

Establishment and verification of a prognostic signature associated with fatty acid metabolism in endometrial cancer

LU PENG^{1-3*}, RUI SUN^{1-3*}, TINGTING HAO^{1,2*}, YULONG MU³, QING ZHANG^{1,2},
JIE JIANG^{1,2}, HELGI B. SCHIÖTH⁴ and RUIFEN DONG^{1,2}

¹Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, Shandong 250012, P.R. China;

²Gynecology Oncology Key Laboratory, Qilu Hospital of Shandong University, Jinan, Shandong 250012, P.R. China;

³Department of Clinical Medicine, Medical School of Shandong University, Jinan, Shandong 250012, P.R. China;

⁴Department of Surgical Sciences, Functional Pharmacology and Neuroscience, Uppsala University, Uppsala SE-751 05, Sweden

Received July 23, 2024; Accepted December 13, 2024

DOI: 10.3892/mmr.2025.13444

Abstract. Endometrial carcinoma (EC) is one of the leading causes of mortality in women. Metabolic disorders, such as abnormal fatty acid metabolism (FAM), are considered to be indicators of tumorigenesis. However, to the best of our knowledge, the relationship between EC and FAM remains unclear. The process of FAM is associated with the function of immune cells, thus samples from The Cancer Genome Atlas were grouped according to immune infiltration levels. Subsequently, prognostic gene signatures were constructed based on selected FAM-associated genes. The signature effect was validated, and enrichment analyses were conducted based on sample classification. Nomograms were used to predict survival, merging clinical data and the gene signature. Samples were divided into high- and low-risk groups based on the gene signature. The survival status, clinical characteristics, enrichment analysis and immune infiltration were significantly different between high- and low-risk groups. According to the nomogram, low microsatellite instability-high as well as a high tumor mutation burden can be observed in the low-nomo-score group. Immune checkpoint inhibitor-associated genes were differentially expressed between groups and 35 sensitive compounds were identified. Comprehensive bioinformatics analysis in EC revealed potential roles of FAM in tumorigenesis, the tumor microenvironment and prognosis, suggesting that FAM-associated signatures are promising biomarkers for EC. These findings may improve the understanding of FAM

in EC and pave the way for a more accurate assessment of prognosis and immunotherapy outcomes.

Introduction

Endometrial carcinoma (EC) is the sixth most prevalent cancer among female patients worldwide and the second most common gynecologic malignancy after cervical cancer with notable morbidity (1). In 2022, the estimated number of new cases of EC was 420,242, and the estimated number of deaths was 97,704 (2), which is an upward trend compared with 2020. In 2020, there were estimated to be 417,367 new cases and 97,370 mortalities due to EC worldwide (3). The prevalence of EC has increased with increasing rates of obesity among women globally, suggesting that obesity is a risk factor for this disease (4,5). Excess weight can be attributed to ~41% of cases of uterine cancer (6). Women whose body mass indices are in the normal range have a 3% risk of developing EC in their lifetime. However, the risk of EC increases by >50% for every 5-unit increase in BMI in obese women (6). Prognosis and treatment options for patients with EC are often determined by histological subtyping (7). The recurrent and metastatic nature of this type of cancer is the main reason for the reduced survival rates of patients (7). As a result, early detection, diagnosis and treatment of EC are important for improving the prognosis of EC.

The development of several prognostic models has improved clinical management by evaluating survival, exploring pathogenesis and predicting drug sensitivity. Signatures have been established based on immune-associated (8), methylation-driven (9), autophagy-associated (10) and glycolysis-associated (11) genes. Current research primarily focuses on investigating the possible underlying pathogenesis of EC and the improvement of innovative, cutting-edge treatments. However, conventional treatments such as curative surgery, chemotherapy and radiotherapy are also still being improved.

Recently, immune checkpoint inhibitor (ICI) therapy has become a key element of EC treatment, since immune dysregulation is likely to occur in EC (12). The microsatellite instability-high (MSI-H) subtype accounted for ~30%

Correspondence to: Professor Ruifen Dong, Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, 107 Wenhua Xilu, Jinan, Shandong 250012, P.R. China
E-mail: ruifen.dong@sdu.edu.cn

*Contributed equally

Key words: fatty acid metabolism, endometrial cancer, tumor microenvironment, prognostic signature

of primary ECs. The proportion of MSI-H or mismatch repair-deficient (dMMR) in recurrent ECs is ~13-30% (12-14). It is well known that immunotherapy has more favorable results in patients with EC with MSI-H (15). Embrolizumab, which inhibits the programmed cell death protein 1 (PD-1) receptor, was approved for the treatment of MSI-H or dMMR solid tumors in 2017 by the United States Food and Drug Administration, regardless of the type of cancer (16,17). A recent clinical trial revealed that 44% of patients presenting sporadic MSI-H with recurrent EC responded to pembrolizumab (18). ICIs are transforming EC treatment, with the potential for broader efficacy through combination therapies and biomarker insights (17). Therefore, it is becoming clear that novel predictive biomarkers are needed to guide clinical decision making.

Abnormal metabolism is a hallmark of cancer. Fatty acid metabolism (FAM) contributes to carcinogenesis, progression of numerous diseases, as well as treatment tolerance and resistance through enhancement of anabolism and catabolism of lipids (19). The mechanism associated with the reprogramming of lipid metabolism and cancer development has been investigated. The involvement of enzymes in the pathways of fatty acid production and catabolism (cholesterol and phospholipids) are upregulated in ovarian cancer (20,21). The abnormal metabolism of fatty acids has been implicated in the development of hepatocellular carcinoma (22) and nasopharyngeal carcinoma (23). Renal cell carcinomas and hepatocellular carcinomas often exhibit deregulation of lipid metabolism, including increased *de novo* biosynthesis and breakdown of fatty acids, allowing cancer cells to proliferate and invade the tissue (24).

The utilization of lipids by cancer cells is often influenced by complex interactions between the tumor microenvironment (TME) and the adjacent stroma. Obesity induced by high-fat diet (HFD) reduces the number and impairs the function of CD8⁺ T cells in the TME of mice, accelerating tumor growth (25). As tumor cell-stromal cell interactions intensify during tumor progression, fatty acids secreted into the microenvironment can influence the function and phenotype of infiltrating immune cells (26). FAM also contributes to therapy resistance, including resistance to chemotherapy, radiation therapy, and therapies targeting tumors with complex and diverse mechanisms (19). In response to oxidative stress induced by numerous chemotherapeutic agents, toxic lipid peroxidation can trigger apoptosis and ferroptosis (27,28). Accordingly, FAM-associated genes may serve as novel potential therapeutic targets for numerous types of cancer. To the best of our knowledge, how abnormal fatty acid programming in EC affects tumor and immune cells or how it affects prognosis is currently unknown.

In the present study, a total of 518 tumor samples and 23 normal samples from The Cancer Genome Atlas (TCGA) were divided into high- and low-immune groups based on the immune scores calculated by the single-sample gene set enrichment analysis (ssGSEA) algorithm, and the prognosis-associated FAM differential genes between the two groups were screened to construct a prognostic gene signature. The tumor samples were then divided into high- and low-risk groups based on the FAM-gene signature. A nomogram was established to predict survival, and the relationship between

the nomogram and TME were further explored. In summary, the present study is intended to demonstrate the effect of FAM-associated genes in the development of EC and the influence on prognosis.

Materials and methods

Research data collection. Expression profiles, RNA sequencing data, mutation information and clinical features of samples were downloaded from the open TCGA database (<https://portal.gdc.cancer.gov/>). Samples with incomplete data were excluded. Furthermore, samples with survival times <20 days or >8 years were removed from the present study since their survival results were not relevant to EC. For survival time <20 days, the death was considered to be associated with postoperative complications; for survival time >8 years, the present study considered the cause of death to be unrelated to EC as patients with EC with a survival of ≥5 years are defined clinically cured. Thus, 518 EC samples and 23 normal samples were enrolled for further study. Copy number variation (CNV) data was acquired from the UCSC Xena database (<http://xena.ucsc.edu/>). The MSI data of the samples was downloaded from the Cancer Imaging Archive database (<https://tcia.at/home>).

Acquisition of FAM-associated genes. FAM-associated genes were obtained from two online gene sets and one published gene set. The HALLMARK_FATTY_ACID_METABOLISM gene set, consisting of 158 genes, was downloaded from the Molecular Signatures Database (<http://www.gsea-msigdb.org/gsea/msigdb/index.jsp>). Using ‘fatty acid metabolism’ as the keyword, a total of 543 genes with relevance scores >20 were downloaded from GeneCards (<https://www.genecards.org/>). A total of 92 FAM-associated genes were obtained from a previous study (29), which introduced metabolism-associated signatures based on fatty acid degradation, elongation and biosynthesis. The feasibility of this genetic source has been established (30). A total of 669 genes were obtained after excluding duplicate genes (Table SI).

