. 7A,B). ROC curve analysis was performed on external data sets to verify the model's accuracy. The results showed that the model has a strong predictive effect on the prognosis of patients (GSE44001-C-index = 0.68, and GSE52903-C-index = 0.73), as shown in Fig. 7C,D.

3.7 Clinical Utility of the Model

The samples were divided into high and low risk groups based on the risk core. Univariate and bivariate analyses of CESC patients showed that the risk score was an independent prognostic factor, as shown in Fig. 8A, B. Nomograms were created to present the results of the regression analysis, the different staging patterns of cervical cancer were significantly correlated with the distribution of risk score values obtained by our model, shown in Fig. 9A. At the same time, the three-year and five-year OS of CESC patients were also predicted and analyzed, and the results showed that there was little difference between the predicted OS and the observed OS, suggesting that the nomogram model had a good prediction effect (Fig. 9B).

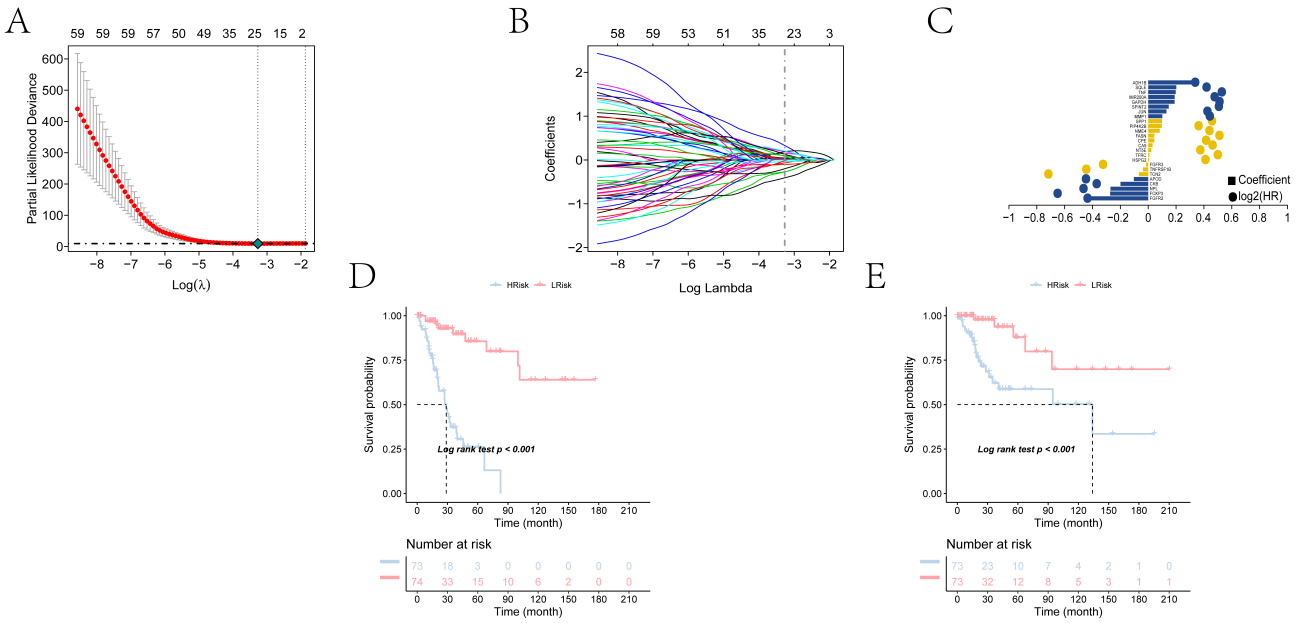

Fig. 3. Prognostic risk model construction. (A,B) Lasso regression. (C) The regression coefficient of differentially expressed genes. (D,E) Training and test set survival analysis.

