An inflammation-related prognostic model risk score predicts the prognosis of Clear cell renal cell carcinoma

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Research Article

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Abstract

Background: Clear cell renal cell carcinoma (ccRCC) is one of the most common malignant tumors of the urinary system, and the prognosis of patients with advanced stage is really poor. Existing evidence suggests that inflammation and inflammation-related genes play complex roles in different tumors, but their role in ccRCC has rarely been studied as a primary research object.

Methods: we used The Cancer Genome Atlas (TCGA) database to established a prognostic model risk score for ccRCC and inflammation-related genes and verified its predictive effect on the prognosis of ccRCC.

Result: We screened 10 inflammatory differentially expressed genes (DEGs) with independent prognostic value for ccRCC and constructed a prognostic model risk score: (-0.0153×APLNR) + (-0.0073×BTG2) +0. 0225×CSF1+ (-0.0107×CX3CL1) +0. 1888×GABBR1+0. 1528×HAS2+0. 0088×ICAM1+0. 3952×P2RY2+ (-0.0442×SPHK1) +0. 0006×TIMP1. The survival analysis showed that ccRCC with a higher risk score implies shorter survival and worse prognosis. Then we used univariate and multivariate Cox regression analysis and Receiver Operating Characteristic (ROC) curve to confirm that the risk score has a good and stable independent prognostic value. and performed an internal validation of the risk score. Gene set enrichment analysis (GSEA) showed that high risk groups were involved in many pathways related to the occurrence and development of tumors. we also found that the expression levels of immune checkpoints including PD-1, CTLA-4, LAG3, TIGIT in ccRCC in the high risk group were significantly higher than those in the low risk group, and the ESITIMATE tool showed that the high risk group had lower tumor purity and greater heterogeneity.

Conclusion: Our study initially revealed the role of inflammatory genes in ccRCC, and provided a prognostic model risk score that could predict the prognosis of ccRCC well, and may provide more information for future research and treatment of ccRCC.

Introduction

Kidney cancer is one of the most common malignant tumor of the urinary system, and its incidence is second only to bladder cancer, and its main component is renal cell carcinoma. According to its cell origin and genetic characteristics, approximately 70% of renal cell carcinoma is ccRCC[1]. The prognosis of ccRCC varies greatly with different histological grades and disease stages, the prognosis of early ccRCC is much better than that of ccRCC with advanced or distant metastasis. However, due to the insidiousness of early symptoms and the lack of specific signs, a number of patients have already metastasized at the time of initial diagnosis, the long-term survival rate of these patients is relatively low, with a five-year survival rate of only about 12%[2], therefore, the mortality rate of ccRCC patients is still high[3]. Currently, the treatment of advanced ccRCC is primarily targeted therapy, immunomonotherapy, targeted combined immunotherapy and combined immunotherapy, which offers a variety of options for unresectable and metastatic ccRCC[4, 5]. However, multiple factors including the singleness of targeted

drug treatment targets and the emergence of drug resistance, differences in response to immunotherapy and targeted combination immunotherapy in ccRCC patients with different risk stratification, the uncontrollable toxicity and high-grade adverse events brought by combined immunotherapy, and limitations of back line treatment options have gradually become key factors affecting the prognosis of advanced or metastatic ccRCC[6]. Due to the high complexity of the mechanism of tumor occurrence and development, the mining of new targets, new signaling pathways and related biomarkers may explain the mechanisms of drug resistance and immune escape, it also brings more diverse options for the treatment and prognosis of ccRCC[7].

According to the genetic origin of tumors, tumors caused by germline mutations are still only a minority, most tumors arise from mutations in somatic cells[8], therefore, any factor that promotes or causes somatic mutation is a potential risk factor for the occurrence and development of tumor. Inflammation is one of the body's defense measures, secondary to tissue lesions of various causes, is a recognized risk factor for cancer[9]. Research has shown that inflammation is closely associated with the occurrence and development of tumors[10]. For example, active inflammation of the gastric mucosa caused by Helicobacter pylori infection increases the risk of gastric cancer and gastric lymphoma[11], EB virus infection induces the occurrence of nasopharyngeal cancer and Burkitt lymphoma[12, 13], and inflammatory bowel disease leads to a high incidence of colorectal cancer[14]. In the process of tumor occurrence and development, inflammation is involved in various pathways[15], including cell transformation, proliferation, invasion, angiogenesis, and metastasis, and has the effects of destroying immune responses, intervening in tissue repair, participating in epigenetic changes, and affecting drug efficacy[16]. It has been proved that inflammation is involved in the growth of ccRCC and the occurrence of immune escape. Individual studies have explored the relationship between some inflammation-related genes and ccRCC. Hypoxia-inducible factor 1A (HIF-1A) is an important factor involved in inflammation, which proven may be indispensable in the occurrence of ccRCC[17]. Interleukin-10 (IL-10) is thought to be involved in inflammation and immunosuppression, and in a study mining gene associated with the ccRCC microenvironment and prognosis, IL-10 was associated with poorer prognosis in ccRCC[18, 19]. However, the number of related genes involved in inflammatory response is huge, and its predictive effect and mechanism in ccRCC have not been clearly explained yet, establishing a new prognostic model with independent prognostic value by screening inflammation-related genes has implications for the prediction of ccRCC prognosis and the discovery of new therapeutic targets and biomarkers.

In this study, we firstly analyzed the ccRCC samples in TCGA database, identified inflammatory DEGs that were differentially expressed relative to normal samples, and performed prognostic analysis on them to screen out genes with independent prognostic value and constructed a prognostic model risk score. To evaluate its feasibility, we explored the independent prognostic value of this risk score and validated it, and did the gene enrichment correlation analysis. Focusing on the current hot spots of ccRCC and tumor treatment, we also performed a differential analysis of the tumor microenvironment, including immune checkpoints and tumor heterogeneity for this risk score and do a preliminary drug susceptibility analysis of inflammatory DEGs in the prognostic model according to FDA-approved drugs.

Materials And Methods

2.1 Data collection and processing

Download the transcriptional data and clinical data of renal clear cell carcinoma from the TCGA database (https://portal.gdc.cancer.gov/)[20], and obtained a total of 611 samples, including 539 renal clear cell carcinoma tissues and 72 normal tissues. We used Active Perl (version 5.26, 64-bit) to extract and organize the gene expression data and clinical data.

2.2 Data Analysis

2.21 Differential expression analyses of inflammation-related genes

Use the R packages limma, ggplot2 and pheat map to analyze and visualize the differential expression of inflammation-related genes between the tumor group and the normal group.