Immunocorrelation analysis. The ssGSEA algorithm was used to evaluate the immune cell content and immune function of each sample. Based on the evaluation results, the samples were classified into high- and low-immune groups using the R package ‘limma’ (version 3.26.9) with a false discovery rate (FDR) >0.05 and fold change >2. The CIBERSORT algorithm was used to quantify 22 types of tumor-infiltrating immune cells in high- and low-immune samples using the R package ‘CIBERSORT’ (version 1.04) as previously described (31). Microenvironment Cell Populations-Counter (MCPcounter) analysis is a robust quantification method (32), which is based on the R packages ‘MCPcounter’ and ‘limma’. CIBERSORT, MCPcounter and ssGSEA were used to compare the differences in immune cell types and immune-associated functions between the EC and normal samples. The intersection of the three algorithms was considered more reliable, strengthening the confidence in the evidence. The R packages ‘limma’ (version 3.26.9) and ‘ggpubr’ (version 0.6.0) were used to visualize the differential results. The immune cell estimation of each EC sample was also downloaded from Tumor Immune Estimation Resource (TIMER; version 2.0, <https://cistrome.shinyapps.io/timer/>).

Construction and validation of the signature. The common genes between the union of FAM-associated genes and differential genes of the two immune groups were defined as differentially expressed genes (DEGs). A total of 50 genes were identified. The association between prognosis and these 50 genes was analyzed by univariate Cox regression analysis. A total of 22 genes with prognostic values were obtained. Least absolute shrinkage and selection operator (LASSO) Cox regression analysis of prognosis-associated genes was conducted using the R package ‘glmnet’ (version 4.1-8). The risk score for each sample is the sum of the multiplication of the expression values of genes and correlation coefficients, which can be further explained as follows:

$$\text{Risk score} = \sum_{k=1}^n (\text{Exp}_k \times \text{Coef}_k)$$

Where Exp_k indicates the expression level of genes and Coef_k denotes the risk coefficient of genes. The R package ‘survival’ (version 3.7.0) was used for Kaplan-Meier survival analysis, and univariate and multivariate Cox regression analyses were conducted. Additionally, to determine the accuracy and reliability of the risk assessment model, the R package ‘timeROC’ (version 0.4) was used to plot the receiver operating characteristic (ROC) curve and calculate the area under the curve (AUC). The R packages ‘timeROC’ (version 0.4) and ‘rms’ (version 6.9-0) were used to develop a predictive model that merged gene expression values and clinical features. The R package ‘ggDCA’ (version 1.2) was used for the decision curve analysis to evaluate the predictive ability of the nomogram.

Dimensionality reduction methods. The R package ‘scatterplot3d’ (version 0.3-44) was used to conduct principal component analysis (PCA) to improve data visibility. ‘Rtsne’ R package (version 0.17) was employed to conduct t-distributed stochastic neighbor embedding dimension reduction (t-SNE).

Functional enrichment analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were conducted using the R package ‘clusterProfiler’ (version 4.14.4) for the differential genes between the high- and low-immune groups and differential genes between high- and low-risk groups. The R package ‘GSVA’ (version 2.0.2) was used for enrichment analysis of biological functions. The enrichment analysis results were visualized using the R packages ‘enrichplot’ (version 1.13.1.994) and ‘ggplot2’ (version 0.6.0). Statistical significance was defined as an FDR <0.05.

Immunohistochemical (IHC) staining. Human EC tumor samples and normal endometrium tissues were obtained from female patients aged 25-70 who were treated surgically from 2022 to 2023 at the Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University (Jinan, China). The patients diagnosed with EC were the experimental group and patients who did not have EC or a endometrial precancerous lesion but received hysterectomy for other reasons were set as the normal controls. All methods were conducted in accordance with the relevant guidelines and regulations and the experiment was approved by the Ethics Committee of Qilu Hospital of Shandong University (Jinan, China). Clinical

specimens were fixed in 4% paraformaldehyde for 12 h at room temperature, then embedded in paraffin. The specimens were cut into 4- μ m paraffin sections, dewaxed with xylene, hydrated with a descending alcohol series, and endogenous peroxidase activity was blocked according to the instruction (ZSGB-BIO; cat. no. PV-9000). Subsequently, antigen retrieval was carried at 95°C using pH 6.0 sodium citrate and non-specific binding sites were blocked with goat serum (Beyotime Institute of Biotechnology; cat. no. C0265) for 30 min at room temperature. The samples were then washed with PBS. Rabbit anti-PPARG coactivator 1 α antibody (cat. no. A20995; ABclonal Biotech Co., Ltd.) was administered to the slides at a 1:2,000 dilution and slides were incubated at 4°C overnight. The samples were then incubated with horse-radish peroxidase-labeled secondary antibody, according to the instructions of the kit (Mouse/Rabbit Polymer Detection System; ZSGB-BIO; cat. no. PV-9000), at room temperature for 30 min, and treated with diaminobenzidine for 1 min, followed by hematoxylin staining for 5 min at 37°C. Finally, images were captured with an Olympus light microscope (IX71; Olympus Corporation). The IHC images were analyzed using Image-Pro Plus 6.0 (Media Cybernetics, Inc.) to determine the average optical density (AOD), which can be expressed as $\text{AOD} = \text{integrated optical density} / \text{area}$.

Statistical analysis. Bioinformatic analyses were conducted using R (<https://www.r-project.org/>; version 4.4.2) and the RStudio (<https://posit.co/products/open-source/rstudio/>; version 2023.06.2+561.pro5) software. χ^2 test was used to analyze differences in clinical information. Kaplan-Meier analysis and the weight of ‘aalen’ calculated by the R package ‘timeROC’ (version 0.4) were used to conduct survival analyses and draw the ROC curves. Log-rank test was used to calculate the P-value in the survival analysis. The ‘cph’ function from the ‘rms’ package (version 6.9-0) was used for survival calibration in risk model. Analysis of paired data was performed using the paired t-test. Analysis of group data was performed using the Wilcoxon rank sum test and Kruskal-Wallis test (non-parametric) using the ‘stat_compare_means’ algorithm in the ‘ggpubr’ R package (version 0.6.0). An unpaired Student's t-test was conducted for the statistical analysis of the IHC staining. Linear regression and Spearman's rank correlation analysis was performed. All statistical P-values were two-sided, and $P < 0.05$ was considered to indicate a statistically significant difference. The waterfall function of the R package ‘maftools’ (version 3.20) was used to compare the TMB in high- and low-risk groups. Heat maps were created using the R package ‘ComplexHeatmap’ (version 2.22.0).

Results

Immune cluster according to ssGSEA. The immune cell content in each EC sample was calculated using the ssGSEA algorithm. Using this evaluation, samples were clustered into high- and low-immune groups (Fig. 1A). t-SNE further indicated that the clustering was effective and the difference between groups was notable (Fig. 1B). The TME score of each sample and the content of immune cells computed by ssGSEA in the high- and low-immune groups are displayed in Fig. 1C. The analysis indicated that low-immune groups contained