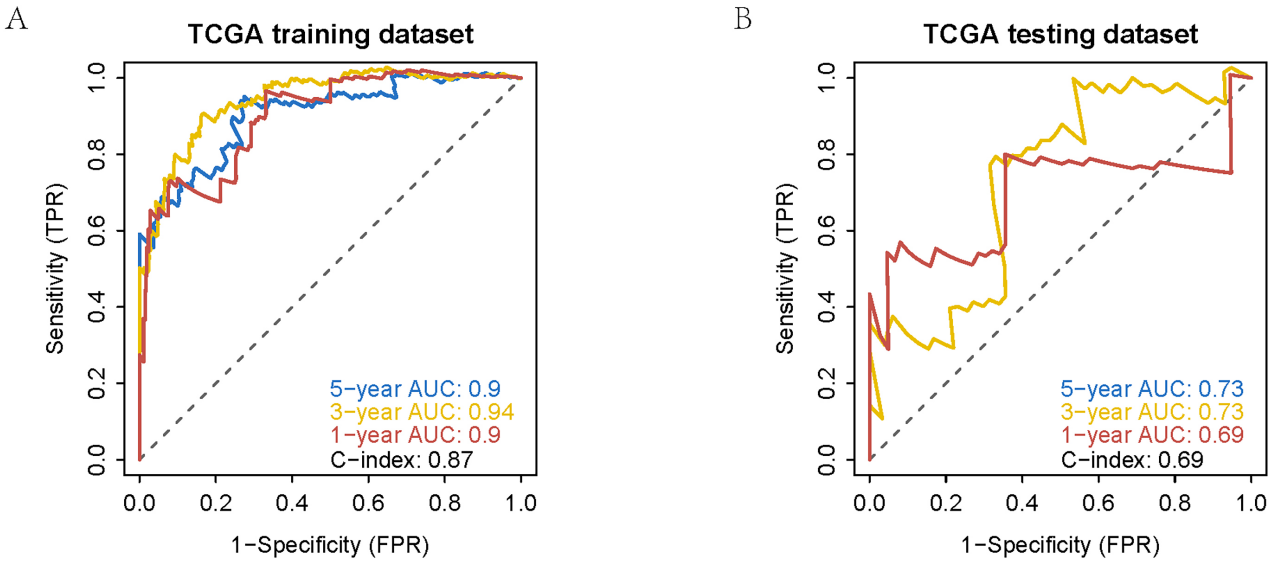

Fig. 4. Validation of the risk model. (A) ROC curves of the training cohort (1-year AUC = 0.88, 3-year AUC = 0.88, 5-year AUC = 0.89). (B) ROC curves of the testing cohort (1-year AUC = 0.83, 3-year AUC = 0.86, 5-year AUC = 0.74).

4. Discussion

Approximately 604,127 new cervical cancer cases are diagnosed in 2020 worldwide [10]. The current Federation International of Gynecology and Obstetrics (FIGO) staging can determine the prognosis initially, and FIGO stage II patients have an overall survival rate of 65%–69%, stage III patients are 40%–43%, and stage IV patients are 15%–20% [11]. The key to treating cervical cancer is early detection, early diagnosis, and early treatment. However, relying on FIGO staging alone to assess and judge the prognosis is not enough. From the perspective of tumor driver genes, abnormal expression of some genes is often associated with poorer prognostic outcome. By establishing risk models, we screened metabolism-related prognostic genes that may be involved in the onset, progression and malignant transformation of cervical cancer, which affect the survival of cervical cancer patients. This provides more information to decipher the role that metabolic reprogramming plays in tumor progression.

The role of abnormal energy metabolism in cancer genesis and progression has been reported by previous studies [12]. As metabolic networks in tumor cells are repro-

