Identification of a novel immune landscape signature for predicting prognosis and response of endometrial carcinoma to immunotherapy and chemotherapy

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Abstract

**Background:** Uterine Corpus Endometrial Carcinoma (UCEC) is the most common gynecological cancer. Here, we have investigated the significance of immune-related genes in predicting the prognosis and response of UCEC patients to immunotherapy and chemotherapy.

**Methods:** Based on TCGA database, the single-sample gene-set enrichment analysis (ssGSEA scores) was utilized to obtain enrichment of 29 immune signatures. Univariate, multivariate Cox regression and LASSO regression analyses were performed to generate an immune-related prognostic signature (IRPS). The biological functions of IRPS-associated genes were evaluated using GSEA, TIMER Database analysis, Mutation analysis, IPS analysis, Gene Expression Profiling Interactive Analysis (GEPIA), Genomics of Drug Sensitivity in Cancer (GDSC) and Immune Cell Abundance Identifier (ImmuCellAI). Potential small molecule drugs for UCEC were predicted using the connectivity map (Cmap). The mRNA and protein expression levels of IRPS-associated genes were tested via quantitative real-time PCR (qPCR) and immunohistology.

**Results:** Two immune-related genes (CCL13 and KLRC1) were identified to construct the IRPS. Both genes were related to the prognosis of UCEC patients (P<0.05). The IRPS could distinguish patients with different prognosis and was closely associated with the infiltration of several types of immune cells. Our findings showed that patients with low IRPS benefited more from immunotherapy and developed stronger response to several chemotherapies, which was also confirmed by the results of ImmuCellAI.
Finally, we identified three small molecular drugs that might improve the prognosis of patients with high IRPS.

**Conclusion:** IRPS can be utilized to predict the prognosis of UCEC patients and provide valuable information about their therapeutic response to immunotherapy and chemotherapy.

**Keywords:** Endometrial Carcinoma, Prognosis, Tumor immune microenvironment, Immunotherapy
1. Introduction

Uterine Corpus Endometrial Carcinoma (UCEC) is the most common gynecological cancer. In 2018, 382,069 new cases and 89,929 deaths were reported worldwide (1). Despite the emergence of targeted therapy and immunological therapy, the incidence and mortality of UCEC have shown a consistent increase (2). The overall 5-year survival rate can reach 75-86% (3), however, the survival time of patients with cancer metastasis or recurrence after treatment may drop below 16 weeks (4). Besides, the therapeutic regimens such as immunotherapy and chemotherapy are mainly designed according to the clinical stage of patients and regardless of the patients’ varying responses. Therefore, it is an urgent need of the scientific community to build a new prognostic model to identify patients that are at a high risk and suitable for certain regimens.

Surgery is the most preferred route to treat UCEC, commonly supported by radiotherapy and chemotherapy that are designed according to histopathologic parameters of the patients. Surprisingly, chemotherapy may exert different or even opposite effects on patients with identical pathological grade. Furthermore, there is limited evidence regarding the type of patients who can draw benefit from chemotherapy. To further complicate this, immunotherapy can trigger strong response in patients with DNA polymerase ε (POLE) mutation, microsatellite instability (MSI) and high-tumor mutational burden (TMB), however, the difficulty of assessing these factors makes them unsuitable as prognostic markers.
Recently, the tumor immune microenvironment (TIME) and infiltration of immune cells have been found to be associated with cancer development, prognosis and therapeutic response (5-8). Immune and stromal cells play critical roles in cancer biology. Immune related genes may regulate the infiltration of immune cells, a process that has close correlation with immunotherapeutic response (9). Therefore, immune-related genes may be utilized predict the prognosis and therapeutic response of UCEC patients. In this study, we identified two immune-related genes with significant prognostic value, and developed a model for predicting the survival and therapeutic response of UCEC patients.
2. Materials and methods

2.1 Data sources and Clustering

Downloaded from the Cancer Genome Atlas (TCGA) database were data about mRNA expression data of UCEC patients and clinical characteristics, including age, tumor grade, histological type and clinical stage from TCGA (http://cancergenome.nih.gov/) on Dec 1, 2019. All the data were derived from 552 tumor cases and 23 normal cases. Cases with incomplete information were excluded. The tumor purity, infiltration level and stromal content were calculated through the ESTIMATE method (10). ssGSEA scores were used to obtain the enrichment level of 29 immune signatures in each UCEC tissue and to perform hierarchical clustering of UCEC (11, 12). The total workflow is as shown in the following figure (Fig. 1).
A total of 15 UCEC specimens and 15 adjacent tissues were obtained from patients at the Wuxi Maternal and Child Health Hospital, the Affiliated Hospital to Nanjing Medical University from 2018-2019 and routine written informed consent was obtained from all patients.
2.2 GO and KEGG analyses

We performed functional enrichment analyses to investigate the potential mechanisms of different hierarchical clustering based on 29 immune signatures. Gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were utilized. Terms with a false discovery rate (FDR) < 0.05 were considered statistically enriched and were listed using R package “ClusterProfiler”.

2.3 Establishment of the immune-related prognostic signature

We divided all the cases into a training set and a testing set at 1:1 ratio. The training set was used to identify the prognostic immune-related genes and to establish the IRPS. The testing set and entire set were used to validate its prognostic capability. First, a univariate Cox regression analysis was used to identify prognosis-related genes in the training set. The inclusion criterion was set at $P<0.05$ and absolute shrinkage and selection operator (LASSO) regression was utilized to minimize the overfitting. We then utilized multivariate Cox model to verify the correlation and developed an immune risk score model using the coefficients of multivariate Cox analysis. The risk score for patients in training set, testing set and total set was calculated using the following equation:

$$Risk \ score = Expression \ of \ the \ 1^{st} \ gene \cdot \ coefficient + Expression \ of \ the \ 2^{nd} \ gene \cdot \ coefficient + Expression \ of \ the \ n^{th} \ gene \cdot \ coefficient$$

Patients were then divided into high-risk and low-risk groups according to the risk score.
2.4 Validation of the IRPS

The receiver operating characteristic (ROC) curve was plotted to validate the prognostic value of IRPS. The area under the curve (AUC) was calculated using R package “survivalROC”. The survival analyses were conducted using Kaplan-Meier survival curves and “survival” R package. We also used the decision Curve Analysis (DCA) curve to obtain the predictive power of the IRPS and other clinical characteristics.

2.5 Construction and validation of a predictive nomogram

To fully expand the predictive power of a prognostic model, a nomogram was constructed based on the clinical characteristics of UCEC, including age, stage, grade and histological type. Validation of the nomogram was evaluated using calibration plot.

2.6 Gene set enrichment analysis

To identify potential biological mechanism-related IRPS, we performed GSEA and GO analysis. KEGG terms with FDR ≤ 0.05 were considered enriched. Based on IRPS, patients were divided into high-risk and low-risk group, the different expression genes with a fold change (FC) >1 and an adjusted P-value <0.05 were identified using R package “limma”. The GO analysis was then performed using the clusterProfiler R package.

2.7 Estimate of tumor-infiltrating immune cells (TIICs)
We used the CIBERSORT tool to quantify 22 types of immunocyte fractions based on TCGA RNA-sequencing data. P<0.05 was set as the threshold.