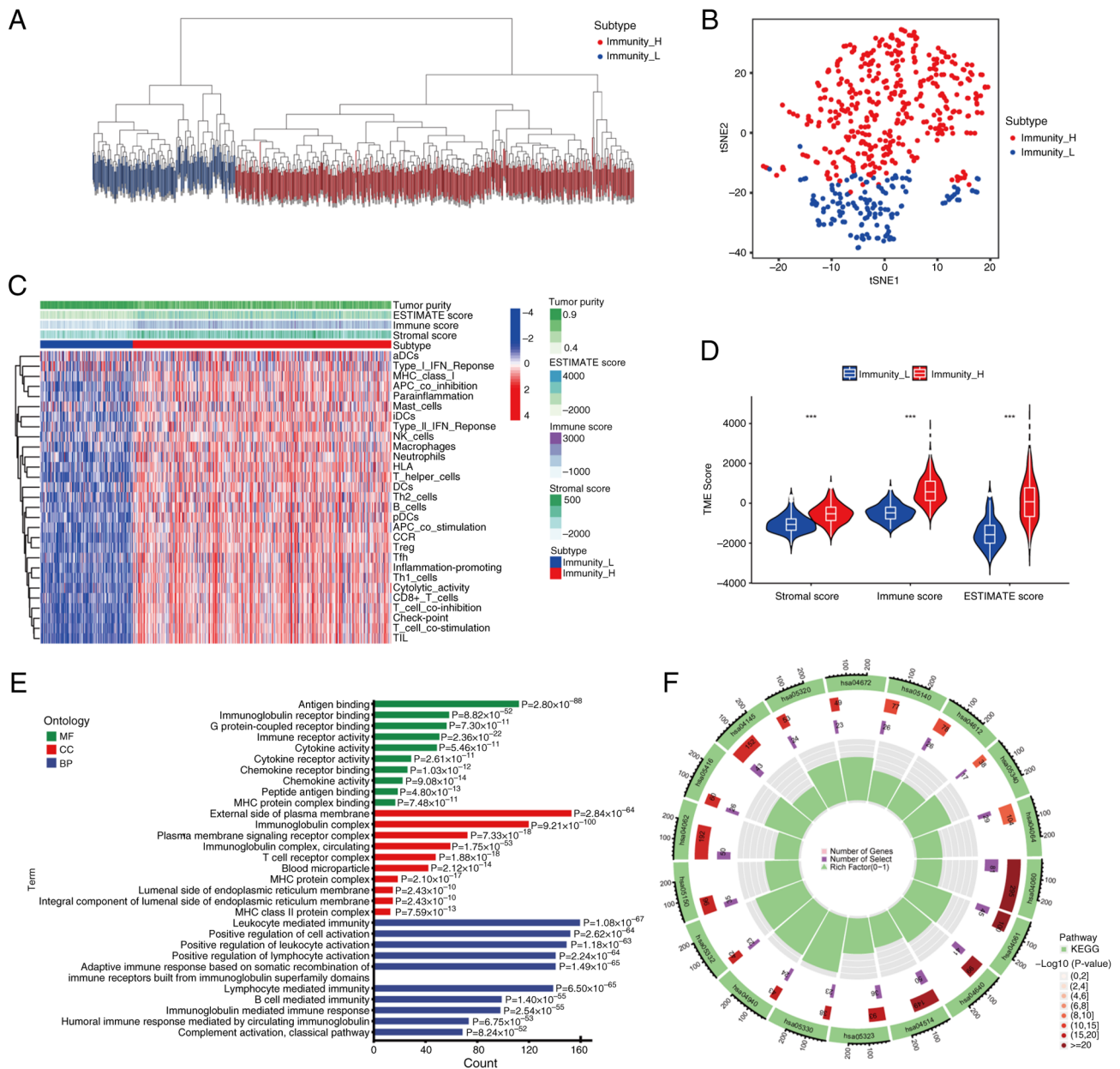


Figure 1. Immune subgroups of samples and their characteristics in the TME. (A) Endometrial carcinoma samples were divided into high- and low-immune groups based on the immune content calculated by single-sample gene set enrichment analysis. (B) tSNE verified the efficiency of grouping indicated by the clustering of the dots in both groups. (C) TME analysis and the immune cell content of each sample arranged by grouping. (D) A violin plot was used to quantify the TME difference between groups. *** $P < 0.001$ (E) Gene Ontology enrichment analysis of the DEGs for MF, CC and BP terms. (F) KEGG enrichment analysis of differentially expressed genes. hsa04672, 'Intestinal immune network for IgA production'; hsa05140, 'Leishmaniasis'; hsa04612, 'Antigen processing and presentation'; hsa05340, 'Primary immunodeficiency'; hsa04064, 'NF-kappa B signaling pathway'; hsa04060, 'Cytokine-cytokine receptor interaction'; hsa04061, 'Viral protein interaction with cytokine and receptor'; hsa04640, 'Hematopoietic cell lineage'; hsa04514, 'Cell adhesion molecules'; hsa05323, 'Rheumatoid arthritis'; hsa05330, 'allograft rejection'; hsa04940, 'Type I diabetes mellitus'; hsa05332, 'Graft-vs.-host disease'; hsa05150, 'Staphylococcus aureus infection'; hsa04062, 'Chemokine signaling pathway'; hsa05416, 'Viral myocarditis'; hsa04145, 'Phagosome'; hsa05320, 'Autoimmune thyroid disease'; tSNE, T-distributed stochastic neighbor embedding; TME, tumor microenvironment; MF, molecular function; CC, cellular component; BP, biological process; Immunity_H, high-immune group; Immunity_L, low-immune group; ESTIMATE, estimation of stromal and immune cells in malignant tumor tissues using expression data; KEGG, Kyoto Encyclopedia of Genes and Genomes.

lower immune cell infiltration and response, resulting in lower immune scores in the TME. There was also a lower stromal score in the low-immune group, which led to a lower ESTIMATE score since this is the sum of the immune and stromal scores. Therefore, the low-immune group had a higher tumor purity. The opposite was true in the high-immune group. For the estimated TME score, quantitative analysis demonstrated the difference in TME between the high- and

low-immune groups in immune, stromal and ESTIMATE scores ($P < 0.001$; Fig. 1D).

Enrichment analysis between groups revealed that the enriched GO molecular function terms were associated with receptor binding. The enriched GO cellular component terms were associated with the plasma membrane, and the enriched biological process terms were associated with 'positive regulation of cell activation' and immunity (Fig. 1E).

KEGG enrichment analysis of DEGs was also conducted, and 'cytokine-cytokine receptor interaction' and 'cell adhesion molecules' were detected (Fig. 1F).

Construction and validation of the prognostic signature. Between the high- and low-immune groups, 2,609 DEGs were identified (Fig. 2A). A total of 669 FAM-associated genes were obtained after deleting duplicate genes from the two online gene sets and one published gene set. Differentially expressed FAM-associated genes were obtained by intersecting the total FAM-associated genes and DEGs from the aforementioned immune cluster, leaving 50 genes (Fig. 2B). Based on Cox regression analysis, 22 prognostic genes were selected (Fig. 2C). Tumor samples from TCGA were randomly divided into training and test cohorts, with an equal number of samples in both groups. Subsequently, clinical manifestation analysis was conducted for patients with clinical manifestations in the two cohorts. Any patients in the unknown group were not analyzed. The analysis revealed that there was no difference in clinical manifestations between the two cohorts (Table I). After LASSO analysis, three genes with the best λ value were chosen to construct the optimum signature (Fig. 2D and E). The risk score of each sample was calculated according to the following formula: Risk score=(expression level of PPARGC1A $\times$ 0.653085171388467) + (expression level of RBP4 $\times$ 0.323298560719871) + (expression level of CRABP2 $\times$ 0.647075550554985).

A series of analyses on the constructor genes, *CRABP2*, *RBP4* and *PPARGC1A*, were conducted. According to TCGA, *CRABP2* had lower expression levels and *RBP4* had higher expression levels in normal samples, but the P-value did not reach significance (Fig. 2F and G). *PPARGC1A* had significantly higher expression levels in normal tissue compared with tumor tissue (Fig. 2H). The mRNA expression levels of *PPARGC1A* were found to be significantly reduced in both carcinoma and adjacent tissue within the same patient (Fig. 2I). The difference in expression levels of *PPARGC1A* among different grades of EC is displayed in Fig. 2J, and the difference in expression levels of *PPARGC1A* among different stages of EC is shown in Fig. 2K. Clinical samples were used to confirm the difference in expression levels of *PPARGC1A* between EC and normal tissue. IHC analysis of 103 EC and 17 endometrium samples revealed higher *PPARGC1A* levels in normal samples (unpaired Student's t-test; Fig. 2L and M). Together, these results suggested that *PPARGC1A* may be an indicator of EC.

Based on the median risk score of 0.93, samples were divided into high-risk and low-risk groups in the training cohort. A significant difference was shown between risk groups in terms of overall survival (OS) and progression-free survival; the low-risk group had an increased survival rate (Fig. 3A and B). The risk curve suggested that as the risk increased, the number of mortalities increased (Fig. 3C). A significant but weak correlation was found between the OS and the risk score based on correlation analysis (Fig. 3D). The time-dependent ROC curve of the prognostic signature in the training cohort indicated a reliable prediction of prognosis in terms of 1-, 3- and 5-year survival (Fig. 3E). An evaluation of the calibration curve also confirmed the effectiveness of the signature (Fig. 3F). The test cohort and the whole tumor

samples were split into high- and low-risk groups based on the same cut-off value as the training cohort. The same analysis was applied in the test cohort (Fig. S1A-F) and all samples (Fig. S2A-F), and the results were consistent with the aforementioned results.

Disruption of FAM can also lead to changes in body weight (6). Therefore, the BMI of each sample in the high- and low-risk groups was investigated. There was no significant difference between the high- and low-risk groups of the training cohort (data not shown); however, a significant difference was observed in the test cohort (Fig. S1G) and all samples (Fig. S2G), indicating that patients in the low-risk group had a higher BMI score. The relationship between abnormal FAM and BMI and EC needs to be further verified.

PCA was conducted on all EC samples. Compared with other classification methods (Fig. 3G-I), the gene signature established displayed differences between groups as the distribution of dots were separated (Fig. 3J), confirming that grouping and demarcation points of the risk score had been selected effectively. Based on univariate logistic analysis, the signature was associated with a poor prognosis (Fig. 3K). According to multivariate logistic analysis, grade and International Federation of Gynecology and Obstetrics (FIGO) stage were independent prognostic factors, while the signature did not have statistical validity (Fig. 3L). Thus, the evidence was insufficient to indicate that the signature was an independent prognostic factor.

Enrichment analysis. Gene set variation analysis (GSVA) of GO and KEGG terms was conducted in high- and low-risk groups. GSVA of GO terms between groups was mainly associated with dendritic regulation and metabolic processes (Fig. S3A; Table SII). The top three pathways of GSVA enrichment on KEGG between groups were 'fatty acid metabolism', 'alpha linolenic acid metabolism' and 'maturity onset diabetes of the young' (Fig. S3B; Table SIII). All enrichment analyses indicated that metabolism and diabetes mellitus were closely associated with EC groups, suggesting that these factors may have an impact on EC progression.