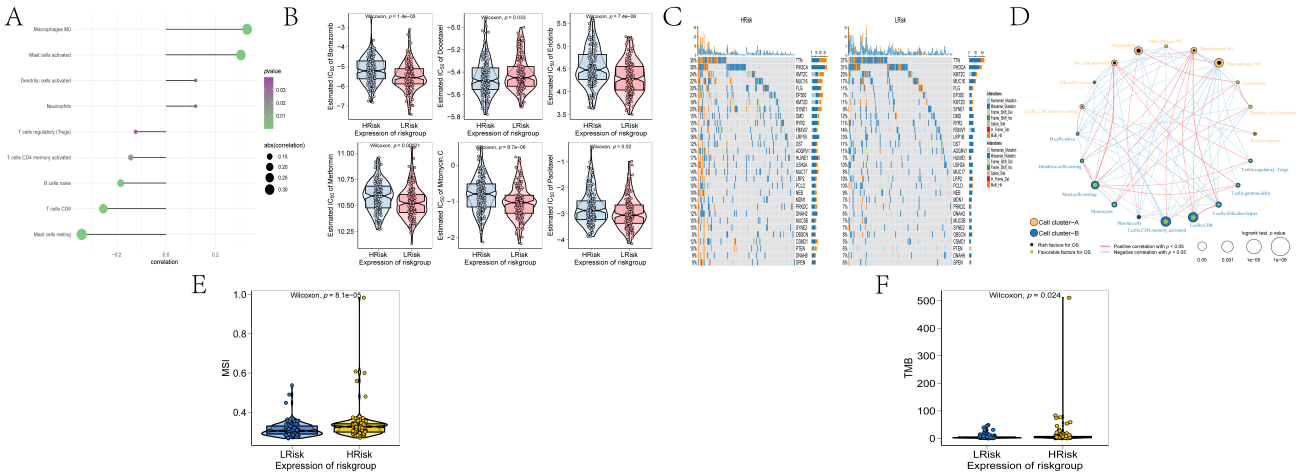

Fig. 5. Multiple omics maps of the risk model. (A,D) Immune infiltration and risk score. (B) Relationship between risk score and drug sensitivity. (C) Relationship between model and SNP mutation. (E,F) TMB and MSI were significantly different between the two groups.