2.22 Establish a prognostic model

We performed univariate Cox regression analysis of inflammatory DEGs by the R package "survival". And used multivariate Cox regression analysis to establish a prognostic prediction model and calculate the risk score (RS), and then divide the patients into two groups according to the median. In order to enhance the visualization of the prognostic model, use the r package "regplot" to draw a nomogram.

2.23 Evaluation of prognostic models

First, we used the Kaplan-Meier (KM) survival curve to analyze the difference in survival time between the high risk group and the low risk group. Risk score and clinical factors such as TNM stage, histological grade, pathological stage, age (≤65 years, >65 years) and gender were involved in univariate Cox regression analysis and multivariate Cox regression analysis to analyze the factors affecting the survival of ccRCC patients. And we used the ROC curve to evaluate the accuracy of the prognostic model.

2.24 Validation of the prognostic model

Use SPSS23.0 to divide ccRCC samples into a training set and test set randomly, then divided them into the high risk group and low risk group, according to the risk score, and used KM survival curve and the ROC curve to verify the prognostic model.

2.25 Functional Analysis

Go (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analyses were performed by the GSEA (http://www.gsea-msigdb.org/gsea/index.jsp) software to compare the differences in gene functions and pathways among the subgroups classified by the prognostic model[21].

2.26 Immunoassay

Analyze the differences in immune cell function between subgroups based on the R package "GSVA". Then we performed differential analysis of immune checkpoints between subgroups based on the Cibersort algorithm. Finally, we used the ESTIMATE tool to count stromal and immune cell content in tumor tissue to calculate stromal score, immune score, and tumor purity.

2.27 Drug Sensitivity Analysis

Download information about FDA-approved drugs through the CellMiner database (https://discover.nci.nih.gov/cellminer/home.do)[22].And then correlated them with DEGs in prognostic models using the Impute and limo packages.

Results

Identification of inflammatory DEGs between ccRCC tissue and normal tissue

First, we extract the 611 samples from TCGA database data, including 539 ccRCC tissue samples and 72 normal tissue samples, and compared the expression levels of 188 genes related to inflammation, and identified 113 differentially expressed genes ($|log2FC|>1$, $p<0.05$) including ABCA-1 and ADM, among them 96 inflammatory genes were up-regulated and 17 were down-regulated, as shown in Figure 1A. We also made a heat map of the distribution of these 113 inflammatory DEGs in the tumor and normal groups, as shown in Figure 1B.

Enrichment analysis of inflammatory DEGs and construction of PPI network

Next, we performed GO and KEGG enrichment analysis on 113 inflammatory DEGs. The GO analysis results are divided into three aspects: BP (biological process), CC (cell component), and MF (molecular function)[23], then select the five most closely related pathways or life activities in each type according to the p-value, the results showed that the BP group mainly regulated the positive regulation of cytokine production, response to molecule of bacterial origin, response to lipopolysaccharide, leukocyte migration, and cell chemotaxis. The CC group was mainly enriched in the external side of plasma membrane, secretory granule membrane, membrane region, membrane microdomain, and membrane raft, while the MF group was enriched in receptor ligand activity, cytokine receptor binding, cytokine activity, G protein-coupled receptor binding, and cytokine receptor activity, as shown in Figure 2A. And KEGG analysis showed that these inflammatory genes were mainly involved in Cytokine-cytokine receptor interaction, Neuroactive ligand-receptor interaction, Viral protein interaction with cytokine and cytokine receptor, TNF signaling pathway, and JAK-STAT signaling pathway, as shown in Figure 2B. The analysis suggests that these genetic pathways are all associated with the inflammatory process and may be implicated in the onset of ccRCC. In addition, in order to further study the interaction of these DEGs, we used the String database to construct a PPI network which containing these genes, as shown in Figure 2C, and the red represents the up-regulated inflammation-related genes, and the green represents the down-regulated inflammatory genes.

Establishment of prognostic model risk score based on inflammatory DEGs

To ensure the integrity of the follow-up data, we deleted the samples within 0 days of follow-up and obtained a total of 526 ccRCC samples. Next, we used univariate Cox regression to analyze the correlation between 113 inflammatory DEGs and the prognosis of ccRCC ($p < 0.01$), and to screen out the DEGs related to the survival of the ccRCC preliminarily, as shown in Figure 3A, a total of 39 inflammatory genes associated with the prognosis of ccRCC were obtained, 7 genes, including APLNR, BTG2, CALCRL, CX3CL1, EDN1, TACR1, and TLR3, were associated with better prognosis of ccRCC (HR<1) which may protective genes, while 32 genes including AQP9 and AXL are associated with poorer survival in ccRCC and may be high risk genes.

Next, we performed multivariate Cox regression analysis on these 39 inflammatory genes, and constructed a risk score formula. The results are shown in Figure 3B, 10 inflammatory DEGs may be independent prognostic factors of ccRCC, among which APLNR, BTG2, CX3CL1, SPHK1 are the independent predictors of good prognosis of ccRCC, while CSF1, GABBR1, HAS2, ICAM1, P2RY2, TIMP1 are the independent predictors of poor prognosis in ccRCC. The risk score formula is as follows: (-0.0153×APLNR) + (-0.0073×BTG2) +0. 0225×CSF1+ (-0.0107×CX3CL1) +0. 1888×GABBR1+0. 1528×HAS2+0. 0088×ICAM1+0. 3952×P2RY2+ (-0.0442×SPHK1) +0. 0006×TIMP1. We then scored 526 ccRCC samples according to this formula, used the median as a threshold, and divided all samples into two groups of high and low risk, with 263 samples in each group. And we performed a Kaplan-Meier survival analysis on the two groups, and the results showed that the survival rate of the high risk group was significantly lower than that of the low risk group ($p < 0.001$), as shown in Figure 3C, suggesting that the prognostic model risk score can effectively predict survival. As shown in Figure 3D, according to the risk score, the samples were divided into high risk and low risk groups, and compared with the low risk group, as the risk score increased, the high risk group had more deaths and shorter survival time, as Figure 3E shown. clinical characteristics such as risk score, Grade (G1-G3), age (≤65 years old or >65 years old), stage, TNM stage and the expression of inflammatory genes among different subgroups are presented in the heat map in Figure 3F, among which APLNR, BTG2 and CX3CL1 were enriched in the low risk score group, and seven genes including CSF1 tended to be highly expressed in the high risk group, and higher risk score corresponds to higher TMN stage, Grade and Stage.

Meanwhile, in order to enhance the visualization of the results of the prediction model, a nomogram (Fig. 3G) of clinical factors and risk score was constructed. The results showed that the risk score of the high risk group corresponds to a higher score, which means a shorter survival time and worse prognosis.