Immunoinfiltration and immune function analysis. An immune-associated analysis was conducted to determine whether high- and low-risk groups had different immune compositions. The MCPcounter method was used to assess the immune cell contents of each group. The levels of three types of immune cells were found to be statistically different between the two groups. Levels of B lineage cells and cytotoxic lymphocytes were higher in the low-risk group, while the level of T cells was higher in the high-risk group (Fig. S3C-E). The immune cell estimation of each EC sample was downloaded from TIMER version 2.0 (<https://cistrome.shinyapps.io/timer/>) (33). The results indicated that B cells, CD4 T cells, CD8 T cells, dendritic cells and macrophages were weakly negatively associated with the risk score, and a significant difference was observed (Fig. S3F-J). CIBERSORT was then used to analyze the differences in immune cell infiltration between high- and low-risk groups. The levels of six immune cell types were different between the groups. The levels of five cell types, including resting dendritic cells, were higher in the low-risk group and the level of activated dendritic cells

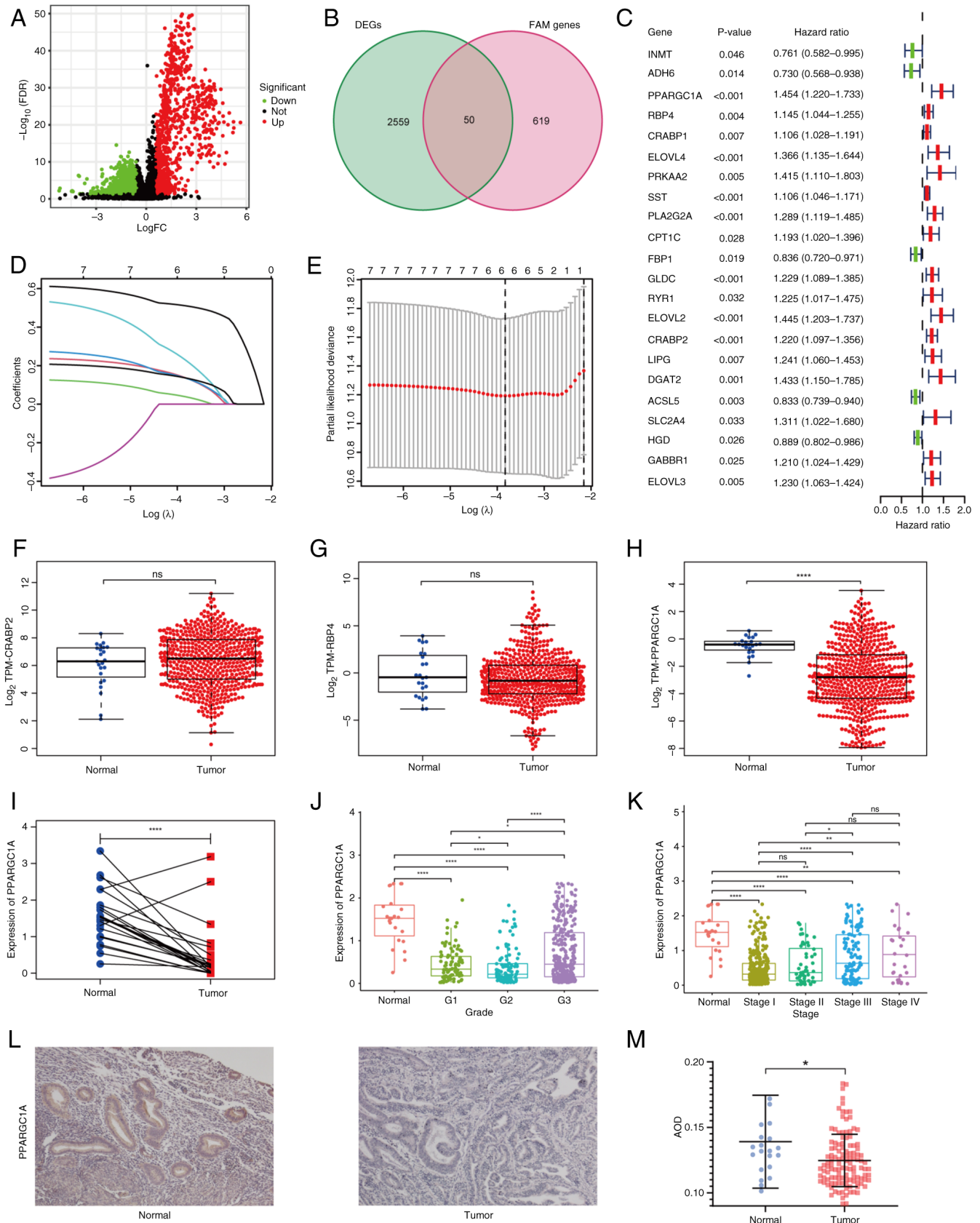


Figure 2. Construction of the prognostic signature. (A) A volcano plot of DEGs between high- and low-immune groups. (B) A Venn diagram of the overlap between DEGs and FAM-associated genes. (C) Prognostic differential FAM-associated genes determined by Cox analysis. (D) LASSO coefficient of differentially expressed FAM-associated genes. (E) Relationship of partial likelihood deviance and $\log(\lambda)$. Expression levels of the signature genes (F) *CRABP2*, (G) *RBP4* and (H) *PPARGC1A* in The Cancer Genome Atlas datasets. (I) *PPARGC1A* expression of paired samples. Relationship of *PPARGC1A* expression and clinical features, including (J) grade and (K) stage. (L) Immunohistochemical staining of *PPARGC1A* in normal and endometrial carcinoma tissue (magnification, $\times 100$). (M) AOD score of *PPARGC1A*. * $P < 0.05$; ** $P < 0.01$; **** $P < 0.001$; DEGs, differentially expressed genes; FAM, fatty acid metabolism; LASSO, least absolute shrinkage and selection operator; AOD, average optical density; FDR, false discovery rate; FC, fold change; TPM, transcripts per million; ns, not significant.

Table I. Comparison of the clinical manifestations between the training and test cohorts.

Clinical features	Test, n (%)	Training, n (%)	P-value
Age, years			
≤60	98 (37.84)	102 (24.88)	0.7863
>60	161 (62.16)	157 (75.12)	
Grade			
1	52 (20.08)	44 (16.99)	0.3752
2	52 (20.08)	64 (24.71)	
3	155 (59.85)	151 (58.30)	
FIGO stage			
I	165 (63.71)	157 (60.62)	0.4354
II	29 (11.20)	23 (8.88)	
III	54 (20.85)	62 (23.94)	
IV	11 (4.25)	17 (6.56)	

χ^2 test was used to analyze the difference in clinical manifestations. FIGO, International Federation of Gynecology and Obstetrics.

was higher in the high-risk group (Fig. S3K). The immune cell infiltration of each sample in the high- and low-risk groups is displayed in Fig. S3L. Furthermore, ssGSEA was conducted to compare immune cell types between high- and low-risk groups (Fig. 4A). A total of 16 immune cell types were found to be significantly different between the low-risk and high-risk groups, with increased clustering in the low-risk group. The results of immune infiltration analysis assessed by four independent algorithms are summarized in Table SIV. The majority of the correlations were negative, which indicated that immune cells were gathered in low-risk groups. T cells counted using MCPcounter and dendritic cells assessed by CIBERSORT exhibited positive correlations. The positive correlation of activated dendritic cells was consistent with the negative correlation of resting dendritic cells. However, the positive correlation of T cells calculated by MCPcounter was contradictory to the finding that all types of T cells exhibited a negative correlation according to the four algorithms and this warrants further investigation.

In addition to immune cells, the association between immune-associated functions and the risk score was also examined. Based on the ssGSEA algorithm, each sample was scored for immune-associated functions, and the scores of samples were displayed based on the high- and low-risk groups (Fig. 4B). Distinct differences in the score were observed between the two groups. The qualitative differences in scores between groups are shown in Fig. 4C. Based on the results, it was concluded that several immune-associated functions were more concentrated in the low-risk group, with the exception of antibody-drug conjugates and type I IFN response. Further investigation of the expression level difference of human leukocyte antigen subtypes between high- and low-risk groups was carried out (Fig. 4D). Of the 24 subtypes studied, 13 were expressed differently between groups and all 13 subtypes were highly expressed in the high-risk group.

The aforementioned results indicated differences in immune infiltration between the high- and low-risk groups. The low-risk group had higher immune content, which indicated that immunotherapy may be beneficial in these patients with EC. The findings also suggested that the created signature could discern between immunity levels, and the cut-off points that were set could identify these differences.

Clinical validation. To determine the effect of age, samples were divided into two groups, <60 and ≥60 years, as a study has shown that the incidence of EC increases significantly over the age of 60 (4). The risk scores were significantly different between the two groups; the ≥60 years group had higher risk scores compared with the <60 years group (Fig. 4E). The differences between grades 1 and 3, and grades 2 and 3 were significant, while there was no difference between grades 1 and 2. Therefore, if grades 1 and 2 are joined into a low-grade group and grade 3 is denoted as a high-grade group, the difference between the high- and low-grade groups is significant (Fig. 4F). Stage I of the FIGO stage was classed as the early stage, and stages II-IV were classed as the late stage. A difference in risk score was observed between the early and late stages; the higher the stage, the higher the risk score (Fig. 4G). The aforementioned results indicated the relationship between the risk score and clinical traits, and they also redefined and divided clinical traits according to the prognostic model to improve alignment of clinical traits with the FAM prognostic signature.