2.8 TIMER Database Analysis

TIMER is a comprehensive resource for systematical evaluations of the clinical impact of different immune cells on diverse cancer types (https://cistrome.shinyapps.io/timer). We analyzed the expressions of KLRC1 and CCL13 in UCEC and evaluated their correlation with the infiltration of immune cells, including B cells, CD4 T cell, CD8 T cell, neutrophils, macrophages, dendritic cell and tumor purity. Besides, correlations of KLRC1 and CCL13 expression with markers of several immune cells were also statistically evaluated using Spearman’s correlation and represented via scatterplots.

2.9 TISIDB Database Analysis

The TISIDB online platform was used to analyze the correlation of KLRC1 and CCL13 expression with 28 immune infiltrating cells (http://cis.hku.hk/TISIDB/index.php).

2.10 Mutation analysis

We downloaded the mutation data of UCEC patients from the TCGA database (https://portal.gdc.cancer.gov) and utilized the maftools to analyze the mutation data. The tumor mutational burden (TMB) score was calculated using following formula:

\[ TMB = \frac{Total \ \text{mutation}}{Total \ \text{covered base}} \cdot (10)^6 \]
2.11 IPS analysis

IPS can be generated in an unbiased manner using machine learning based on four major gene categories that determine immunogenicity. The IPS was calculated with z-scores of representative genes associated with immunogenicity. The IPSs of patients with UCEC were obtained from the Cancer Immunome Atlas (TCIA) (https://tcia.at/home).

2.12 Immunotherapy Response Prediction

The response to immunotherapy was predicted using an online tool called Immune Cell Abundance Identifier (ImmuCellAI) (13), which can estimate the abundance of 24 immune cells from gene expression datasets, including RNA-Seq and microarray data, and predict the patient’s response to an existing immune checkpoint blockade therapy.

2.13 Verification of gene correlation in GEPIA

To further verify the correlation of KLRC1 and CCL13 expression with immune cells markers, the Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) database was employed. GEPIA database is an interactive tool which can be used to analyze the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples to form TCGA and GTEx databases. Statistical analysis was performed using Spearman’s correlation.
2.14 Chemotherapy response and candidate small molecule drugs prediction

The response of chemotherapy in UCEC patients was determined using a public database called Genomics of Drug Sensitivity in Cancer (GDSC; https://www.cancerrxgene.org). The half-maximal inhibitory concentration (IC50) was estimated which represented the drug response. The potential small molecule drugs for UCEC were predicted using Connectivity map (CMap) (https://www.broadinstitute.org/connectivity-map-cmap). These small molecule drugs were predicted based on 382 DEGs between high-risk and low-risk group with |log2fold change (FC) | >1 and FDR <0.05. The 3D structures of the three most significant drugs were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/).

2.15 Quantitative real-time RT-PCR (qRT-PCR)

Total RNA (tRNA) from 15 UCEC samples and 15 adjacent tissues was extracted using TRIzol reagent (Invitrogen) and RNA 6000 Nana kit. PrimeScirpt® RT reagent kit was used to synthesize the complementary RNA. The SYBR® Premix Ex Taq™ Kit (TaKaRa DRR041) was utilized to perform real-time quantification. The relative expression levels of target genes were estimated using the 2^\(-\Delta\Delta Ct\) method. The PCR primers are listed in Table S1.

2.16 Immunohistochemical staining and evaluation

The protein expression levels of CCL13 and KLRC1 were tested via immunohistochemical (IHC) staining. Briefly, the tissues slides were deparaffinized, rehydrated and treated with 3% H2O2 for 15 minutes to eliminate endogenous peroxidase. Then, antigen retrieval was performed by heating the slides in sodium
citrate buffer for 3 minutes. Next, the slides were incubated with rabbit anti-CCL13 or anti-KLRC1 primary antibodies (Affinity, Biosciences, 1:200) at 4°C overnight. The slides were washed and incubated with HRP-conjugated donkey anti-rabbit secondary antibodies (Abcam) for 15 minutes. The staining was visualized using DAB solution and samples were counterstained with hematoxylin.

Immunostaining of CCL13 and KLRC1 were analyzed by two pathologists who were blinded to the same information. The staining intensity score was defined on a scale of 0 to 3 in which 0 means no staining, 1 means mild staining, 2 means medium staining and 3 means intense staining. The percentage score of stained cells were also calculated on a scale of 1 to 4 in which 1 represents (0-25%), 2 = (26%-50%), 3 = (51%-75%) and 4 = (76%-100%). In order to obtain the final score, the intensity score and percentage score were multiplied to reach the final score ranging from 0 to 12.

2.17 Statistical analysis

The R project (version 3.6.2; R Foundation) was adopted for all analysis (http://www.r-project.org/). The following R packages was adopted in this study (R package: pheatmap, ggplot2, rms, glmnet, preProcessCore, forest plot, limma, GSVA, survminer, survival ROC, beeswarm, ggstatsplot). Two-side statistical analyses were performed and samples with P-value < 0.05 were considered statistically significant.

3. Results
3.1 Construction of UCEC subgrouping

We used the ssGSEA method to assess the infiltration of immune cells by analyzing the mRNA expression of UCEC patients. On the basis of the ssGESA scores of 29 gene sets, we divided the patients into three groups: Immunity High (Immunity_H), Immunity Medium (Immunity_M), and Immunity Low (Immunity_L). We found that the Immunity_H had higher ESTIMATE Score, Immune Score and Stromal Score but lower Tumor Purity (Fig. 2, Fig. 3 A-C) as compared to other groups. Besides, the expression levels of most HLA genes were significantly higher in Immunity_H group than that in Immunity_L group (Fig. 3D). The type of immune cells was different among three groups (Fig. 3E). We also compared the expression of several immune regulators, including PD-1, PD-L1, TIM-3, LAG-3 and CTLA4. The expression levels of these immune regulators in Immunity_H group were all higher than those in Immunity_L group (Fig. 3F-J). We then conducted Kaplan-Meier survival analysis which highlighted that patients in three groups had distinct clinical outcomes (P=0.027, Fig. 3K).
Figure 2. Hierarchical clustering of UCEC patients

Figure 3. Three UCEC subtypes displayed different phenotypes. (A-C) The difference in Tumor Purity, Immune Score and Stromal Score in three UCEC subtypes. (D) The expression of HLA genes among UCEC subtypes. (E) The proportion of different
immune cells in three UCEC subtypes. (F-J) The expression of 5 immune checkpoint molecules (CTLA4, CD274, HAVCR2, LAG3 and TIGHT) in three UCEC subtypes. (K) The survival curve exhibited that the prognosis of patients among UCEC subtypes is different. *0.01≤P<0.05; *0.001≤P<0.01; ***P<0.001.

3.2 Extraction of differentially expressed genes and immune-related genes

To identity the differentially expressed genes (DEGs) among all of the three groups, we first compared the DEGs between Immunity_L and Immunity_H, Immunity_L and Immunity_M, and Immunity_M and Immunity_H and identified 2314, 411 and 1378 DEGs (Fig. S1A-C). Then, according to the data from the Immunity_H and Immunity_L groups, we identified 1811 immune-related genes using “limma” package based on the threshold of |log2FC| ≥ 1 and FDR ≤ 0.05. Venn analysis was applied to obtain the overlapping genes. Finally 89 overlapping genes were selected for further analysis (Fig. S1D).

3.3 Identification of potential biological function-related genes

Go and KEGG analyses were performed which highlighted 89 biological function-related key genes in UCEC. We found that biological functions like such as T cell activation, leukocyte cell-cell adhesion, positive regulation of leukocyte activation, etc. were associated with the identified 89 genes. Furthermore, these genes participated in the KEGG pathways including Cytokine-cytokine receptor interaction, Natural killer
cell mediated cytotoxicity, and Viral protein interaction with cytokine and cytokine receptor etc (Fig. S2A-D).