Independent prognostic value of prognostic model risk score

Next, to evaluate whether this prognostic model risk score is an independent prognostic factor for ccRCC, we performed univariate and multivariate Cox regression analyses. Univariate Cox regression analysis showed that age, grade, stage, TMN stage, and risk score ($p<0.001$, HR=1.157) were all associated with poorer prognosis in ccRCC (Fig. 4A). And multivariate Cox regression analysis showed that the risk score

was an independent prognostic factor for poor prognosis in ccRCC (p<0.01, HR=1.100), as shown in Figure 4B.

ROC is a reliable method for identifying the prognostic value of this risk score for ccRCC[24]. Therefore, we evaluated the predictive value of the risk score using the ROC curve, and its area under the curve (AUC) was 0.770, indicating a good predictive value. To evaluate the predictive accuracy of risk score, we performed ROC analysis on the 1st, 3rd, and 5th years of survival of ccRCC patients, and the AUC were 0.770, 0.742, and 0.757, as shown in Figure 4C. Multi-indicator ROC curve analysis was used to analyze the predictive value of various clinical factors on the 5-year survival of ccRCC, among them the prognostic model risk score had the highest AUC value, which was 0.757, indicating that the prognostic model risk score had a good ability to predict the prognosis of ccRCC, as shown in Figure 4D.

Validation of prognostic model risk scores

In order to verify the predictive ability of prognosis of the risk score, 526 ccRCC samples were randomly divided into training and test sets and then analyzed them separately. We calculated the risk scores of ccRCC patients in the training set and test set according to the risk score formula, and divided the patients in the training set and test set into high risk or low risk groups according to the median risk score value (Fig. 5A, 5B), and analyzed the survival status of the training set and the test set samples separately (Fig. 5C, 5D), the distribution of the survival status of the training set and the test set was basically consistent with the whole sample, that the low risk group showed better survival rate and longer survival time than the high risk group after a period of follow-up. The KM survival curve analysis showed that the overall survival rates between the two risk groups in the training set and the test set were significantly different (p < 0.001) (Fig. 5E, 5F), and the survival rate of the high risk group was significantly lower than that of the low risk group. The AUC of the ROC curve for 1, 3, and 5 year survival were 0.763, 0.705, 0.730 in the training set, and 0.780, 0.784, 0.781 in the test set (Fig. 5D), similar to the overall result obtained above. All of this demonstrated that the prognostic model has a good predictive value for the prognosis of ccRCC.

GSEA based on the prognostic model risk score

We used GSEA to further explore the differences in gene function and pathways among the subgroups classified by the prognostic model. The Gene Ontology (GO) analysis showed (Fig. 6A), in the high risk group, cilium movement (NES=1.94, p=0.002)、microtubule bundle formation (NES=1.93, p=0.006), Cytokine activity (NES=1.75, p=0.012), cilium or flagellum dependent cell motility (NES=1.64, p=0.015), meiosis 、cell cycle process (NES=1.75, p=0.017) were enriched、In contrast, the lipid oxidation apical part of cell (NES=-2.28, P =0.000), apical plasma membrane (NES=-2.27, P =0.000), Microbody (NES=-2.24, P =0.000) and renal system process (NES=-2.21, P =0.000) were enriched in the low risk group. In addition, we also identified the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, as shown in Figure 6B, we found homologous recombination (NES=1.82, p=0.018), alpha-linolenic acid metabolism (NES=1.63, p=0.033), glycerophospholipid metabolism (NES=1.62, p=0.029) were enriched in the high risk group, And vasopressin regulated water reabsorption (NES=-2.24, p=0.000), peroxisome (NES=-2.23,

p=0.000), proximal tubule bicarbonate reclamation (NES=-2.21, p=0.000), valine leucine and isoleucine degradation (NES=-2.21, p=0.000) =-2.16, p=0.002), fatty acid metabolism (NES=-2.15, p=0.002) and other pathways were found to be enriched in the low risk group.

Differences in tumor microenvironment and tumor heterogeneity of ccRCC between high risk and low risk group

The tumor microenvironment includes immune cells, stroma, blood vessels, and lymphatic vessels, which are the material basis for the growth, metabolism and metastasis of malignant tumors[25]. Therefore, in order to assess the differences in the tumor microenvironment between the high risk and low risk groups in the prognostic model, we conducted the following analyses. In the analysis of immune cell function, as shown in Figure 7A, the high risk group and the low risk group showed significant differences in APC co stimulation, CCR, Checkpoint, Cytolytic activity, HLA, Inflammation promoting, Parainflammation, T cell co inhibition, T cell co stimulation, and Type II IFN Reponse. Except for Type II IFN Response, the activities of the other 9 immune pathways in the high risk group were higher than those of the low risk group. And we drew a heat map of immune response based on TIMER, CIBERSORT, QUANTISEQ, MCP counter, XCELL, EPIC (Fig. 7B).

Immune checkpoints are molecular markers expressed on immune cells, which are part of the tumor microenvironment. Given the importance of immune checkpoint inhibitors in the current ccRCC treatment, we further analyzed the differences in immune checkpoint expression between the two groups, as shown in Figure 7C, the results showed that there were significant differences in the expression of 36 immune checkpoints between the high and low risk groups. Except for HHLA2, KIR3DL1, TNFSF18, and NRP1, which were higher in the low risk group, the expression levels of the remaining 32 immune checkpoints, including PD-1, CTLA-4, LAG3, BTLA, and TIGIT were higher in the high risk group.

Differences in the ccRCC tumor microenvironment constitute the macroscopic heterogeneity of tumor. To further judge the heterogeneity of ccRCC samples and to determine the differences in the infiltration of non-tumor cells in the tumor microenvironment, we used the ESTIMATE tool to evaluate the tumor purity in samples from high risk and low risk groups. ImmuneScore showed that the high risk group had a higher content of immune cells than the low risk group ($p < 0.001$) (Fig. 7D), while the StromalScore showed no difference in stromal cells between the two groups (Fig. 7E). The ESTIMATE score showed that the composite score was significantly higher in the high risk group (Fig. 7F). Tumor purity was calculated by ESTIMATE score, and the results showed that the high risk group had lower tumor purity and greater heterogeneity (Fig. 7G).

Drug susceptibility analysis of inflammatory DEGs in a prognostic model

CellMiner is a database based on the 60 cancer cells listed by the National Cancer Institute (NCI), and is widely used to study the relationship between identified oncogenes and compounds of which approved by the US Food and Drug Administration (FDA) or Drug molecules in clinical trials[22]. We use the CellMiner database to obtain information about FDA-approved drugs, and correlated it with 10

inflammatory DEGs in the prognostic model risk score. The analysis showed that the expression of inflammatory DEGs was significantly correlated with the sensitivity of 149 drugs ($p < 0.05$). The expression of APLNR was positively correlated with the drug sensitivity of Fluphenazine, Isotretinoin, Megestrol acetate and Imiquimod, but negatively correlated with Irofulven. The expression of CSF1 was positively correlated with the drug sensitivity of Midostaurin, JNJ−42756493, Zoledronate, IPI−145, and Dasatinib, the expression of TIMP1 was positively correlated with the sensitivity of Simvastatin, and the expression of CX3CL1 was positively correlated with the drug sensitivity of Vemurafenib but negatively with Docetaxel, and BTG2 is also positively correlated with Fluphenazine, as shown in Figure 8.