Establishment of the nomogram and comparisons with previous models. Based on the aforementioned analysis, a nomogram, a fusion model of gene expression levels and clinical traits, including age, grade and stage information, was established (Fig. 5A). ROC curves demonstrated considerable prognostic effects for 1, 3 and 5 years with an AUC of 0.772, 0.780 and 0.814, respectively (Fig. 5B). The calibration curve also confirmed the validity of the prediction (Fig. 5C). Numerous prognostic models have been developed to predict factors that may affect prognosis from different perspectives. To compare the effectiveness of the models created in the present study with previously established models, five established models were chosen at random. These were from the studies of Chen *et al* (34), Liu *et al* (35), Yin *et al* (36), Zhang and Yang (37) and Zhang *et al* (38). In terms of 1-, 3- and 5-year predictions, the nomogram of the present study ranked highest and in terms of 1- and 5-year predictions, the signature created in the present study ranked second (Fig. 5D-F). Both the FAM gene-based signature and nomogram model performed well in prognosis prediction, suggesting that FAM serves an important role in EC.

Therapy prediction based on the nomogram. According to the median number of total scores from the nomogram, the samples were evenly divided into high- and low-nomo-score groups. Genes were mutated at different frequencies in samples. For the 20 genes with the highest mutation rates, the mutation percentage of the low-nomo-score group was higher than that of the high-nomo-score group, except for *TP53* (Fig. 5G). The *TP53* mutation occurred at a rate of 14% in the low-nomo-score samples, whereas the mutation rate was 56%

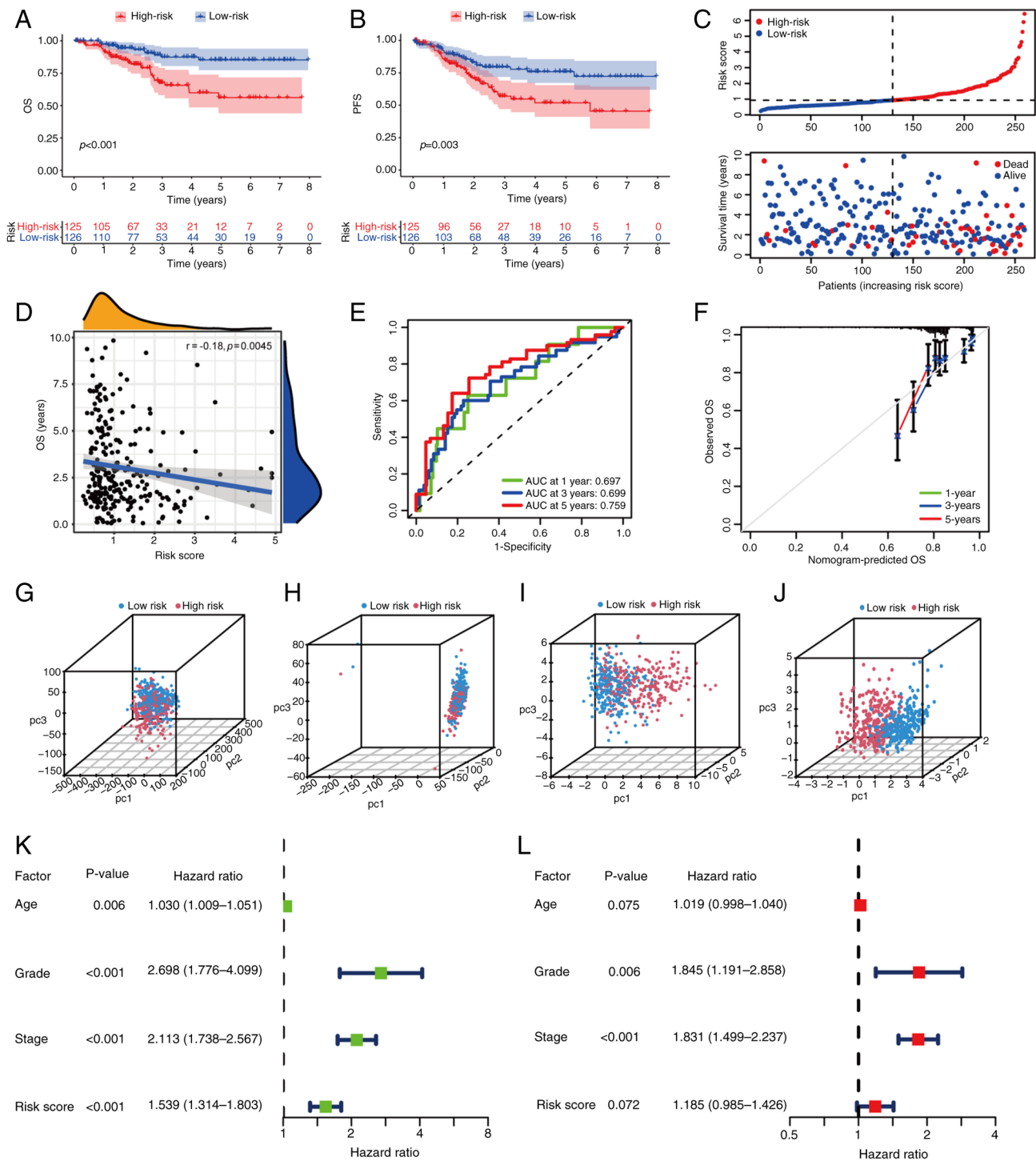


Figure 3. Clinical characteristics and prognosis analysis of the training group. (A) OS and (B) PFS comparison between high- and low-risk score groups in the training cohort. (C) Risk score distribution in high- and low-risk groups. (D) Correlation analysis of OS and risk score. (E) Receiver operating characteristic curve of the signature at 1, 3 and 5 years in the training group. The AUC indicates the accuracy of prediction. (F) Calibration curve of the prediction at 1, 3 and 5 years in the training group. PCA of all patients with EC for (G) all genes, (H) all 2,609 differentially expressed genes and (I) all 669 FAM-associated genes. (J) PCA of all patients with EC in the gene signature. (K) Univariate and (L) multivariate analyses of common clinical characteristics and risk scores. OS, overall survival; PFS, progression-free survival; AUC, area under the curve; PCA, principle component analysis; EC, endometrial carcinoma; pc, principal component; FAM, fatty acid metabolism.

in the high-nomo-score samples, with missense mutations accounting for the majority of mutations. The three genes with the highest mutation rates were *PTEN*, *PIK3CA* and *ARID1A* in both high and low nomo-score groups. The TMB of samples from different nomo groups was then calculated. In general, the low-nomo-score group had a higher TMB, with a statistically

significant difference (Fig. 5H). Samples were divided into high- and low-TMB groups according to the median value of TMB and survival analysis demonstrated that the high-TMB group had a higher survival probability (Fig. 5I), which was consistent with the established conclusion that a high TMB is associated with prolonged survival after immune checkpoint