3.4 Development and validation of the IRPS

To construct the IRPS (IRPS) based on 89 identified overlapping genes, we used univariate Cox regression analysis to identify prognosis-related genes (Table S2). 3 prognosis-associated overlapping genes were reserved with the criterion of $P \leq 0.05$. Subsequently, LASSO Cox analysis with ten-fold cross-validation was conducted to minimize overfitting (Fig. S3A-B). After analysis, three genes were all reserved, including KLRC1, CCL13 and LTA. All these genes were associated with the overall survival of UCEC patients (Fig. S4). We then performed multivariate Cox proportional hazards regression analysis to build the IRPS (Table 1). Two hub genes were reserved. The mRNA and protein expression of these two gens were presented in Fig. 4. The mRNA expression of CCL13 and KLRC1 in tumor tissues were significantly lower than that in the normal tissues (Fig. 4A, B). Similarly, the protein expression of CCL13 and KLRC1 was consistent with their mRNA expression (Fig. 4C, D). The risk score for each sample was calculated according to the expression levels of 3 hub genes and corresponding coefficients. Risk score was calculated using the following formula:

$$Risk\ factor = [(-2.163) \cdot KLRC1 + (-0.398) \cdot CCL13]$$

A cutoff Risk score value of 1.40 was selected based on the median value of the risk score in the training set, and this value was used to divide the patients into low-risk (Risk score $\leq 1.4028$) and high-risk (Risk score $> 1.4028$) groups.
Table 1. Multivariate Cox analyses based on 2 hub genes

| Gene  | Coef | HR  | 95%CI     | p value |
|-------|------|-----|-----------|---------|
| CCL13 | -0.398 | 0.672 | 0.437-1.034 | 0.0709  |
| KLRC1 | -2.163 | 0.115 | 0.008-1.645 | 0.1111  |
Figure 4. The mRNA and protein expression level of CCL13 and KLRC1 in UCEC tumor and the adjacent tissues. (A-B) The mRNA expression of CCL13 and KLRC1 in UCEC tumor and the adjacent tissues. (C-D) The protein expression of CCL13 and KLRC1 in UCEC tumor and the adjacent tissues observed using IHC.
The capacity of the IRPS to distinguish risk score, survival status and expression of 2 hub genes in the training set are displayed in Fig. 5A-C. Kaplan-Meier survival analysis displayed statistical difference between two risk groups (Fig. 5D), and the areas under the ROC curves (AUC) were 0.599, 0.649, and 0.661 for 1-, 3-, and 5-year survival, respectively (Fig. 5E). We used the testing set cohort and the entire cohort to further validate the prognostic power of the IRPS model with the same risk score. The distribution of risk score, survival status and expression of two hub genes in the testing and entire sets are presented in Fig. 5F-H and Fig. 5K-M. Similar to the training set, the prognosis of patients in high-risk group was significantly worse than that in the low-risk group in both testing and entire sets (Fig. 5I, N). Time-dependent ROC analysis revealed the prognostic accuracy of the IRPS in testing and entire sets as well (Figs. 5J, O).

Figure 5. Construction of the IRPS. (A-C) The distribution of Risk score, survival status and expression of 2 hub genes in training set. (D) Kaplan-Meier survival curves of
overall survival between high-risk and low-risk patients in training set. (E) 1-year, 3-year, and 5-year ROC curve of the predictive power of the IRPS in training set (Fig 5F-J). Figure 5 K-O display similar analyses which were conducted in the test set and the entire set.

Furthermore, we analyzed the prognostic power of the IRPS with different clinical features in the entire set. We first represented the data in a heatmap to obtain the general distribution of risk score, hub genes and other clinical features (Fig. S5A) and then utilized subgroup Kaplan-Meier analysis to evaluate the prognostic value of IRPS in some specific conditions (Fig. S5B-G). We found the IRPS reached satisfactory prognostic discrimination in patients with age ≤ 60 (Fig. S5B), grade G1&G2 (Fig. S5C), grade G3&G4 (Fig. S5D), histological type endometrial (Fig. S5E), stage I&II (Fig. S5F) and stage III&IV (Fig. S5G).

3.5 Construction and validation of a prediction nomogram

We used the univariate and multivariate Cox regression analyses to determine whether the IRPS was an independent prognostic indicator for UCEC. In univariate Cox regression analysis, the hazard ratio (HR) of risk score and 95% confidence interval (CI) were 2.717 (1.407-5.248), 1.783 (1.013-3.140), and 2.191 (1.425-3.370) in training, testing, and entire sets, respectively (Table 2). In multivariate Cox regression analysis, the HR of risk score and 95% CI were 2.224 (1.137-4.350) and 1.949 (1.273-2.983) in
training and entire set, respectively. However, in test sets, the HR of risk score and 95% CI were 1.653 (0.956-2.859) (Table 2). According to the univariate and multivariate Cox regression analyses, the age, stage, histological type, grade and risk score are significant prognostic factors and should be involved in the construction of the prognostic models.

| Variables     | Univariate analysis |           | Multivariate analysis |           |
|---------------|---------------------|-----------|-----------------------|-----------|
|               | HR                  | 95% CI    | P-value               | HR        | 95% CI    | P-value |
| Train sets    |                     |           |                       |           |           |         |
| Age           | 1.303               | 0.674-2.517 | 0.431                | 1.037     | 0.505-2.130 | 0.921  |
| Stage         | 4.044               | 2.179-7.506 | <0.001               | 2.902     | 1.496-5.629 | 0.002  |
| Histological type | 3.443               | 1.870-6.340 | <0.001               | 1.597     | 0.729-3.500 | 0.242  |
| Grade         | 2.869               | 1.327-6.202 | 0.007                | 1.881     | 0.774-4.568 | 0.163  |
| RiskScore     | 2.717               | 1.407-5.248 | 0.003                | 2.224     | 1.137-4.350 | 0.020  |
| Test sets     |                     |           |                       |           |           |         |
| Age           | 2.465               | 1.253-4.851 | 0.009                | 2.346     | 1.159-4.751 | 0.018  |
| Stage         | 4.126               | 2.306-7.383 | <0.001               | 3.852     | 2.030-7.310 | <0.001 |
| Histological type | 2.682               | 1.506-4.777 | <0.001               | 0.918     | 0.462-1.822 | 0.806  |
| Grade         | 3.996               | 1.864-8.565 | <0.001               | 2.521     | 1.085-5.857 | 0.032  |
| RiskScore     | 1.783               | 1.013-3.140 | 0.045                | 1.653     | 0.956-2.859 | 0.072  |
| Entire sets   |                     |           |                       |           |           |         |
| Age           | 1.778               | 1.112-2.843 | 0.016                | 1.563     | 0.952-2.567 | 0.078  |
| Stage         | 4.116               | 2.700-6.275 | <0.001               | 3.400     | 2.152-5.371 | <0.001 |
| Histological type | 3.044               | 2.003-4.624 | <0.001               | 1.193     | 0.716-1.989 | 0.498  |
|                | HR    | CI           | p-value   | HR    | CI           | p-value   |
|----------------|-------|--------------|-----------|-------|--------------|-----------|
| Grade          | 3.397 | 1.976--5.840 | <0.001    | 2.137 | 1.163--3.928 | 0.014     |
| RiskScore      | 2.191 | 1.425--3.70  | <0.001    | 1.949 | 1.273--2.983 | 0.002     |