Discussion

ccRCC is the most common subtype of renal cell carcinoma, but due to its natural resistance to conventional treatments such like radiotherapy and chemotherapy, targeted therapy and immunotherapy are currently the main treatments for advanced ccRCC[26]. And these treatments have partially improved the survival of patients, however due to the lack of Post-line treatment and suboptimal efficacy, the mortality rate of advanced ccRCC remains high[3]. The development of molecular biology and cancer genomics has brought new hope for the treatment of advanced ccRCC, but still lacks a good prognostic model to predict the prognosis and bring enlightenment for the screening of potential therapeutic targets[27]. ccRCC is a highly heterogeneous tumor, compared with a single biomarker, incorporating multiple genetic markers into one prognostic model may improve the accuracy of prognosis prediction[28, 29]. Currently, inflammation has been found to play an important role in the occurrence and development of cancer[10], studies have shown that cancer cells and interstitial cells can react with inflammatory cells to form an inflammatory tumor microenvironment (TME), because of its strong plasticity, it can dynamically intervene in the occurrence, development and metastasis of tumors[30]. The role of its family genes as biomarkers in ccRCC is gradually being revealed

In this study, we first use the TCGA database to screen 113 inflammation-related genes with differential expression between ccRCC and normal tissues, and explored the mechanism of action of these inflammatory DEGs in ccRCC by GO-KEGG analysis, the results showed that the inflammatory DEGs obtained from the preliminary screening were enriched in Cytokine-cytokine receptor interaction, Neuroactive ligand-receptor interaction, Viral protein interaction with cytokine and cytokine receptor, TNF signaling pathway, JAK-STAT signaling pathway, and use these inflammatory DEGs to construct a PPI network to visualize the expression and connection strength between them. Studies have shown that genes with strong connectivity, such as IL-10, are one of the important cytokines in inflammatory diseases, and participate in the occurrence of tumors including ccRCC by inhibiting the killing effect of cytotoxic lymphocytes[19, 31]. In addition, TLR2 makes the body susceptible to infectious inflammation, and its expression in ccRCC tissue is significantly higher than that in peripheral tissue, suggesting that it is related to tumorigenesis[32, 33]. And another example is HIF1A, as one of the hypoxia-inducible factor family, which has long been considered to be closely related to the occurrence of inflammation and can inhibit the occurrence of ccRCC as a tumor suppressor gene[34, 35].

Secondly, we constructed a ccRCC prognostic model risk score based on inflammation-related genes, we used univariate and multivariate risk regression to screen out 10 independent prognostic inflammatory DEGs of ccRCC, among them, four genes, including APLNR, BTG2, CX3CL1, and SPHK1 are independent predictors of good prognosis in ccRCC, while CSF1, GABBR1, HAS2, ICAM1, P2RY2, and TIMP1 are independent adverse prognostic factors in ccRCC. Currently, studies have revealed the relationship between some of these genes and the prognosis of ccRCC, for example, the expression of APLNR is decreased in high grade, high stage and metastatic ccRCC, which is negatively correlated with tumor aggressiveness[36]; BTG2 is negatively correlated with the prognosis of ccRCC, over expressed BTG2 can inhibit the proliferation, migration and invasion of ccRCC[37]; and SPHK1 can reduce the activity of HIF-2 in ccRCC to reduce tumor proliferation and invasion[38]; the high expression of CSF1 may lead to poor prognosis and recurrence of the ccRCC patients after nephrectomy[39]; the down regulation of miR-125a can target the over expression of HAS2, which causes the invasion and metastasis of ccRCC[40]; The high expression of ICAM1 and TIMP1 indicates poor prognosis of ccRCC[41], which may be an independent predictive marker of ccRCC prognosis, however, some studies have also revealed that the incapacitation of double positive T cells for CXCL13(+) and CD8(+) is considered to be the cause of immune escape in ccRCC, which is different from the results of our analysis[42]. Then, a risk score formula was constructed based on these inflammatory genes: (-0.0153×APLNR) + (-0.0073×BTG2) +0. 0225×CSF1+ (-0.0107×CX3CL1) +0. 1888×GABBR1+0. 1528×HAS2+0. 0088×ICAM1+0. 3952×P2RY2+ (-0.0442×SPHK1) +0. 0006×TIMP1. And we used the formula to calculate the prognostic risk score of each sample, with the median as the threshold, we divided the ccRCC samples into high and low risk groups, and then we did a stratified analysis, the results showed that the low risk group tended to have longer survival, and there were significant differences in Grade, stage and TNM stage between the high risk group and the low risk group. Besides, we built a nomogram based on age, grade, stage, T, M, N stages, risk score to enhance its visualization. Secondly, in order to prove the independent prognostic value of the prognostic model risk score, we used univariate and multivariate Cox analysis to prove its independent value on the prognosis of ccRCC, and used ROC curve and multi-indicator ROC curve analysis to prove that the prognostic model has a relatively stable predictive value for the prognosis of ccRCC and is superior to other clinical features in predicting the long term prognosis of ccRCC. Then, in order to verify this risk score, we randomly divided the ccRCC samples in the TCGA database into the training set and test set, and divided the two groups into high risk group and low risk group according to the median as the threshold, and also performed KM analysis, the results show that the prognosis of the high risk group is significantly worse than that of the low risk group in both the training set and the test set. The results are similar to the conclusions of the KM analysis of the total sample. Similarly, we performed ROC analysis on the training set and test set to evaluating 1 year, 3 year and 5 year survival rates, the AUC results are basically the same in the training set, the test set or the overall samples, reflecting its good predictive value for prognosis.