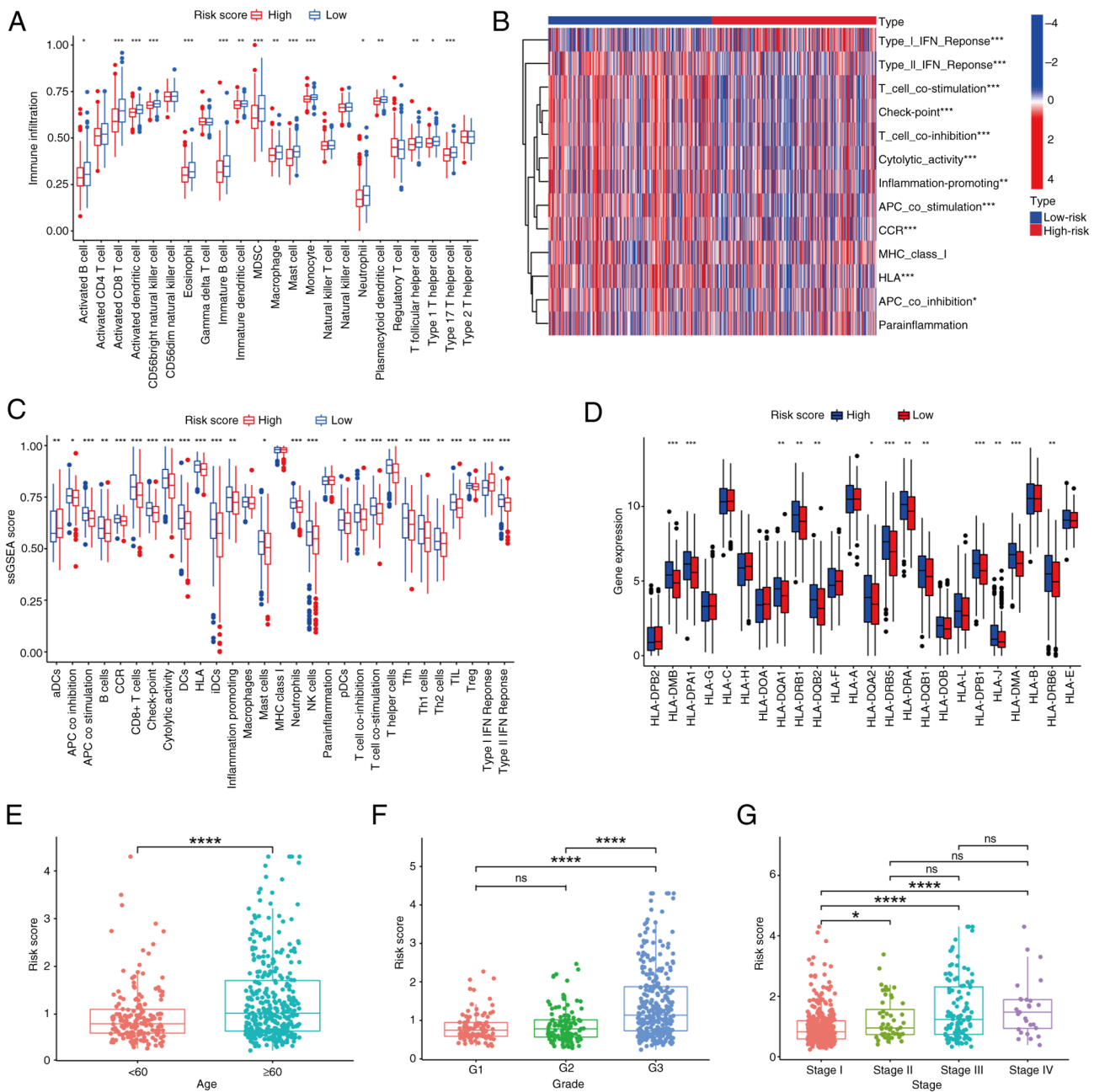


Figure 4. Immune distribution between groups. (A) Immune cell infiltration calculated by ssGSEA. (B) Content of immune cells in each sample calculated by ssGSEA. (C) Samples in the high- and low-risk groups scored according to the ssGSEA algorithm to compare the score difference between the two groups. (D) Expression levels of HLA in high- and low-risk groups. Distribution of risk scores in different (E) age, (F) grade and (G) stage groups. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$. ns, not significant; HLA, human leukocyte antigen; ssGSEA, single-sample gene set enrichment analysis.

inhibitor (ICI) treatment in several types of cancer (39). The combination of TMB classification and nomo-risk showed a higher survival rate for patients with a high TMB and a low nomo-risk score. Furthermore, patients with low TMB and high nomo-risk score had the least favorable outcomes (Fig. 5J). In summary, the aforementioned results indicated that low-nomo-score groups have a higher TMB, thus predicting an improved outcome of immunotherapy.

As ICI therapy strategies have been discussed previously (17), the relationship between the nomo-score and ICI genes was investigated. A total of 34 ICI-associated genes were identified and the results of correlation analysis revealed that ICI genes were correlated with the nomogram

(Fig. 5K). Furthermore, the expression levels of ICI genes in the high- and low-nomo-risk groups were calculated. There were 17 genes with different expression levels between groups (Fig. 5L). Thus, a prevalent relationship between the ICI genes and the nomogram was observed. The MSI of EC also affected the efficacy of immunotherapy. The MSI-H group had a lower nomo-score, indicating improved immunotherapy efficacy for the low-risk group (Fig. 5M and N). All results indicated that low-nomo-score groups had improved survival rates and responded effectively to immunotherapy. A prediction of drug sensitivity was conducted based on gene signatures and nomo-score groups using the R package 'pRRophetic'. There were 35 predicted sensitive drugs in both categories,

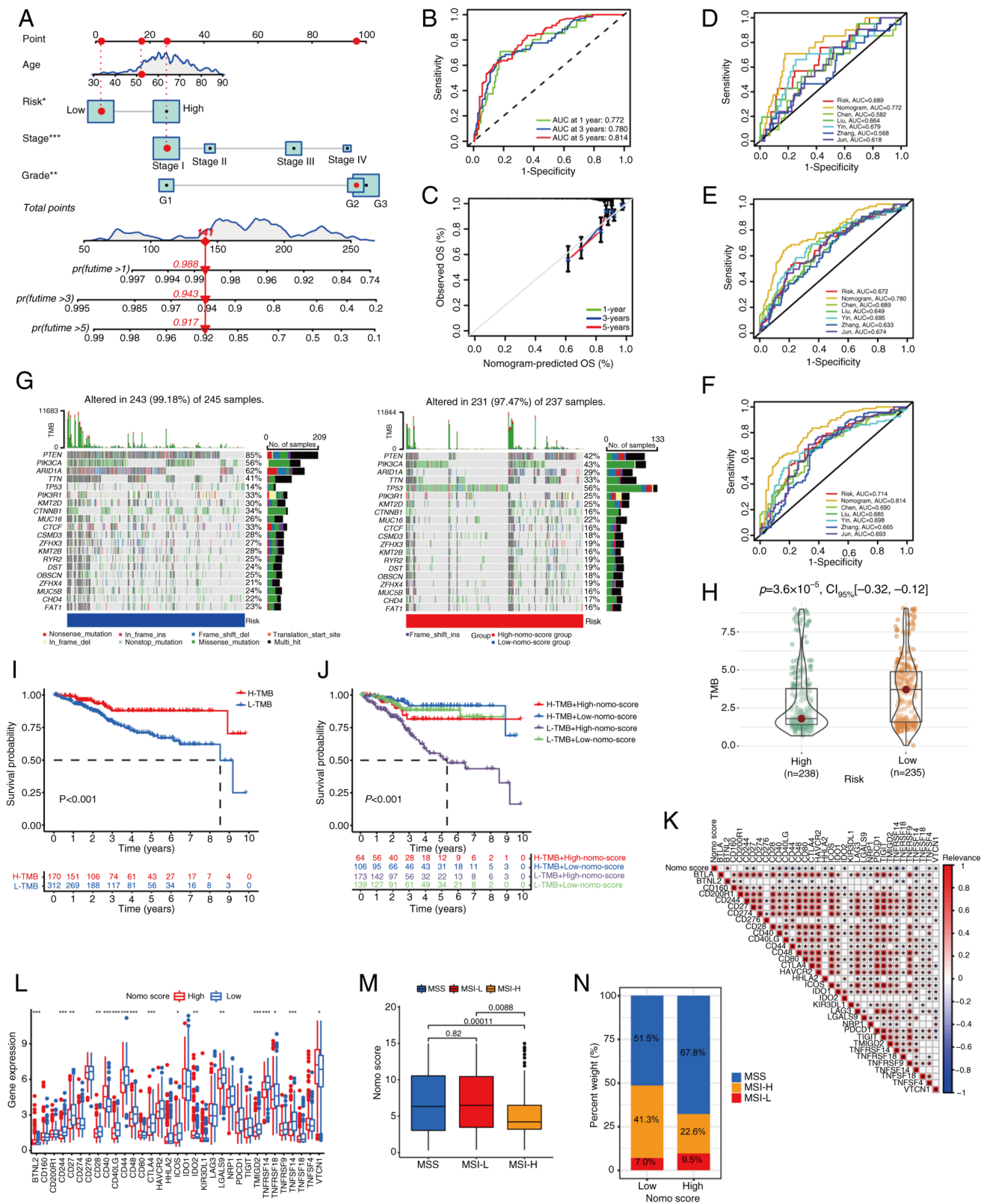


Figure 5. Establishment of a nomogram and therapy prediction based on the nomogram. (A) Nomogram comprising age, grade, stage and risk score. Patients received a score based on their clinical information and risk score to predict 1-, 3- and 5-year survival rates. (B) Receiver operating characteristic curves for the prediction of 1-, 3- and 5-year survival rates. (C) Calibration curve to explicate the prediction accuracy. Comparison between the models built in the present study and existing models for (D) 1, (E) 3 and (F) 5 years. (G) Gene mutation rate of each sample in the high-nomo-score and low-nomo-score groups. The top 20 genes with the highest mutation rate were listed. (H) Scatter plot of the TMB of samples in the two groups. (I) Survival analysis of samples with high and low TMB. (J) Samples were divided into four groups according to the nomo-score and TMB. The survival status was significantly different among groups. (K) The relevance between ICI-associated genes and nomo score. (L) Differences in ICI-associated gene expression levels between the high- and low-nomo-score groups. (M) MSI subtypes of samples and nomo scores. (N) MSI subtype composition in high- and low-nomo-score groups. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ OS, overall survival; TMB, tumor mutation burden; ICI, immune checkpoint inhibitor; MSI, microsatellite instability; AUC, area under the curve; nomo, nomogram; ins, insertion; del, deletion; H-, high; L-, low; MSI-H, high microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stability.

and 29 of them were identified in both the gene signature and nomo-score groups (Table SV). These results demonstrated that the nomo-score evaluation system could predict the survival status and therapy effect, and that a low nomo-score indicated improved outcomes.