HR: hazard ratio; CI: confidence interval

Table 2. Univariate and multivariate Cox regression analyses of the prognosis-related factors

To expand the prognostic power of the IRPS and other clinical characteristics, we constructed a nomogram that integrated age, clinical stage, grade, histological type, and risk score. Each parameter was assigned with a score and their total score was calculated. To validate the performance of the nomogram (Fig. 6A), we constructed 1, 3, and 5-year calibration curves (Fig. 6B–D), which revealed a close association between the predicted and actual curves. We further compared the AUC of IRPS and other clinical characteristics for 1, 3, and 5-year survival (Fig. 7A-C) and found that the results were not suitable for clinical usage. However, when these factors were combined, the AUC reached 0.736, 0.746 and 0.796 for 1, 3, and 5-year survival, respectively (Fig. 7D-F), suggesting the combination of IRPS and other clinical characteristics was highly reliable. This methodology was further confirmed by the decision curve analysis (DCA) (Fig. 7G-H).
Figure 6. Construction and validation of a nomogram. (A) Nomogram to predict the probability of 1, 3 and 5-year OS of UCEC patients. (B-D) Calibration curves of the nomogram to predict the probability of OS at 1, 3 and 5 years.
Figure 7. The predictive power of the IRPS and other clinical characteristics. (A-C) 1, 3 and 5-year ROC of IRPS and the other clinical characteristics. (D-E) 1, 3 and 5-year ROC of the combination of IRPS and the existing clinical factors. (G, H) The decision curve analysis (DCA) curves showed that the prognostic power of combining IRPS and clinical factors was superior to the existing prognostic factors.

3.6 Potential biological pathways associated with IRPS
To further identify the potential biological pathways related to the IRPS, and to elucidate enriched KEGG pathways, GSEA was performed. We identified that pathways, such as Kegg_axon_guidance, Kegg_basal_cell_carcinoma, Kegg_glycosaminoglycan_biosynthesis_chondroitin_sulfate, were enriched in the high-risk group, whereas Kegg_autoimmune_thyroid_disease, Kegg_B_cell_receptor_signaling_pathway, and Kegg_chemokine_signaling_pathway were enriched in the low-risk group (Fig. S6A-B). In the GO analysis, we also identified several immune-related GO terms such as T cell activation, regulation of leukocyte activation, regulation of lymphocyte activation, regulation of T cell activation and leukocyte cell-cell adhesion (Fig. S6C-D).

3.7 Correlation between IRPS and immune cell infiltration

We used CIBERSORT to obtain the proportion of the 22 immune cells (Fig. 8A) and found that the proportions of several types of immune cells, including plasma cells, CD8+ T cells, CD4+ memory T cell, follicular helper T cells and M1 macrophages, were higher in the low-risk group, but those of immune cells like, CD4+ memory T cells, M0 macrophages and mast cells were lower in the high-risk group. Besides, we also investigated the correlation between IRPS and different types of immune cells. The IRPS showed positive correlation with memory B cells (r=0.18, P=0.01), activated dendritic cells (r=0.13, P=0.04), (r=, P=), M0 macrophages (r=0.41, P=0), mast cells (r=0.18, P=0), CD4+ memory T cells (r=0.29, P=0), and negative correlation with M1 macrophages (r=-0.26, P=0), NK cells (r=-0.21, P=0), CD4+ memory T cells (r=-0.48,
P=0), CD8+T cells (r=-0.44, P=0) and follicular helper T cell (r=-0.18, P=0; Fig. 8B-C).

Figure 8. Correlation between IRPS and immune cell infiltration. (A) The landscape of immune cell infiltration in low-risk and high-risk groups. Th low-risk and high-risk groups are represented via blue and red violin, respectively. (B) The association between IRPS and immune cell infiltration. (C) The association between IRPS and each type of immune cell.

3.8 Correlation analysis between 2 hub genes and immune infiltration level

To investigate the relationship between the two hub genes, tumor purity and the immune
filtrating cells, we utilized the TIMER database to obtain the correlations. We first analyzed the correlations between CCL13 expression, tumor purity and immune infiltration level of 6 immune cells. The results showed that CCL13 expression level had negative correlation with tumor purity ($r=−0.136$, $P=0.0198$). Besides, CCL13 expression level had significant positive correlations with infiltrating levels of B cell ($r=0.166$, $P=0.00473$), CD8+ T cell ($r=0.298$, $P=2.41e-07$), CD4+ T cell ($r=0.145$, $P=0.013$), Macrophage ($r=0.103$, $P=0.0775$), Neutrophil ($r=0.266$, $P=3.95e-06$) and Dendritic cell ($r=0.198$, $P=0.000659$) (Fig. S7A). Similarly, the KLRC1 expression level had negative correlation with tumor purity ($r=−0.231$, $P=0.0000647$) and had positive correlation with infiltrating levels of B cell ($r=0.471$, $P=2.23e-17$), CD8+ T cell ($r=0.394$, $P=3.83e-12$), CD4+ T cell ($r=0.373$, $P=4.99e-11$), Macrophage ($r=0.327$, $P=1.10e-08$), Neutrophil ($r=0.346$, $P=1.17e-09$) and Dendritic cell ($r=0.492$, $P=3.17e-19$) (Fig. S7B). The above results were also validated using the TISIDB dataset (Fig. S8A-C). These results suggest that CCL13 and KLRC1 play a specific role in immune infiltration in UCEC.