Based on the prognostic model risk score, we performed GSEA, the GO analysis showed that the high risk group was mainly enriched in the formation of organelles and regulation of cell cycle, current study believes that the fiber is made of a microtube core axon, which can cause the progression of the tumor

and the appearance of the tumor[43]; a variety of tubulin such as Tubulin Cofactor A[44], participate in the formation of microtubules, the latter prove participate in tumor invasion and metastasis. The role and mechanism of cytokine in cancer occurrence and treatment is gradually revealed, some evidence demonstrate that cytokines such as IL-30 can promote the appearance of tumors[45], and hypoxia inducible factor-1 (HIF-1) can participate in tumor growth, metabolism, immune escape and resistance by regulating the target genes[46]. And the KEGG analysis of the high-risk group showed that it was enriched in Homologous Recombination, α-Linolenic acid metabolism, Glycerol phospholipid metabolism, current research proves that Homologous Recombination Deficiency (HRD) can lead to the occurrence of various tumors including ccRCC[47, 48], PARP inhibitors developed based on this mechanism have shown a powerful pan-anticancer effect and have been approved for the treatment of multiple tumors; and α-Linolenic acid can inhibit tumor by preventing tumor colonization, destroying nutrient supply, competing with enzymes, and it can also inhibit the inflammatory response caused by M1-like macrophages[49, 50]; glycerophospholipids are considered to have potential anti-inflammatory effects and may have the potential to inhibit tumors[51]. Current research tends to believe that oxidative stress caused by intracellular factors such as inflammation can damage DNA and interfere with its repair, leading to HRD and eventually lead to the occurrence and development of tumors[52]. Therefore, inflammation-related genes may affect cell metabolism, participate in DNA damage and repair obstacles, and ultimately lead to the occurrence of ccRCC by changing the expression of cytokines and cell cycle.

Furthermore, given that the role of the immune microenvironment in the occurrence and development of tumors has been gradually revealed[53], and the current status that immune checkpoint inhibitors as the most popular immunotherapy in solid tumors such as ccRCC[54], we focused on analyzing the association of this prognostic model with immune cells and related functions, immune checkpoint expression, and the tumor microenvironment. The results showed that the expression of immune checkpoints such as PD-1, CTLA-4, and LAG3 in the high risk group were higher than those in the low risk group, indicates that the high risk group may have better curative effect when applying PD-1, CTLA-4, and LAG3 inhibitors, this also partly explains that the latest guidelines only recommend targeted combination immunotherapy or combined immunotherapy as the first level recommendation for the first-line treatment of metastatic or unresectable medium and high risk ccRCC, while the first level recommendation for low risk groups is still targeted therapy[5]. Meanwhile, we used the ESTIMATE tool to find that the high risk group had higher ccRCC immune cell content, higher ESTIMATE score, lower tumor purity, and higher heterogeneity, study have shown that with the progression of ccRCC, including later stages and higher histological grades, its tumor purity decreases and corresponds to a poorer prognosis, which is consistent with our findings[55].

Finally, in order to demonstrate the significance of this risk model for clinical drug selection, we used the CellMiner database to screen out the relevant drugs approved by FDA, and conducted a correlation analysis between drugs and inflammatory DEGs of risk score, to evaluate the relationship between their expression and drug sensitivity. The results showed that there were statistical differences between the expression of inflammatory DEGs and the sensitivity of various drugs. For example, the higher the expression of APLNR, the higher sensitivity of cells to Fluphenazine, Isotretinoin, Megestrol acetate, and

Imiquimod, but the lower sensitivity to Irofulven. The increased expression of CSF1 increases the sensitivity of cells to Midostaurin, JNJ−42756493, Zoledronate, IPI−145, and Dasatinib. Among them, JNJ−42756493 is a pan-FGFR family inhibitor which can binds to CSF1R to increase drug sensitivity, and enhance the therapeutic effect of metastatic urothelial carcinoma by targeting immune escape and angiogenesis in the tumor microenvironment[56]; and the high expression of CSF1 can induce osteoclasts in triple-negative breast cancer to be sensitive to bone-targeting drugs such as Zoledronate[57]. Based on these results, we believe that the inflammatory DEGs in the risk score are related to the sensitivity of certain antineoplastic drugs, which may have guiding significance for clinical medication.

Conclusion

In this study, we screened important inflammatory DEGs in ccRCC, and established a prognostic model risk score and validated it internally. We grouped ccRCC samples according to prognostic model risk scores and explored their differences in gene and pathway enrichment, immune cells, immune function, immune checkpoints, and tumor microenvironment, revealed that high risk ccRCC has lower tumor purity, greater heterogeneity, and have better benefit of selecting immunotherapy than lower risk patients. In conclusion, our study established a prognostic model of ccRCC based on multiple inflammatory genes, which has independent prognostic value for ccRCC, it can guide the use of drugs, and provides a basis for further exploration of relevant therapeutic targets and revelation of the role that inflammatory genes in ccRCC and pan-cancer.

However, our study also has some limitations, we may have flaws in the process of screening inflammation-related genes, secondly, we conduct this study on the basis of the TCGA database, the results of the study are best verified by external databases or further experiments.

Declarations

Data Availability

The data presented in this study are openly available in TCGA (https://portal.gdc.cancer.gov/).

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Authors' contributions

Research idea and study design: CWJ, CBH, ZYX, CH, SJJ; data acquisition: ZYX, CH; data analysis/interpretation: ZYX, CH; analysis: ZYX, CH, SJJ. Each author contributed important intellectual content drafting and/or revising the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interest