Discussion

Abnormal FAM can affect the progression of EC, which may be mediated by the gut microbial profile (40); however, its interaction with immunity is rarely described. In the present study, a comprehensive analysis of the role of FAM-associated genes in EC samples was conducted. The impact was discussed from perspectives such as CNVs, somatic mutations, expression levels, interactions between genes and influence on survival. Tumor samples were grouped into high- and low-immune groups according to the ssGSEA score. A common feature of DEGs between immune groups and FAM-associated genes was the presence of 50 differentially expressed FAM-associated genes, 22 of which were associated with prognosis. Three genes, *PPARGC1A*, *RBP4* and *CRABP2*, were selected to establish prognostic signatures. Following the categorization of tumor samples into high- and low-risk groups based on the signature formula, a comprehensive set of analyses were undertaken to validate the efficacy of the signature. Significant differences were observed in the distribution of risk scores and survival rates between the groups, where the low-risk group exhibited more pronounced immune infiltration. Furthermore, differential expression of ICI-associated genes was observed between the groups, which led to the identification of 35 sensitive compounds.

PPARGC1A promotes apoptosis and inhibits proliferation in breast cancer (41). *RBP4*, a fatty acid-binding protein (42), is implicated in the pathogenesis of endometriosis by enhancing endometrial stromal cells viability, proliferation and invasion (43). In endometrioid endometrial adenocarcinoma, *RBP4* has been identified as one of the six hub genes associated with survival and may serve as a potential target of immune therapy (44). *CRABP2* directs retinoic acid toward the retinoic acid receptor (RAR), resulting in growth arrest and apoptosis (45). Resveratrol interferes with the reprogramming of the retinoic acid signaling pathway in decidualized human endometrial stromal cells (HESCs) by accelerating the downregulation of cellular *CRABP2* and RAR (46). HESCs express *CRBP1*, an intracellular carrier protein for retinol, and *RBP4*, a blood carrier protein for retinol, which can function as a paracrine messenger (47). The aforementioned studies suggest that decidual transformation, the *CRBP1*-RAR pathway and retinoic acid may contribute to the pathogenesis and progression of EC.

Tumor samples were separated into high- and low-risk groups according to the signature formula. The difference in risk score distribution and survival rates between groups were significant. The 5-year survival rate of patients was sufficiently predicted by this model. Samples in the two groups had different clinical manifestations. In the majority of cases, immune cells were negatively correlated with the signature, which means that low-risk score groups exhibited increased immune infiltration. An IHC study on endometrioid adenocarcinoma demonstrated that the expression levels of

CD3 (T lymphocytes), CD57 (natural killer cells) and CD68 (macrophages) were higher in the optimal outcome group compared with the poor outcome group. The two groups did not significantly differ in terms of CD20 (B lymphocytes) and S100 (dendritic cells) expression (48). To the best of our knowledge, no studies have confirmed the relationship between immune cell activation status and EC, which needs further exploration.

The model in the present study was made more practical by including age, grade and stage of EC, thus a nomogram was established. According to bioinformatics analysis, the TMB is an important prognostic factor for EC (49). Thus, the TMB was compared between the two nomo-score groups. The low-score group had a higher TMB and improved survival rates. However, contradictory to this, *TP53* had a lower mutation rate in the low-score group. Pan-cancer analysis on the mutation rate of *TP53* indicated that *TP53* mutations result in poor survival prognosis in uterine EC (50). A high mutation rate of *TP53* was observed in the high-score group, which indicated poor survival rates. Analysis of MSI also demonstrated that samples in the low-score group were more inclined to MSI-H and thus had improved responses to immunotherapy. As for ICI, it can be used both as a monotherapy and in combination with cytotoxic chemotherapy, other immunotherapy or as targeted agents (51). The *PD-L1* (also known as CD274) and cytotoxic T-lymphocyte-associated antigen 4 (*CTLA-4*) pathways are the two main targets of ICI (52,53). The expression levels of *PD-L1* and *CTLA-4* were significantly different between the high- and low-nomo-score groups. The correlation between gene expression levels and the nomogram was also validated.

Both the gene signature and nomogram model were associated with 35 sensitive drugs, 29 of which were associated with both. Clinically common anticancer drugs, such as dasatinib and tamoxifen, were identified among the 29 sensitive components. Foretinib was also identified, and this inhibits hepatocyte growth factor/Met signaling in EC cell lines. This pathway is typically stimulated in an autocrine manner and is relevant for cell survival, inducing p53-dependent apoptosis in EC cell lines *in vitro* (54). Since *TP53* had an unusual mutation rate in samples, this finding should be investigated in future studies. These findings demonstrated that the model constructed in the present study could be feasible for clinical applications, providing appropriate treatment options and relatively accurate prediction of prognosis.

In conclusion, the present study demonstrated that different FAM modification patterns contributed to the heterogeneity and complexity of individual TMEs. In patients with EC, the FAM score is a promising biomarker for determining prognosis, molecular subtypes, TME infiltration characteristics and immunotherapy effects. The present study has limitations, despite extensive analysis. The data in the Gene Expression Omnibus lack survival information, thus only TCGA data were used, limiting the sample size. In addition, the genes found in the present study have not been fully verified *in vitro*.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Nature Science Foundation of China (grant no. 81902657), Natural Science Foundation of Shandong Province (grant no. ZR2020MH271) and China Postdoctoral Science Foundation (grant no. 2021M691939).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LP and RD designed the present study. LP conducted the bioinformatics analysis, while RS and TH were responsible for the immunohistochemistry. LP and YM collaborated on writing the content, creating the figures and conducting the experiments. QZ funded the present study and was responsible for clinical data collection. JJ and HBS were responsible for the interpretation of data and revising the manuscript. All authors read and approved the final version of the manuscript. LP, YM and QZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The human research was approved by the Ethics Committee of Qilu Hospital of Shandong University (approval no. KYLL-202210-055-1; Jinan, China). All patients participating in the present study provided written informed consent.

Patient consent for publication

All patients provided written consent for their information to be published.

Competing interests

The authors declare that they have no competing interests.

References

- Makker V, MacKay H, Ray-Coquard I, Levine DA, Westin SN, Aoki D and Oaknin A: Endometrial cancer. *Nat Rev Dis Primers* 7: 88, 2021.
- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A: Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 74: 229-263, 2024.
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71: 209-49, 2021.
- Lu KH and Broaddus RR: Endometrial cancer. *N Engl J Med* 383: 2053-2064, 2020.
- Morice P, Leary A, Creutzberg C, Abu-Rustum N and Darai E: Endometrial cancer. *Lancet* 387: 1094-1108, 2016.
- Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA and Smeeth L: Body-mass index and risk of 22 specific cancers: A population-based cohort study of 5.24 million UK adults. *Lancet* 384: 755-765, 2014.
- Urick ME and Bell DW: Clinical actionability of molecular targets in endometrial cancer. *Nat Rev Cancer* 19: 510-521, 2019.
- Cheng Y, Li X, Dai Y, Dong Y, Yang X and Wang J: Identification of an immune-related risk signature and nomogram predicting the overall survival in patients with endometrial cancer. *J Gynecol Oncol* 32: e30, 2021.
- Liu J, Ji C, Wang Y, Zhang C and Zhu H: Identification of methylation-driven genes prognosis signature and immune microenvironment in uterus corpus endometrial cancer. *Cancer Cell Int* 21: 365, 2021.
- Wang H, Ma X, Liu J, Wan Y, Jiang Y, Xia Y and Cheng W: Prognostic value of an autophagy-related gene expression signature for endometrial cancer patients. *Cancer Cell Int* 20: 306, 2020.
- Yang X, Li X, Cheng Y, Zhou J, Shen B, Zhao L and Wang J: Comprehensive analysis of the glycolysis-related gene prognostic signature and immune infiltration in endometrial cancer. *Front Cell Dev Biol* 9: 797826, 2021.
- Cancer Genome Atlas Research Network; Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, *et al*: Integrated genomic characterization of endometrial carcinoma. *Nature* 497: 67-73, 2013.
- Prendergast EN, Holman LL, Liu AY, Lai TS, Campos MP, Fahey JN, Wang X, Abdelaal N, Rao JY, Elvin JA, *et al*: Comprehensive genomic profiling of recurrent endometrial cancer: Implications for selection of systemic therapy. *Gynecol Oncol* 154: 461-466, 2019.
- Soumerai TE, Donoghue MTA, Bandlamudi C, Srinivasan P, Chang MT, Zammarin D, Cadoo KA, Grisham RN, O'Ceirbhail RE, Tew WP, *et al*: Clinical utility of prospective molecular characterization in advanced endometrial cancer. *Clin Cancer Res* 24: 5939-5947, 2018.
- O'Malley D, Bariani GM, Cassier PA, Marabelle A, Hansen AR, De Jesus Acosta A, Miller WH Jr, Safra T, Italiano A, Mileskin L, *et al*: Pembrolizumab in patients with microsatellite instability-high advanced endometrial cancer: Results from the KEYNOTE-158 study. *J Clin Oncol* 40: 752-761, 2022.
- du Rusquec P, de Calbiac O, Robert M, Campone M and Frenel JS: Clinical utility of pembrolizumab in the management of advanced solid tumors: An evidence-based review on the emerging new data. *Cancer Manag Res* 11: 4297-4312, 2019.
- Mutlu L, Harold J, Tymon-Rosario J and Santin AD: Immune checkpoint inhibitors for recurrent endometrial cancer. *Expert Rev Anticancer Ther* 22: 249-258, 2022.
- Bellone S, Roque DM, Siegel ER, Buza N, Hui P, Bonazzoli E, Guglielmi A, Zammataro L, Nagarkatti N, Zaidi S, *et al*: A phase II evaluation of pembrolizumab in recurrent microsatellite instability-high (MSI-H) endometrial cancer patients with Lynch-like versus MLH-1 methylated characteristics (NCT02899793). *Ann Oncol* 32: 1045-1046, 2021.
- Hoy AJ, Nagarajan SR and Butler LM: Tumour fatty acid metabolism in the context of therapy resistance and obesity. *Nat Rev Cancer* 21: 753-766, 2021.
- Ji Z, Shen Y, Feng X, Kong Y, Shao Y, Meng J, Zhang X and Yang G: Deregulation of lipid metabolism: The critical factors in ovarian cancer. *Front Oncol* 10: 593017, 2020.
- Yoon H and Lee S: Fatty acid metabolism in ovarian cancer: Therapeutic implications. *Int J Mol Sci* 23: 2170, 2022.
- Darwish NM, Elshaer MMA, Almutairi SM, Chen TW, Mohamed MO, Ghaly WBA and Rasheed RA: Omega-3 polyunsaturated fatty acids provoke apoptosis in hepatocellular carcinoma through knocking down the STAT3 activated signaling pathway: In vivo and in vitro study. *Molecules* 27: 3032, 2022.
- Tang M, Dong X, Xiao L, Tan Z, Luo X, Yang L, Li W, Shi F, Li Y, Zhao L, *et al*: CPT1A-mediated fatty acid oxidation promotes cell proliferation via nucleoside metabolism in nasopharyngeal carcinoma. *Cell Death Dis* 13: 331, 2022.
- Chen J, Chen J, Huang J, Li Z, Gong Y, Zou B, Liu X, Ding L, Li P, Zhu Z, *et al*: HIF-2 α upregulation mediated by hypoxia promotes NAFLD-HCC progression by activating lipid synthesis via the PI3K-AKT-mTOR pathway. *Aging (Albany NY)* 11: 10839-10860, 2019.
- Ringel AE, Drijvers JM, Baker GJ, Catozzi A, García-Cañaveras JC, Gassaway BM, Miller BC, Juneja VR, Nguyen TH, Joshi S, *et al*: Obesity shapes metabolism in the tumor microenvironment to suppress anti-tumor immunity. *Cell* 183: 1848-1866, 2020.
- Corn KC, Windham MA and Rafat M: Lipids in the tumor microenvironment: From cancer progression to treatment. *Prog Lipid Res* 80: 101055, 2020.