We further revealed the relationship between the two hub genes and several immune markers, including CD8+ T cells, T cells (general), B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells, DCs and several functional T cells. After adjustment by purity, the results showed that CCL13 expression level was associated with most immune marker sets of immune cells and different T cells except for the M1 Macrophage and Dendritic cell (Table 3). In addition, the KLRC expression level was
associated with nearly all of the immune cells. These results were also verified using the GEPIA database (Table 4). In general, these findings suggested that CCL13 and KLRC1 play a remarkable role in immune regulation in UCEC.
| Description          | Gene markers | CCL13 |    |    |    | KLRC1 |    |    |    |
|----------------------|--------------|-------|----|----|----|-------|----|----|----|
|                      |              | Cor   | P  | Cor |    | P    | Cor|    |    |
| CD8+ T cell          | CD9A         | 0.452 | ***| 0.435| ***| 0.597| ***| 0.581| ***|
|                      | CD9B         | 0.248 | ***| 0.196| ** | 0.384| ***| 0.369| ***|
| T cell (general)     | CD3D         | 0.444 | ***| 0.444| ***| 0.605| ***| 0.623| ***|
|                      | CD3E         | 0.422 | ***| 0.412| ***| 0.634| ***| 0.660| ***|
|                      | CD2          | 0.468 | ***| 0.466| ***| 0.624| ***| 0.624| ***|
| B cell               | CD19         | 0.157 | ** | 0.119| 0.041| 0.385| ***| 0.399| ***|
|                      | CD79A        | 0.307 | ***| 0.296| ***| 0.454| ***| 0.436| ***|
| Monocyte             | CD86         | 0.343 | ***| 0.301| ***| 0.542| ***| 0.543| ***|
|                      | CD115 (CSF1R)| 0.159 | ** | 0.110| 0.060| 0.443| ***| 0.470| ***|
| TAM                  | CCL2         | 0.303 | ***| 0.272| ***| 0.282| ***| 0.253| ***|
|                      | CD68         | 0.312 | ***| 0.289| ***| 0.459| ***| 0.438| ***|
|                      | IL10         | 0.136 | *  | 0.125| 0.032| 0.236| ***| 0.199| ** |
| M1 Macrophage        | INOS (NOS2)  | -0.019| 0.659| -0.039| 0.502| 0.119| *  | 0.034| 0.563|
|                      | IRF5         | 0.027 | 0.531| 0.030| 0.605| 0.241| ***| 0.207| ** |
|                      | COX2(PTGS2)  | -0.015| 0.732| -0.084| 0.154| -0.033| 0.446| -0.036| 0.535|
| M2 Macrophage        | CD163        | 0.376 | ***| 0.324| ***| 0.393| ***| 0.365| ***|
|                      | VSIG4        | 0.260 | ***| 0.195| ** | 0.409| ***| 0.388| ***|
|                      | MS4A4A       | 0.377 | ***| 0.335| ***| 0.492| ***| 0.466| ***|
| Neutrophils          | CD66b (CEACAM8)| -0.092| 0.031| -0.082| 0.162| 0.068| 0.113| 0.090| 0.125|
|                      | CD11b (ITGAM)| 0.228 | ***| 0.158| *  | 0.441| ***| 0.466| ***|
|                      | CCR7         | 0.330 | ***| 0.344| ***| 0.482| ***| 0.518| ***|
| Natural killer cell  | KIR2DL1      | 0.164 | ** | 0.091| 0.120| 0.445| ***| 0.423| ***|
|                      | KIR2DL3      | 0.200 | ***| 0.165| *  | 0.514| ***| 0.514| ***|
|                      | KIR2DL4      | 0.290 | ***| 0.322| ***| 0.693| ***| 0.696| ***|
|                      | KIR3DL1      | 0.241 | ***| 0.240| ***| 0.525| ***| 0.577| ***|
|                      | KIR3DL2      | 0.193 | ***| 0.210| ** | 0.485| ***| 0.493| ***|
|                      | KIR3DL3      | 0.154 | ** | 0.126| 0.031| 0.364| ***| 0.386| ***|
|                      | KIR2DS4      | 0.182 | ***| 0.136| 0.020| 0.465| ***| 0.512| ***|
| Dendritic cell       | HLA-DPB1     | 0.208 | ***| 0.110| 0.060| 0.472| ***| 0.442| ***|
|                      | HLA-DQB1     | 0.152 | ** | 0.071| 0.226| 0.418| ***| 0.375| ***|
|                      | HLA-DRA      | 0.182 | ***| 0.084| 0.152| 0.475| ***| 0.435| ***|
|                      | HLA-DPA1     | 0.234 | ***| 0.143| 0.014| 0.539| ***| 0.519| ***|
|                      | BDCAl-(CD1C)| 0.027 | 0.529| 0.010| 0.868| 0.345| ***| 0.344| ***|
|                      | BDCAl-(NRP1)| 0.081 | 0.059| 0.075| 0.203| 0.304| ***| 0.238| ***|
|                      | CD11c (ITGAX)| 0.188 | ***| 0.140| 0.016| 0.556| ***| 0.569| ***|
| Th1                  | T-bet (TBX21)| 0.450 | ***| 0.411| ***| 0.631| ***| 0.617| ***|
|                      | STAT4        | 0.282 | ***| 0.240| ***| 0.493| ***| 0.460| ***|
|                      | STAT1        | 0.270 | ***| 0.248| ***| 0.296| ***| 0.244| ***|
|                      | IFN-γ (IFNG) | 0.495 | ***| 0.489| ***| 0.498| ***| 0.481| ***|
|                      | TNF-α (TNF)  | -0.031| 0.464| 0.009| 0.876| 0.086| 0.044| 0.068| 0.245|

| Description          | Gene markers | Cor   | P  | Cor   |   | Cor   | P  | Cor   | P  |
|----------------------|--------------|-------|----|-------|---|-------|----|-------|---|
|                      |              | None  | P  | None  |   | None  | P  | None  |   |

| Description          | Gene markers | Cor   | P  | Cor   |   | Cor   | P  | Cor   |   |
|----------------------|--------------|-------|----|-------|---|-------|----|-------|---|
|                      |              | None  | P  | None  |   | None  | P  | None  |   |

| Description          | Gene markers | Cor   | P  | Cor   |   | Cor   | P  | Cor   |   |
|----------------------|--------------|-------|----|-------|---|-------|----|-------|---|
|                      |              | None  | P  | None  |   | None  | P  | None  |   |

| Description          | Gene markers | Cor   | P  | Cor   |   | Cor   | P  | Cor   |   |
|----------------------|--------------|-------|----|-------|---|-------|----|-------|---|
|                      |              | None  | P  | None  |   | None  | P  | None  |   |
|                  | GATA3   | 0.195*** | 0.164*  | 0.237*** | 0.190*  | 0.048  | 0.265 | −0.103 | 0.079  | 0.065  | 0.129 −0.030 | 0.604*** |
|------------------|---------|----------|---------|----------|---------|---------|-------|--------|---------|---------|-------------|-----------|
| Th6              | STAT6   | −0.048   | 0.265   | −0.103   | 0.079   | 0.065   | 0.129 | −0.030 | 0.604*** |
|                  | STAT5A  | 0.119    | *       | 0.053    | 0.367   | 0.274   | ***   | 0.303  | ***     | 0.193   | ***         | 0.226*** |
|                  | IL13    | 0.193    | ***     | 0.226    | ***     | 0.116   | *     | 0.084  | 0.153   |         |             |           |
| Th17             | BCL6    | −0.085   | 0.046   | −0.088   | 0.132   | −0.005  | 0.902 | 0.054  | 0.356   |         |             |           |
|                  | IL21    | 0.314    | ***     | 0.316    | ***     | 0.207   | ***   | 0.245  | ***     |         |             |           |
| Treg             | STAT3   | 0.020    | 0.643   | −0.038   | 0.520   | 0.150   | **    | 0.141  | 0.016   |         |             |           |
|                  | IL17A   | 0.279    | ***     | 0.288    | ***     | 0.140   | *     | 0.168  | *       |         |             |           |
|                  | FOXP3   | 0.360    | ***     | 0.376    | ***     | 0.439   | ***   | 0.453  | ***     |         |             |           |
|                  | CCR8    | 0.345    | ***     | 0.366    | ***     | 0.312   | ***   | 0.341  | ***     |         |             |           |
|                  | STAT5B  | 0.065    | 0.129   | 0.044    | 0.449   | 0.107   | 0.013 | 0.111  | 0.058   |         |             |           |
|                  | TGFβ (TGFB1) | 0.103 | 0.016   | 0.114   | 0.052   | 0.223   | ***   | 0.213  | **       |         |             |           |
|                  | PD-1 (PDCD1) | 0.421 | ***     | 0.371    | ***     | 0.466   | ***   | 0.458  | ***     |         |             |           |
|                  | CTLA4   | 0.445    | ***     | 0.412    | ***     | 0.523   | ***   | 0.500  | ***     |         |             |           |
|                  | LAG3    | 0.462    | ***     | 0.447    | ***     | 0.476   | ***   | 0.448  | ***     |         |             |           |
|                  | TIM-3 (HAVCR2) | 0.367 | ***     | 0.314    | ***     | 0.617   | ***   | 0.603  | ***     |         |             |           |
|                  | GZMB    | 0.467    | ***     | 0.478    | ***     | 0.587   | ***   | 0.580  | ***     |         |             |           |

Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity; *P < 0.01; **P < 0.001; ***P < 0.0001.