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

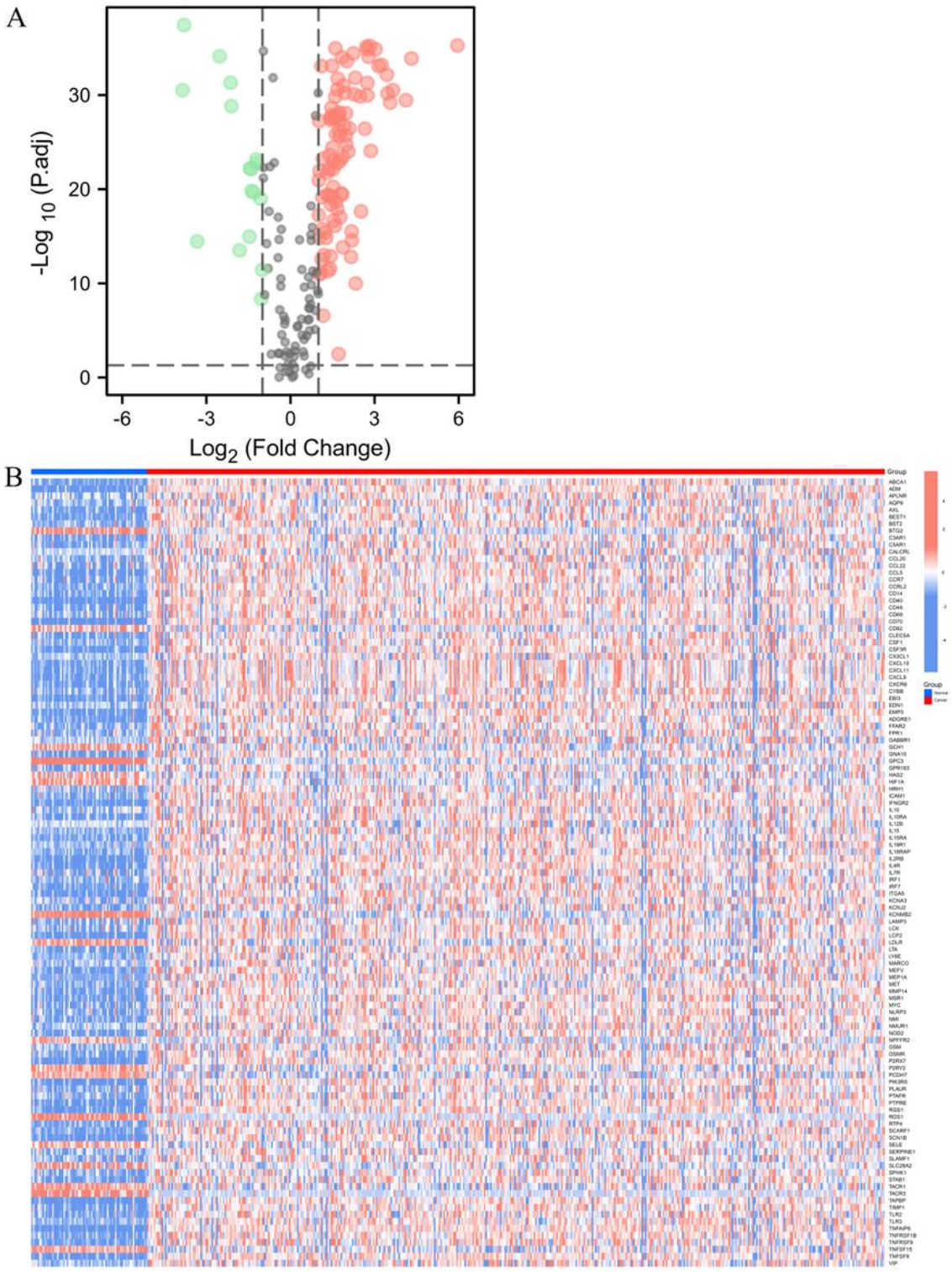

Figure 1

A. The volcano map shows the relationship between normal tissue and inflammatory DEGs in ccRCC, with up-regulated genes in red and down-regulated genes in green.