27. Rysman E, Brusselmans K, Scheys K, Timmermans L, Derua R, Munck S, Van Veldhoven PP, Waltregny D, Daniëls VW, Machiels J, *et al*: De novo lipogenesis protects cancer cells from free radicals and chemotherapeutics by promoting membrane lipid saturation. *Cancer Res* 70: 8117-8126, 2010.
28. Hajjaji N and Bougnoux P: Selective sensitization of tumors to chemotherapy by marine-derived lipids: A review. *Cancer Treat Rev* 39: 473-488, 2013.
29. Yang C, Huang X, Liu Z, Qin W and Wang C: Metabolism-associated molecular classification of hepatocellular carcinoma. *Mol Oncol* 14: 896-913, 2020.
30. Ding C, Shan Z, Li M, Chen H, Li X and Jin Z: Characterization of the fatty acid metabolism in colorectal cancer to guide clinical therapy. *Mol Ther Oncolytics* 20: 532-544, 2021.
31. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M and Alizadeh AA: Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 12: 453-457, 2015.
32. Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, Selves J, Laurent-Puig P, Sautès-Fridman C, Fridman WH and de Reyniès A: Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol* 17: 218, 2016.
33. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B and Liu XS: TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res* 48: W509-W514, 2020.
34. Chen Y, Liao Y, Du Q, Shang C, Qin S, Lee K, Zou Q, Liu J and Yao S: Roles of pyroptosis-related gene signature in prediction of endometrial cancer outcomes. *Front Med (Lausanne)* 9: 822806, 2022.
35. Liu J, Wang Y, Meng H, Yin Y, Zhu H and Ni T: Identification of the prognostic signature associated with tumor immune micro-environment of uterine corpus endometrial carcinoma based on ferroptosis-related genes. *Front Cell Dev Biol* 9: 735013, 2021.
36. Yin W, Liao F, Chen M, Qin X, Lai H, Lin Y and Yao D: Immune infiltration and a ferroptosis-associated gene signature for predicting the prognosis of patients with endometrial cancer. *Aging (Albany NY)* 13: 16713-16732, 2021.
37. Zhang X and Yang Q: A pyroptosis-related gene panel in prognosis prediction and immune microenvironment of human endometrial cancer. *Front Cell Dev Biol* 9: 705828, 2021.
38. Zhang J, Wang Z, Zhao R, An L, Zhou X, Zhao Y and Wang H: An integrated autophagy-related gene signature predicts prognosis in human endometrial cancer. *BMC Cancer* 20: 1030, 2020.
39. Valero C, Lee M, Hoen D, Wang J, Nadeem Z, Patel N, Postow MA, Shoushtari AN, Plitas G, Balachandran VP, *et al*: The association between tumor mutational burden and prognosis is dependent on treatment context. *Nat Genet* 53: 11-15, 2021.
40. Zhao SS, Chen L, Yang J, Wu ZH, Wang XY, Zhang Q, Liu WJ and Liu HX: Altered gut microbial profile accompanied by abnormal fatty acid metabolism activity exacerbates endometrial cancer progression. *Microbiol Spectr* 10: e0261222, 2022.
41. Zu Y, Chen XF, Li Q, Zhang ST and Si LN: PGC-1 α activates SIRT3 to modulate cell proliferation and glycolytic metabolism in breast cancer. *Neoplasma* 68: 352-361, 2021.
42. Perduca M, Nicolis S, Mannucci B, Galliano M and Monaco HL: Human plasma retinol-binding protein (RBP4) is also a fatty acid-binding protein. *Biochim Biophys Acta Mol Cell Biol Lipids* 1863: 458-466, 2018.
43. Lee JC, Kim SH, Oh YS, Kim JH, Lee SR and Chae HD: Increased expression of retinol-binding protein 4 in ovarian endometrioma and its possible role in the pathogenesis of endometriosis. *Int J Mol Sci* 22: 5827, 2021.
44. Chen B, Wang D, Li J, Hou Y and Qiao C: Screening and identification of prognostic tumor-infiltrating immune cells and genes of endometrioid endometrial adenocarcinoma: Based on the cancer genome atlas database and bioinformatics. *Front Oncol* 10: 554214, 2020.
45. Schug TT, Berry DC, Shaw NS, Travis SN and Noy N: Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. *Cell* 129: 723-733, 2007.
46. Ochiai A, Kuroda K, Ozaki R, Ikemoto Y, Murakami K, Muter J, Matsumoto A, Itakura A, Brosens JJ and Takeda S: Resveratrol inhibits decidualization by accelerating downregulation of the CRABP2-RAR pathway in differentiating human endometrial stromal cells. *Cell Death Dis* 10: 276, 2019.
47. Pavone ME, Malpani S, Dyson M and Bulun SE: Altered retinoid signaling compromises decidualization in human endometriotic stromal cells. *Reproduction* 154: 207-216, 2017.
48. Zinovkin D and Pranjal MZ: Tumor-infiltrated lymphocytes, macrophages, and dendritic cells in endometrioid adenocarcinoma of corpus uteri as potential prognostic factors: An immunohistochemical study. *Int J Gynecol Cancer* 26: 1207-1212, 2016.
49. Zhang J, An L, Zhou X, Shi R and Wang H: Analysis of tumor mutation burden combined with immune infiltrates in endometrial cancer. *Ann Transl Med* 9: 551, 2021.
50. Li VD, Li KH and Li JT: TP53 mutations as potential prognostic markers for specific cancers: analysis of data from the cancer genome atlas and the international agency for research on cancer TP53 database. *J Cancer Res Clin Oncol* 145: 625-636, 2019.
51. Green AK, Feinberg J and Makker V: A review of immune checkpoint blockade therapy in endometrial cancer. *Am Soc Clin Oncol Educ Book* 40: 1-7, 2020.
52. Oyewole-Said D, Konduri V, Vazquez-Perez J, Weldon SA, Levitt JM and Decker WK: Beyond T-cells: Functional characterization of CTLA-4 expression in immune and non-immune cell types. *Front Immunol* 11: 608024, 2020.
53. Chaudhri A, Xiao Y, Klee AN, Wang X, Zhu B and Freeman GJ: PD-L1 binds to B7-1 only in cis on the same cell surface. *Cancer Immunol Res* 6: 921-929, 2018.
54. Kogata Y, Tanaka T, Ono YJ, Hayashi M, Terai Y and Ohmichi M: Foretinib (GSK1363089) induces p53-dependent apoptosis in endometrial cancer. *Oncotarget* 9: 22769-22784, 2018.



Copyright © 2025 Peng et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.