Table 3. Correlation analysis between two hub genes and immune cells markers in TIMER
| Description             | Gene markers | CCL13 |   | KLRC1 |   |
|-------------------------|--------------|-------|---|-------|---|
|                         |              | R    | P |       |   |
| CD8+ T cell             | CD8A         | 0.59 | ***| 0.65  | ***|
|                         | CD8B         | 0.37 | ***| 0.52  | ***|
| T cell (general)        | CD3D         | 0.47 | ***| 0.59  | ***|
|                         | CD3E         | 0.49 | ***| 0.65  | ***|
|                         | CD2          | 0.49 | ***| 0.65  | ***|
| B cell                  | CD19         | 0.13 |    | 0.091 | 0.31***|
|                         | CD79A        | 0.31 | ***| 0.45  | ***|
| Monocyte                | CD86         | 0.41 | ***| 0.62  | ***|
|                         | CD115 (CSF1R) | 0.29 | **| 0.52  | ***|
| TAM                     | CCL2         | 0.41 | ***| 0.34  | ***|
|                         | CD68         | 0.42 | ***| 0.52  | ***|
|                         | IL10         | 0.14 |    | 0.071 | 0.3 | ***|
| M2 Macrophage           | CD163        | 0.39 | ***| 0.48  | ***|
|                         | VSIG4        | 0.35 | ***| 0.52  | ***|
|                         | MS4A4A       | 0.42 | ***| 0.57  | ***|
| Neutrophils             | CD66b (CEACAM8) | -0.13 | 0.093 | 0.022 | 0.77***|
|                         | CD11b (ITGAM) | 0.3  | ***| 0.56  | ***|
|                         | CCR7         | 0.38 | ***| 0.51  | ***|
| Natural killer cell     | KIR2DL1      | 0.12 |    | 0.12  | 0.44***|
|                         | KIR2DL3      | 0.3  | ***| 0.54  | ***|
|                         | KIR2DL4      | 0.36 | ***| 0.75  | ***|
|                         | KIR3DL1      | 0.35 | ***| 0.5   | ***|
|                         | KIR3DL2      | 0.21 | **| 0.56  | ***|
|                         | KIR3DL3      | 0.25 | ***| 0.39  | ***|
|                         | KIR2DS4      | 0.2  | **| 0.41  | ***|
| Dendritic cell          | HLA-DPB1     | 0.3  | ***| 0.56  | ***|
|                         | HLA-DQB1     | 0.22 | **| 0.36  | ***|
|                         | HLA-DRA      | 0.28 | ***| 0.53  | ***|
|                         | HLA-DPA1     | 0.36 | ***| 0.6   | ***|
|                         | BDCA-1(CD1C) | 0.12 |    | 0.13  | 0.35***|
|                         | BDCA-4(NRP1) | 0.14 |    | 0.057 | 0.36***|
| Th1                     | T-bet (TBX21) | 0.48 | ***| 0.67  | ***|
|                         | STAT4        | 0.37 | ***| 0.48  | ***|
|                         | STAT1        | 0.24 | *  | 0.37  | ***|
Table 4. Correlation analysis between two hub genes and immune cells markers in GEPIA

|               | IFN-γ (IL10) | TNF-α (TGF) |
|---------------|-------------|-------------|
| T cell exhaustion | **0.56**  | **0.022**  |
| PD-1 (PDCD1)   | **0.45**  | **0.52**  |
| CTLA4          | **0.42**  | **0.4**  |
| LAG3           | **0.4**  | **0.48**  |
| TIM-3 (HAVCR2) | **0.4**  | **0.4**  |
| GZMB           | **0.48**  | **0.48**  |

*P < 0.01; **P < 0.001; ***P < 0.0001

In addition, we further assessed the relationships of the mutants of these 2 hub genes with immune infiltrates in UCEC. Compared with the immune infiltration levels in samples with wild type signatures, diverse forms of mutation in two hub genes could inhibit the immune infiltration levels of several immune cells, including CD8+ T cell, macrophages and dendritic cells (Fig. S7C-D).

### 3.9 Correlation between IRPS and mutation profile

The relationship between IRPS and mutation profile was evaluated in UCEC patients using somatic mutation data. The top 10 mutated genes in high-risk and low-risk group are shown in the Fig. 9A and Fig. 9B. And the most frequently mutated genes in high-risk and low-risk group are presented in Fig. 9C. The results revealed that somatic mutation was more frequently observed in the low-risk group. And the TMB scores in low-risk group were significantly higher than that in high-risk group (P<0.05, Fig. 9D). Further results revealed that TMB score had negative correlation with IROS (P=4.015e-09, Fig. 9E).
Figure 9. The mutation profile and TMB among high-risk and low-risk group. (A, B) The top 10 mutated genes in high-risk and low-risk group. (C) The most frequently mutated genes in high-risk and low-risk group. (D) The TMB in high-risk and low-risk group. (E) The relationship between TMB and IRPS. TMB: tumor mutational burden

3.10 Correlation between IRPS and two therapeutic regimens

We also analyzed the expression of four immune checkpoint molecules (IPS-CTLA4, IPS-PD1-PD-L1-PD-L2 and IPS-PD1-PD-L1-PD-L2-CTLA4) in high-risk and low-risk groups. The results revealed that IRPS was negatively corelated with the listed four immune checkpoint molecules (Fig. 10A). Besides, we performed IPS analysis to acquire immunogenicity. The results showed that four molecules displayed higher scores in the low-risk group (Fig. 10B). Besides, according to the online tool Immune Cell Abundance Identifier (ImmuCellAI), patients in the low-risk group showed higher immunotherapy response rate compared with patients in the high-risk group (Fig. 10C, D), which implied that patients in the low-risk group would benefit from
immunotherapy.

Figure 10. Correlation between IRPS and immune checkpoint molecules and the predicted response to immunotherapy. (A) The gene expression profile of PD1, CTLA-4, PD-L1 and PD-L2 in low-risk and high-risk group. (B) The association between IPS and the IRPS in UCEC patients. (C) The different immunotherapy response rates in low-risk and high-risk group. (D) The relationship between IRPS and immunotherapy response.

Considering that chemotherapy is the most common way to treat UCEC cancer, we used GDSC database to predict the likelihood of response to several common chemotherapy drugs. We estimated the IC50 of each sample and observed a significant difference of IC50 between high-risk and low-risk groups among eight chemo drugs. Patients in the low-risk group were more sensitive to commonly administered chemodrugs (P=
1.467e-05 for cisplatin, P= 4.412e-06 for gemcitabine, P= 0.039 for paclitaxel, P= 0.002 for bleomycin, P= 1.458e-06 for vinblastine, P=0.048 for vinorelbine, P= 4.620e-05 for vorinostat, P= 0.005 for methotrexate) (Fig. 11). In contrast, the chemotherapeutic response of Docetaxel and Doxorubicin was not significantly different between both groups.

Figure 11. Chemotherapeutic response in the high-risk and low-risk groups. *0.01≤ P<0.05; **0.001≤ P<0.01; ***P<0.001.

3.11 Potential small molecular drugs for UCEC

In order to explore new therapeutic regimens for UCEC, the Cmap database was employed (14). This database comprises of genome-wide transcriptional expression data from small molecule drugs, and can discover the connections between drugs, genes and diseases through the variation of gene-expression profiles. In this research, we found eight associated small molecule drugs that are listed in the Table 5.
Among these small molecule drugs, the 3D chemical structures of three most significant small molecule drugs were obtained from PubChem (Fig. 12).