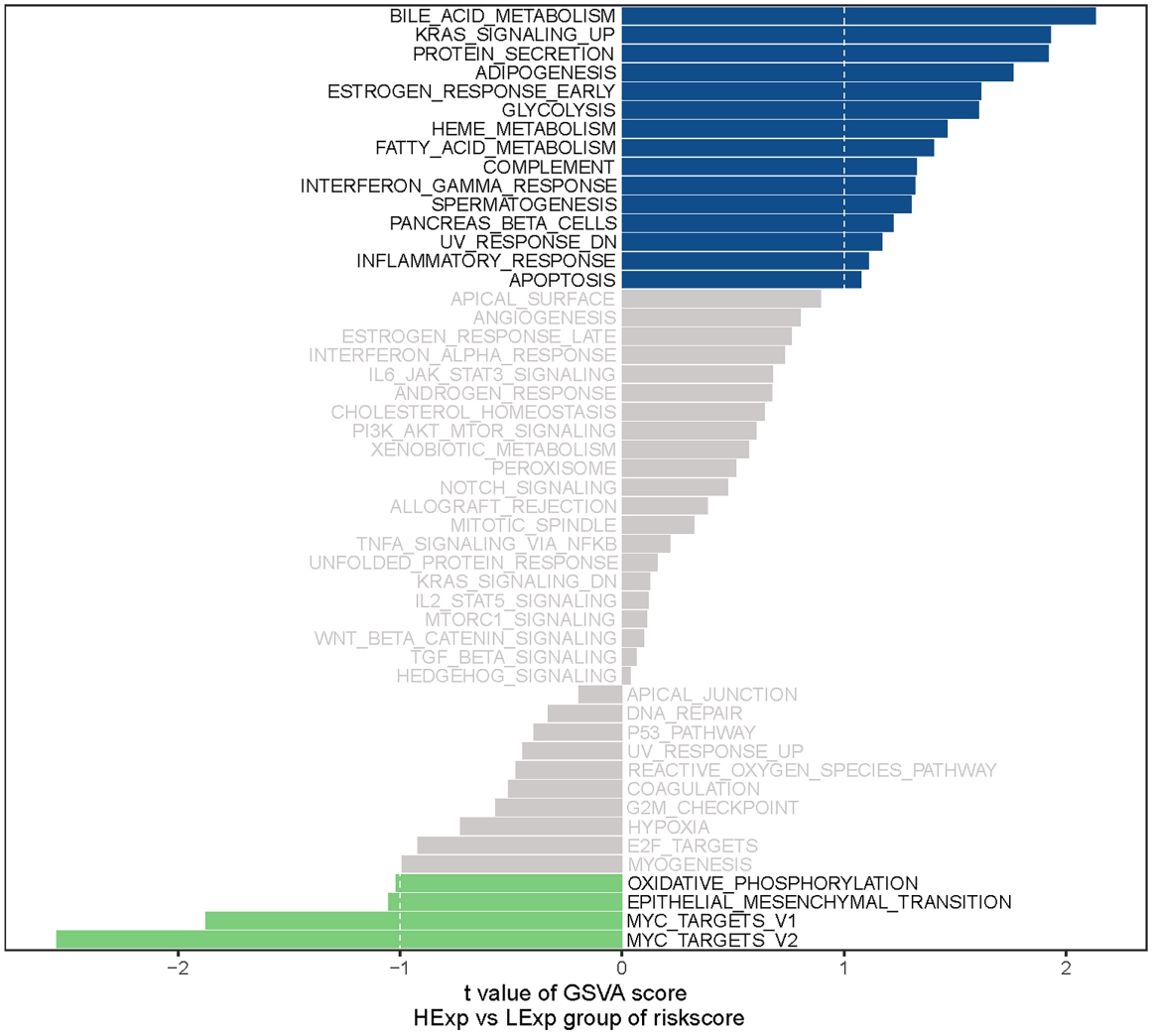

Fig. 6. Signaling mechanisms for prognostic model.

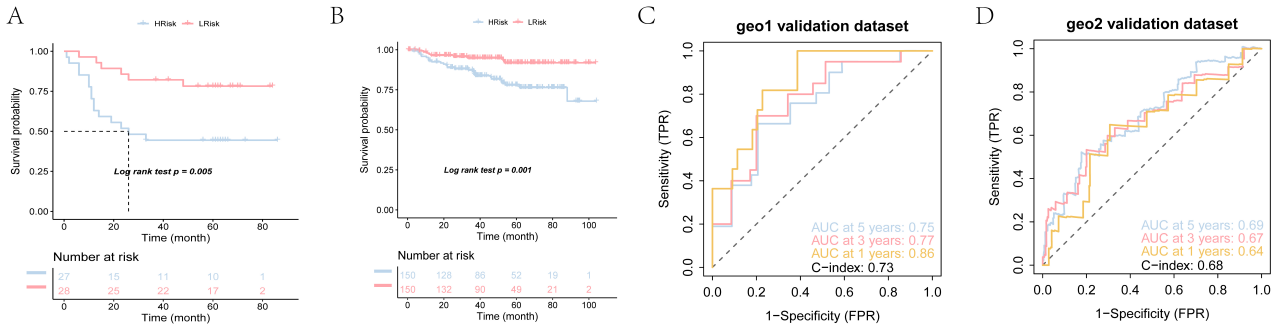

Fig. 7. Robustness of the prognostic model. (A,B) Overall survival (OS) in the external data sets (GSE44001, GSE52903). (C,D) ROC curve analysis in the external data sets.

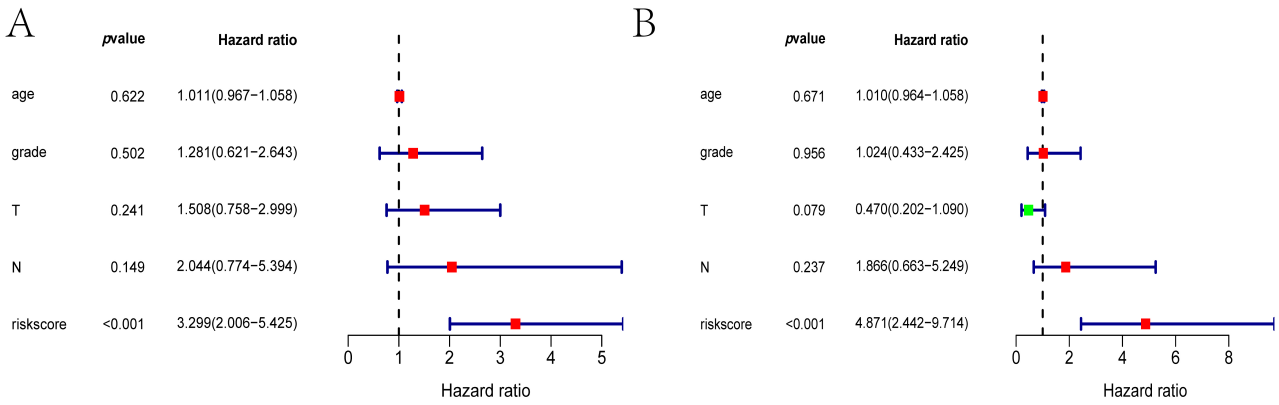

Fig. 8. The model has an independent prognostic value. (A) Univariate analysis. (B) Multivariate Cox analyses.