B. Heat map of the inflammatory DEGs between the normal (N, blue) and tumor tissues (T, red).

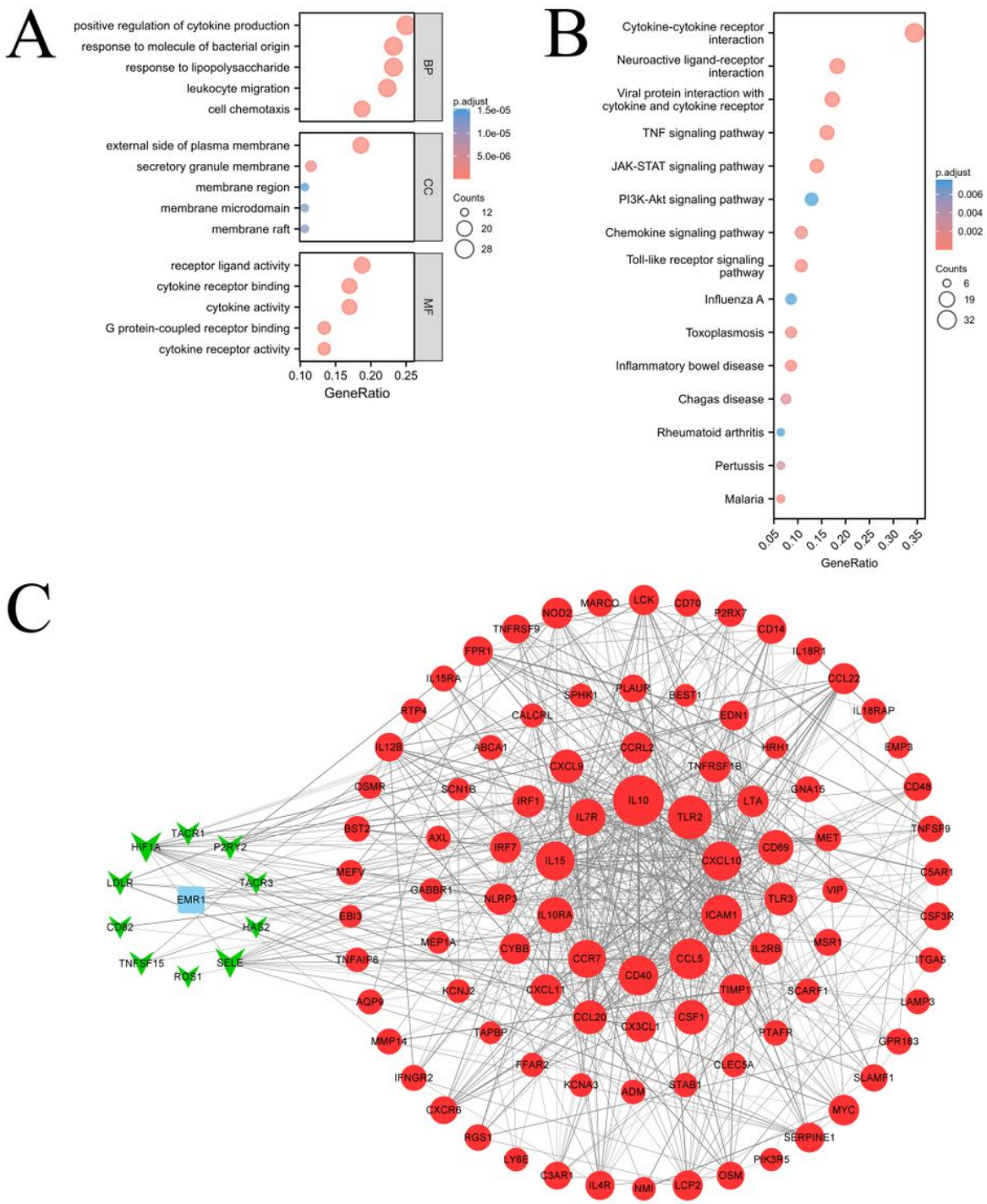

Figure 2

A. GO analyses for inflammatory DEGs.

B. KEGG analyses for inflammatory DEGs.

C. PPI network indicating the interactions of inflammatory DEGs (interaction score = 0.7).

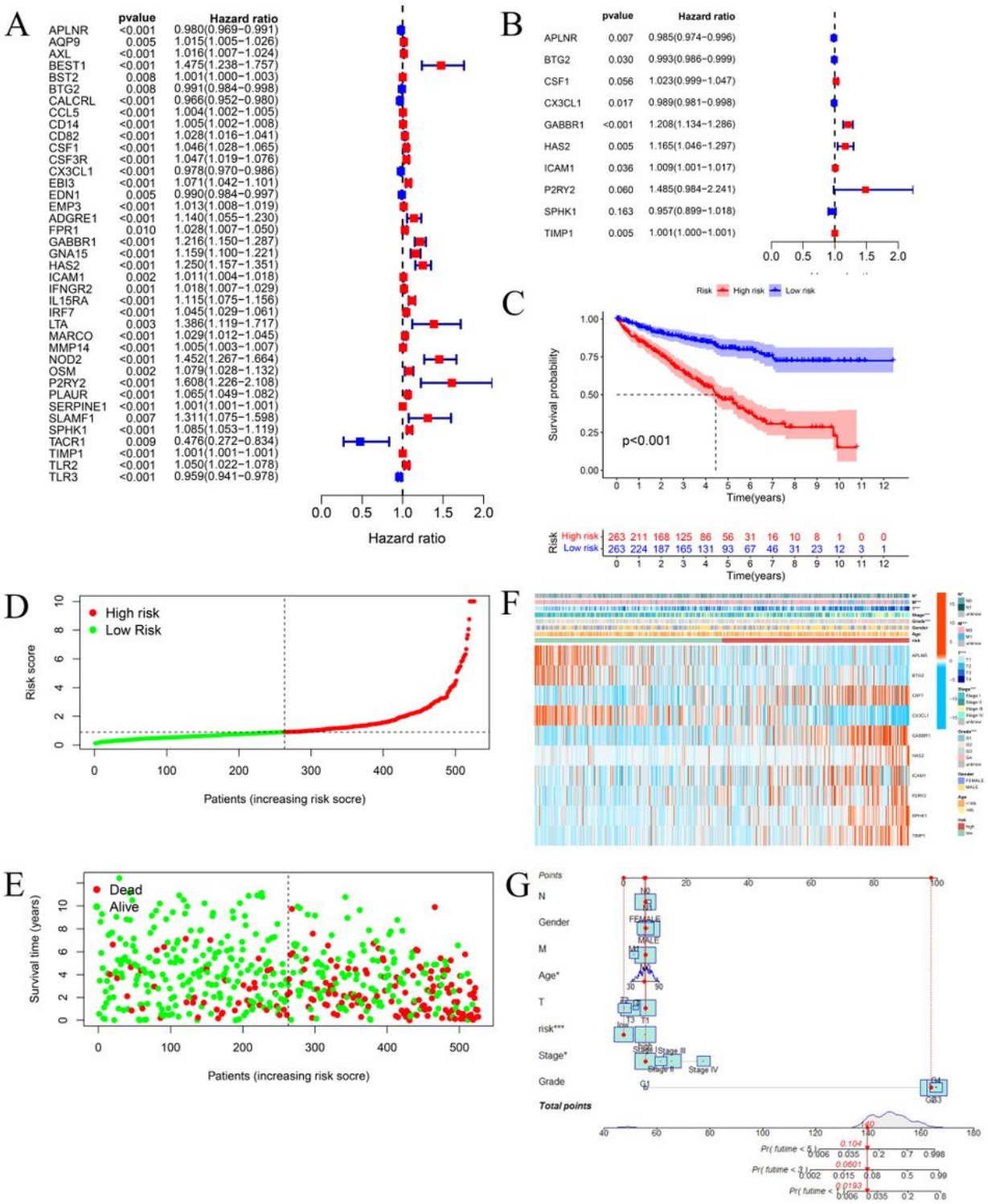

Figure 3

A. Univariate Cox regression analysis of the correlation between inflammatory DEGs and prognosis of ccRCC.

B. Multivariate Cox regression analysis of the correlation between inflammatory DEGs and prognosis of ccRCC.

C. Kaplan–Meier curves for the OS of patients in the high- and low risk groups.

D. Risk curve based on the risk score for each sample.

E. Scatterplot based on the survival status of each sample, green dots and red dots indicate survival and death respectively.

F. The heat map shows the distribution of clinical features and inflammatory DEGs in the high risk and low risk groups. Blue represents the low risk group, red represents the low risk group, while red represents a high expression and green represents a low expression.