Table 5. Results of CMap analysis

| cmap name        | mean | n  | enrichment | p       | specificity | percent non-null |
|------------------|------|----|------------|---------|-------------|-----------------|
| carbenoxolone    | 0.332| 4  | -0.807     | 0.00271 | 0           | 50              |
| emetine          | -0.6 | 4  | -0.713     | 0.01369 | 0.1941      | 75              |
| lovastatin       | 0.367| 4  | -0.704     | 0.01578 | 0.0233      | 50              |
| MG–262           | 0.567| 3  | -0.794     | 0.01787 | 0.1417      | 66              |
| piperlongumine   | 0.368| 2  | 0.897      | 0.02181 | 0.1234      | 50              |
| megestrol        | 0.406| 4  | -0.677     | 0.02425 | 0.0068      | 50              |
| semustine        | 0.398| 4  | -0.653     | 0.03364 | 0.1111      | 50              |
| trimethoprim     | 0.442| 5  | -0.577     | 0.04055 | 0.0449      | 60              |

Among these small molecule drugs, the 3D chemical structures of three most significant small molecule drugs were obtained from PubChem (Fig. 12).

Table 5. Results of CMap analysis

Figure 12. The 3D structure of the three small molecule drugs for UCEC. (A) carbenoxolone, (B) emetine and (C) lovastatin.
4. Discussion

UCEC is the most common tumor affecting female reproductive system, with a 5-year survival rate of 16% in patients with distant metastasis (15). To date, the therapeutic regimens, such as immunological therapy and chemotherapy, are mainly designed according to the clinical stages of the tumor. Due to physiological differences, not all the patients can benefit from the current therapeutic regimens (16). To overcome this challenge, in this research, we developed a model for predicting the survival and therapeutic response of UCEC patients using two immune-related genes.

We first estimated the relative levels of 24 immune cells based on the training set online data, and used hierarchical clustering analysis to profile the infiltration of immune cells. The results revealed that the infiltration of immune cells varied much among UCEC patients. We found different tumor purities, immune scores, stromal scores, fractions of different immune cells, expression of several HLA genes and expression of five immune checkpoint molecules (CTLA4, CD274, HAVCR2, LAG3 and TIGHT) among UCEC subtypes. These results strongly suggest that the TIME has different landscapes
in UECE patients. Emerging evidence demonstrates that TIME is closely associated with the prognosis of several cancers (17). In this research, we found the overall survival of three UCEC subtypes differed significantly. The hierarchical clustering analysis is capable of distinguishing patients with different prognosis. However, this method is complicated and produces irrelevant information, making it less clinically applicable.

To overcome the shortcomings, we filtered out two hub genes (CCL13 and KLRC1) closely related to the prognosis of UCEC patients. The mRNA and protein expression levels of both genes were verified via qPCR and IHC. The patients were divided into high-risk or low-risk groups based on the IRPS value. The survival analyses confirmed the ability of IRPS in distinguishing patients with different prognosis. The prognostic performance of IRPS was also fairly satisfactory in clinical subgroups. In order to enhance the predictive power of the IRPS, we added other clinical characteristics and built a nomogram model. According to the ROC and DCA curves, this nomogram model exhibited remarkable ability in predicting the prognosis of patients.

We further investigated the potential biological function of IRPS in UCEC patients. The GSEA analysis revealed that several immune-related pathways were significantly enriched in the low-risk group. In contrast, the same pathways in the high-risk group were scattered. In addition, the correlation between IRPS and immune cell infiltration was also analyzed. It is widely acknowledged that innate and adaptive immune cells
play a major role in regulating the cancer growth. Increasing evidence shows that some immune cells (like Neutrophils, Macrophage M2, T regulatory cell) can stimulate, while some (like Macrophage M1, CD8+ T cell and Th1 CD4+ T cell) can inhibit cancer growth (18, 19). In the present study, low-risk and high-risk groups differed in the proportion of immune cells in UCEC, including plasma cells, CD8+ T cells, CD4+ memory T cells, CD4+ memory T cells, follicular helper T cells, M0 macrophages, M1 macrophages and activated mast cells. To be specific, M1 Macrophages, CD4+ and CD8+ T cells and plasma cells were activated in the low-risk group, suggesting that they can inhibit cancer growth and improve the prognosis of UCEC patients. From the scatter diagrams (Fig. 11C), we found that IRPS was closely related to several types of immune cells, indicating that IRPS has the ability of describing TIME.

As listed in the methods section, the IRPS was established using the expression profiles of CCL13 and KLRC1. CCL13 is a gene located on chromosome 17q11.2 that encodes monocyte chemoattractant protein 4 (MCP-4), a Cys-Cys (CC) type cytokine characterized by two adjacent cysteines. In the immunoregulatory and inflammatory processes, CCL13 demonstrates chemotaxis to monocytes, lymphocytes, basophils and eosinophils, but not neutrophils, and plays a role in the accumulation of leukocytes during inflammation. Increasing evidence has confirmed that chemokines and their receptors can facilitate the entry of specific immune cells into tumors, thus enhancing anti-tumor response and improving patient prognosis (20, 21).
KLRC1 (Killer Cell Lectin Like Receptor C1) is a protein-coding gene associated with Natural killer (NK) cells. Natural killer (NK) cells are lymphocytes that can mediate the lysis of certain tumor cells and virus-infected cells, and specific humoral and cell-mediated immunity. The protein encoded by KLRC1 belongs to the killer cell lectin-like receptor family, also called NKG2 family, which is a group of transmembrane proteins preferentially expressed in NK cells. KLRC1 can form a complex with KLRL1/CD94, and participate in the recognition of the MHC class I HLA-E molecules in NK cells. A previous study found KLRC1 expression changed with CD8+ T cell infiltration in 34 types of human cancers (22).

It is well known that tumors can escape the immune system via several mechanisms, including expanding T regulatory cells, inducing the production of certain inhibitory cytokines, altering the function of antigen presenting cells (APCs) (18). In this research, we found that the expression of CCL13 and KLRC1 had a positive correlation with the activation of several types of immune cells. Mutations in these two genes can inhibit the infiltration of some immune cells, especially in CD8+ T cells. Thus, IRPS based on both genes can distinguish cellular immunooactivation and immunosuppression.

We also investigated whether IRPS can provide valuable information about the host response to immunotherapy and chemotherapy. Immune checkpoint molecules are traditional biomarkers for evaluating the therapeutic benefit of immunotherapy. In this research, we found that the expression levels of four immune checkpoint molecules
(PD-1, PD-L1, PD-L2 and CTLA4) were significantly low in the high-risk group, compared to those in the low-risk group, suggesting that the patients in the high-risk group might not benefit from immunotherapy based on immune checkpoint inhibitors. Apart from immune checkpoint molecules, tumor mutational burden (TMB) has emerged as a promising predictive biomarker for immunotherapy based on immune checkpoint inhibitors in several tumor types (22). High TMB and high neoantigen load have positive correlation with sensitivity to immunotherapy (23). Multiple studies have proved that TMB may be a surrogate for overall neoantigen load (24, 25). During the cancer onset, somatic cells mutate and express neoantigens (26). These neoantigens can sometimes induce T-cell-dependent immune responses by activating CD8+ T cells that can recognize those neoantigens and initiate tumor cell lysis (27). In this research, we found IRPS had a negative correlation with TMB. Patients in the high-risk group displayed lower TMB scores compared to those in the low-risk group. An online tool called ImmuCellAI has been established to predict immunotherapy response, (13). We employed this tool and found that patients in the low-risk group showed higher immunotherapy response rate, compared to patients in the high-risk group. According to the evidence regarding immune checkpoint molecules, TMB and ImmuCellAI tool, patients in the low-risk group may benefit more from immunotherapy with immune checkpoint inhibitors, but those in the high-risk group may not. Similarly, the patients in the low-risk group were sensitive to chemodrugs such as cisplatin, gemcitabine, paclitaxel, bleomycin, vinblastine, vinorelbine, vorinostat and methotrexate. However, patients in the high-risk group were resistant to these drugs, which may explain their
poor prognosis.