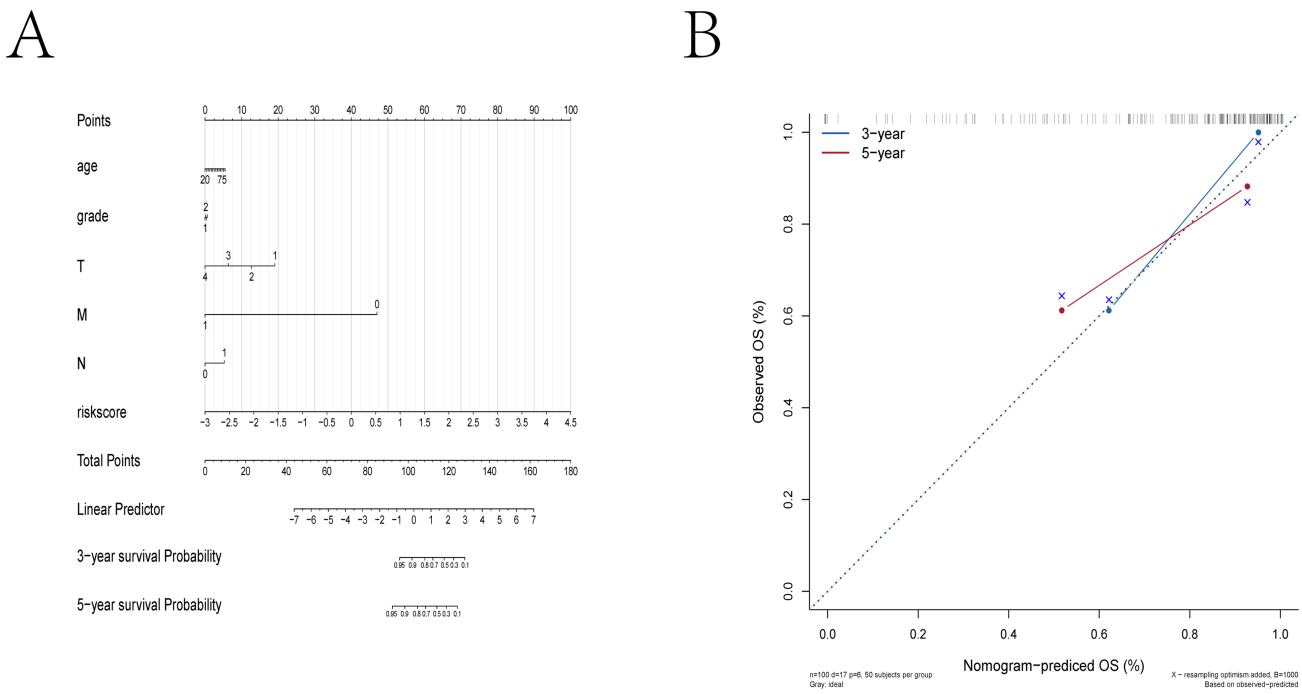

Fig. 9. Nomogram. (A) Indicators of clinical risk are correlated with the risk score. (B) The model accurately predicted 3- and 5-year OS of CESC.

grammed, this leads to reorganization and redirection of nutrient fluxes. We developed and validated a prognostic model based on 25 metabolic genes in this paper. First, these genes may reflect cervical carcinogenesis and contribute to the early diagnosis of cervical cancer. For example, fibroblast growth factor receptor 2 (FGFR2) has been widely studied in a variety of human cancers and may be a potential tumor marker for early screening of cervical cancer [13,14]. Of course, there are some genes that need to be further explored in the pathogenesis of cervical cancer. Secondly, these genes are expected to be new targets for antitumor therapy targeting cellular metabolic enzymes. For example, PIP4K2A (phosphatidylinositol-5-phosphate 4-kinase type 2 alpha), the protein encoded by this gene is a family of enzymes that catalyze the phosphorylation of phosphatidylinositol-5-phosphate at the fourth hydroxyl group of the myo-inositol ring to form phosphatidylinositol-5, 4-bisphosphate. The results of the study [15] showed that through p85/p110 component degradation in PTEN-deficient glioblastoma, PIP4K2A can negatively regulate phosphoinositide 3-kinase (PI3K) signaling. In addition, Jones *et al*. [16] demonstrated that PIP4K2A overexpression reduced clonogenic growth. Interestingly, we found that PIP4K2A downregulation in cervical cancer was associated with better OS, which is consistent with other cancer reports and needs further investigation.