G. A nomogram for both clinic-pathological factors and risk score.

Figure 4

A. Univariate Cox regression analyses of associations between clinical parameters (including risk score) and OS.

B. Multivariate Cox regression analyses of associations between clinical parameters (including risk score) and OS.

C. Time-dependent ROC curves of OS at 1, 3, and 5 years.

D. Multi-indicator ROC curve analysis.

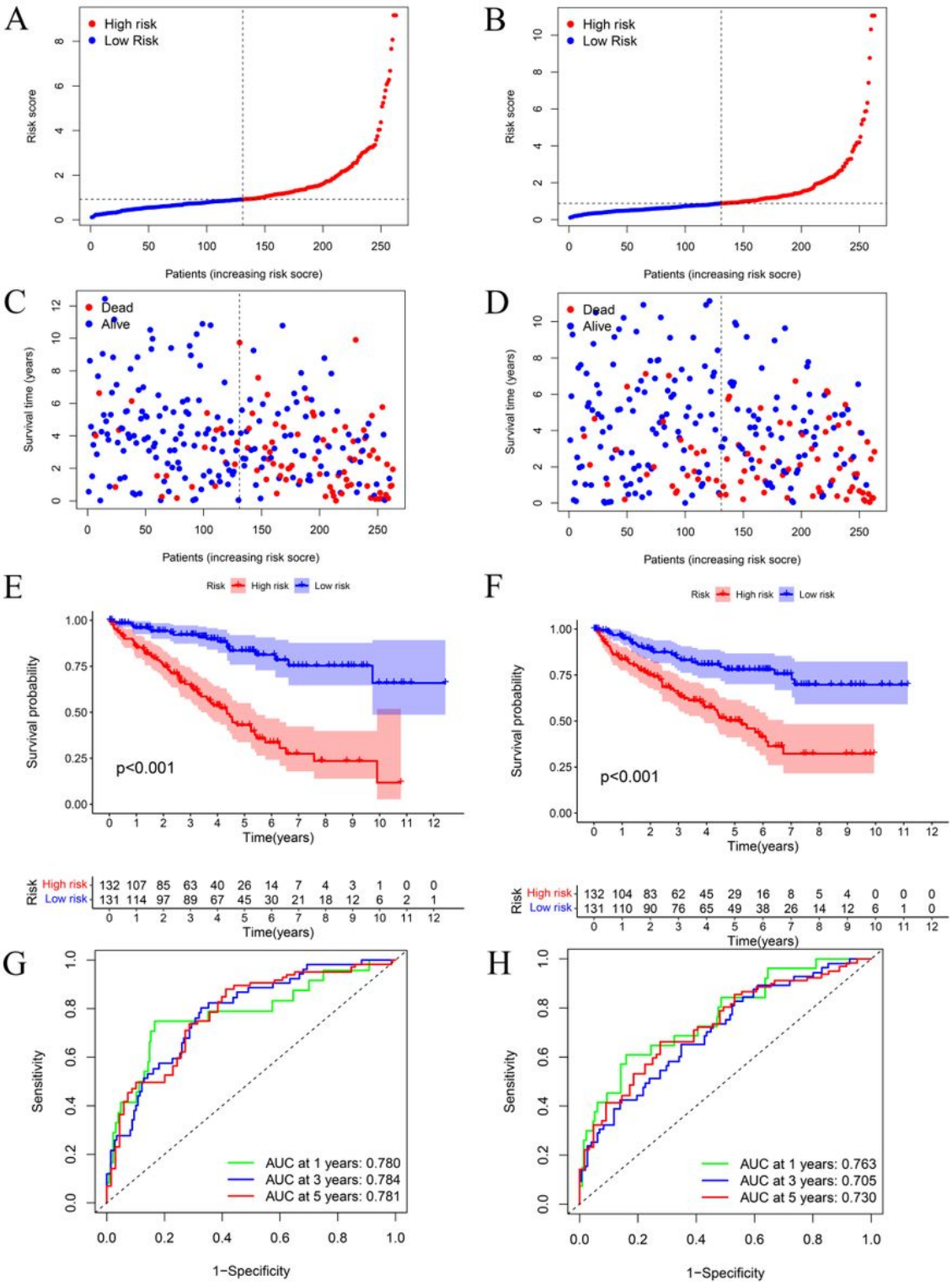

Figure 5

A. Risk curve based on the risk score for training set.

B. Risk curve based on the risk score for test set.

C. The survival status and survival time of training set ranked by risk score.

D. The survival status and survival time of test set ranked by risk score.

E. Kaplan–Meier curves for the OS of training set.

F. Kaplan–Meier curves for the OS of test set.

G. Time-dependent ROC curves of OS for training set.

H. Time-dependent ROC curves of OS for test set.

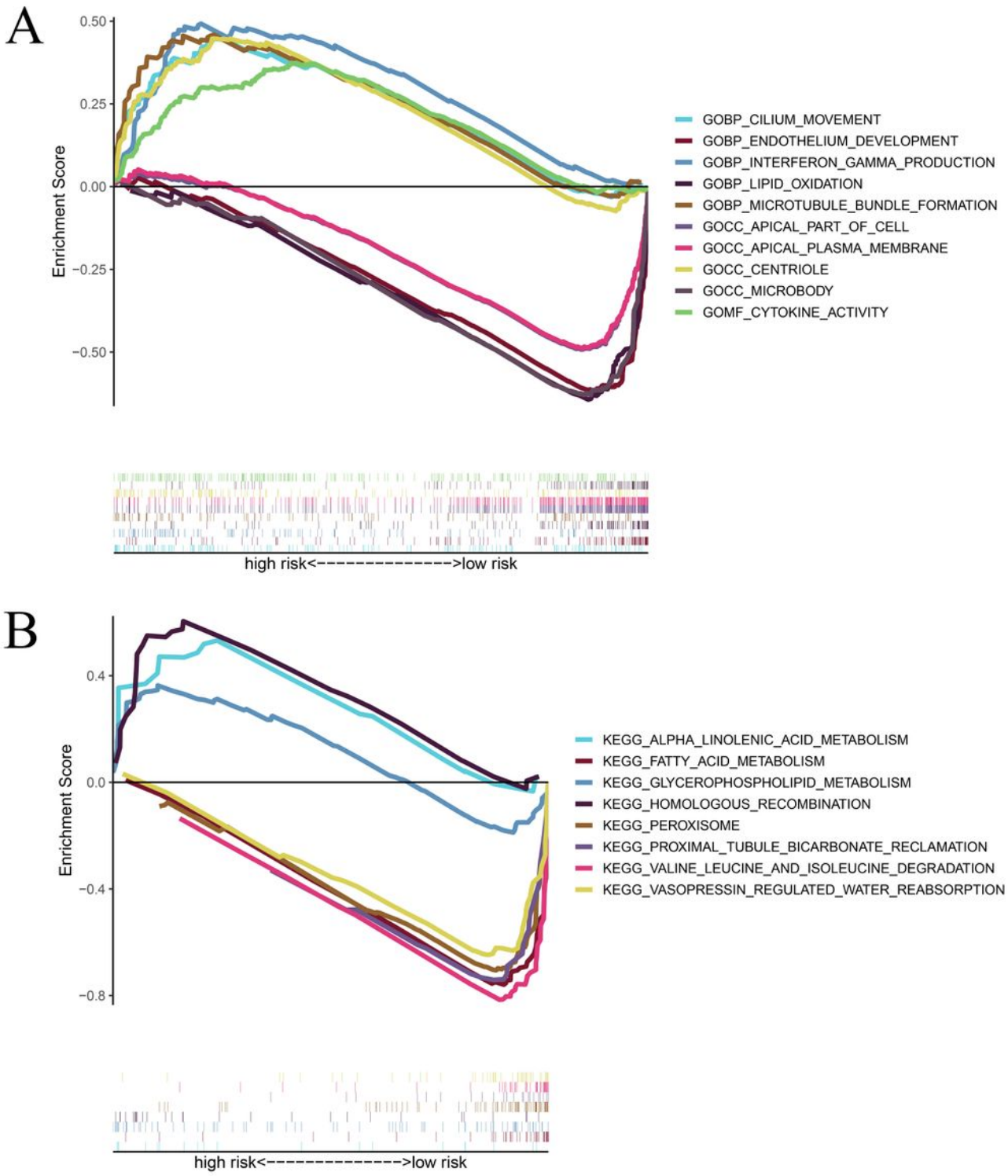

Figure 6

Enrichment plots from GSEA for subgroups based on the risk score

A. GO analyses.

B. KEGG analyses.

Figure 7

A. Enrichment scores of 13 immune pathways in low risk group and high risk group.

B. Heat map of immune response in low risk and high risk groups.

C. Gene expression of 36 immune checkpoints in low risk and high risk groups.

D. Immune scores in the low risk and high risk groups.

E. Stromal score in the low risk and high risk groups.

F. ESTIMATE score in the low risk and high risk groups.

G. Tumor purity in the low risk and high risk groups.

Figure 8

Relationship between drug sensitivity and expression of inflammatory DEGs.