Fortunately, with the help of the Connectivity Map database, we found that several small molecule drugs, such as carbenoxolone, emetine, lovastatin and MG-262, could provide potential benefits for patients in the high-risk group. Interestingly, there is limited research regarding the effects of these drugs on tumor. Carbenoxolone is widely used as an antiulcer drug, but with unknown effect on tumor. Carbenoxolone can also act as the inhibitor of Pannexin 1 (Panx-1), an ATP release channel (28) and its inhibition can suppress the migration and invasion of testicular cancer cells to counter cancer progression and metastasis (29-31). Emetine, a potent anti/protozoal and emetic drug is derived from the ipecac root and recent evidence has verified its anti-malarial, anti-bacterial and anti-amoeic effects (32-35). Over the past decades, emetine has been reported to have anti-tumor effects on leukemia, ovarian cancer, bladder cancer and lung cancer (36-39) by inhibiting tumor growth by regulating multiple mechanisms such as apoptosis and autophagy. Lovastatin, an HMG-CoA reductase inhibitor, can decrease cholesterol biosynthesis and is an ideal medicine for treating coronary heart disease. In a research in 2004, lovastatin was found to be a useful adjuvant drug for breast cancer (40). Coronary events decreased by 43% after pravastatin treatment and by 19% after simvastatin treatment in patients with colon cancer (41, 42). Besides, lovastatin can reduce cancer-related deaths (43). MG-262, also known as Z-Leu-Leu-Leu-B(OH)2, is a proteasome inhibitor that can reversibly and selectively inhibit chymotryptic activity of the proteasome. As we know, the ubiquitin-proteasome system
controls the turnover of regulatory protein in critical biological activities, and proteasome inhibition has emerged as a novel approach to treat cancer. In some studies, MG-262 has exhibited obvious inhibitory effect on the growth of malignant cells (44). The above drugs are untraditional anti-tumor drugs and there is limited evidence of their effects on tumors especially UCEC. However, for patients in the high-risk group, all drugs with potential benefits should be tried. For patients who may not benefit from traditional drugs, adjuvant agents should be tried.

Nevertheless, there are still some limitations in this research. First, this study only includes the immune-related genes and did not take other biomarkers into consideration. Additionally, this research is based on the online data, and large-sample clinical studies are still needed to validate the predictive value of our IRPS model.

5. Conclusion

Our study identified two immune-related genes, CCL13 and KLRC1 in the development of UCEC. The IRPS of both genes can predict the prognosis and immune status of UCEC patients and evaluate their therapeutic response to immunotherapy and chemotherapy.
Abbreviations

UCEE: Uterine Corpus Endometrial Carcinoma
LASSO: least absolute shrinkage and selection operator
IRPS: immune-related prognostic signature
GEPIA: Gene Expression Profiling Interactive Analysis
GDSC: Genomics of Drug Sensitivity in Cancer
TMB: tumor mutational burden
TIME: tumor immune microenvironment
TCGA: The Cancer Genome Atlas
GO: gene ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
FDR: false discovery rate
ROC: receiver operating characteristic
AUC: area under the curve
DCA: decision Curve Analysis
GSEA: gene set enrichment analysis
TIICs: tumor-infiltrating immune cells
KLRC1: Killer Cell Lectin Like Receptor C1

Declarations

Data availability statement

All datasets presented in this study are included in the article/Supplementary Material.

Ethics approval and consent to participate

The study was approved by the Clinical Research Ethics Committee, Wuxi Maternal and Child Health Hospital, the Affiliated Hospital to Nanjing Medical University, and all informed consent were conducted in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

**Availability of data and material**
All data are included in the article.

**Competing interests**
The authors declare that they have no conflict of interest.

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**Author Contributions**
YZ conceived the study. JHL, YCW and JM participated in the design, analysis and draft of the study and they contributed equally to the study. JHL and YCW plotted all figures in this manuscript. JM helped in data analysis. All authors approved the final version of this manuscript and agreed to be accountable for all aspects of the work.

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Supplementary data

Figure S1. Differentially expressed genes (DEG) in three UCEC subtypes. (A-C) The Volcano plots showed the DEGs between different groups (A: Immunity_L vs Immunity_H. B: Immunity_L vs Immunity_M. C: Immunity_M vs Immunity_H). (D) The Venn plot exhibited the intersections between DEGs and immune-related genes. Red points stand for up-regulated genes, while green point stand for down-regulated genes.

Figure S2. Functional enrichment analyses of DEGs. (A, B) Gene ontology (GO) analysis showed the enriched go term of these DEGs. (C, D) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis exhibited the enriched biological pathways of
these DEGs.

Figure S3. The effect of expression level of 3 target genes on overall survival of UCEC patients. (A) Effect of CCL13 expression level on UCEC patient survival. (B) Effect of KLRC1 expression level on UCEC patient survival. (C) Effect of LTA expression level on UCEC patient survival.

Figure S4. Lasso Cox regression analysis of 3 hub genes. (A) Lasso coefficient profiles of 3 hub genes. (B) 10-fold cross-validations results which identified optimal values of the penalty parameter $\lambda$.

Figure S5. The prognostic impact of the IRPS under different clinical features. (A) The heatmap shows the distribution relationship of risk score, hub genes and other clinical features. (B-G) The survival analyses exhibited that IRPS reached satisfactory prognostic discrimination in patients with age $\leq 60$, grade G1&G2, grade G3&G4, histological type endometrial, stage I&II and stage III&IV.

Figure S6. GESA and GO analysis of UCEC based on IRPS. (A) The enriched gene sets in high risk group. (B) The enriched gene sets in low risk. (C) The GO enrichment of the DEGs. (D) The enrichment genes of the core GO terms.

Figure S7. Correlation of 2 hub genes with tumor purity and immune infiltrating cells.
(A) CCL13 expression is inversely related to tumor purity and has significant positive correlations with infiltrating levels of B cell, CD8+ T cell, CD4+ T cell, neutrophils, and dendritic cells in UCEC, other than macrophages. (B) KLRC1 expression is also significantly negatively related to tumor purity and has positive correlation with infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in UCEC. (C) Mutants of CCL13 were associated with low infiltration of CD8+ T cell, macrophage and dendritic cell. (D) Mutants of KLRC1 were associated with low infiltration of B cell, CD8+ T cell, macrophage and dendritic cells.

Figure S8. Correlation between the expression of 2 hub genes and immune infiltrating cells in UCEC. (A) Correlation between the expression of 2 hub genes and the abundance of immune infiltrating cells in UCEC available at TISIDB database. (B) Correlation of CCL13 expression with infiltration levels of B cell, CD4+ T cell, CD8+ T cell, macrophages, neutrophils and dendritic cells in UCEC. (C) Correlation of KLRC1 expression with infiltration levels of B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and dendritic cells in UCEC.

Table S1. Primers used in PCR application
Table S2. Univariate cox analysis of 89 differentially expressed genes