The multi-omics study shows that the chemotherapeutic drug sensitivity of tumors has different expressions in different samples. The drug sensitivity analysis we used provides a new scheme to distinguish different chemotherapeutic drug combinations in the high-risk and low-risk groups. Since the tumor microenvironment (TME) is generally associated with drug resistance in patients, we further analyzed the relationship between risk score and immune infiltration to provide new ideas for differentiating drug resistance mechanisms in patients in both high-risk and low-risk groups. The characteristics of low oxygen and nutrient deficiency in TME lead to the establishment of metabolic competition between tumor cells and immune cells, and the accumulation of toxic metabolites will have a negative impact on immune response [17]. Mitochondrial dysfunction in $CD8^+$ T cells from cancer patients has been demonstrated [18]. In terms of metabolites, higher extracellular lactate levels in the tumor microenvironment were found to inhibit the proliferation and function of $CD8^+$ T cells. Lipid metabolism may contribute to $CD8^+$ T cell depletion, while cholesterol plays a beneficial role in $CD8^+$ T cell immunity, suggesting that lipid accumulation and catabolism may be applicable to opposing $CD8^+$ T cell antitumor responses [19,20]. We found that risk score and CD8+ T Cell content had a negative correlation, which corresponds to previous research. During tumor development, malignant tumor cells constantly adjust their metabolic patterns in order to obtain sufficient nutrients to supply self-renewal and proliferation in the hypoxic and nutrient-deprived tumor microenvironment. This leads to an increase of lactate, reactive oxygen species, carbon monoxide, arachidonic acid and prostaglandins in the TME [21]. Currently, it is believed that tumors affect metabolic reprogramming and subsequently exhibit abnormal biological properties through some specific pathways, including the JAK-STAT [22], p53 [23], TNF [24], Ras [25], and PI3K-AKT-mTOR signaling pathway [26]. In a prospective study of cervical cancer patients with PIK3CA mutations, treatment with 300mg daily of alpelisib resulted in 100% disease control [27]. Several other inhibitor therapies targeting tumor metabolism are in preclinical or clinical trials (Clinicaltrials.gov, NCT02771626, NCT03245489). Our study showed that the pathways enriched to the high expression group such as ADIPOGENESIS, TNFA_SIGNALING_VIA_NFK, MYOGENESIS, KRAS_SIGNALING_DN and other signaling pathways are metabolically related. This phenomenon suggests two facts: on the one hand, it reveals that cervical cancer affects metabolic reprogramming through these potential mechanisms, which in turn affects tumor progression; on the other hand, the results proved that the prognostic model was robustly connected to metabolic systems. The combination of metabolic intervention and immunotherapy will bring hope for the precise treatment of CESC patients.

We first used Cox regression to screen genes before constructing the risk prediction model by Lasso regression. In addition to using the test dataset to validate the predictive efficacy, we also used the GEO dataset to validate the model robustness, both of which achieve accurate prediction performance. Moreover, our study can provide insights for identifying potential diagnostic and prognostic biomarkers for other biologically heterogeneous cancers.

5. Conclusions

In summary, we identify a novel metabolic genetic prognostic model for cervical cancer prognosis prediction based on the TCGA dataset. According to our systematic and comprehensive studies, prognostic models could provide more accurate evaluations of cervical cancer patients' prognoses.

Availability of Data and Materials

Data are contained within the article.

Author Contributions

XL conceived and designed the experiments, performed the experiments, analyzed the data, contributed materials/analysis tools, prepared figures, authored or reviewed drafts of the paper, approved the final draft. RG analyzed the data, authored or reviewed drafts of the paper, approved the final draft. CW and SC analyzed and interpreted the data, supervised and contributed to the writing. All authors have read and approved the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

We thank all members of our study team for their whole-hearted cooperation and all included participants for their wonderful cooperation.

Funding

This project was funded by the Henan Medical Science and Technique Foundation (Grant no. LHGJ20190353).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.ceog4912273.

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