An immune-related signature predicted survival in patients with Kidney clear cell carcinoma

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Abstract

Objective

The main aim of this study was to identify immune-related genes in Kidney clear cell carcinoma (ccRcc) patients.

Methods

We downloaded RNAseq data and clinical information of ccRcc patients from the TCGA database and retrieved the immune-related genes list from the web of Immport. We performed Cox and LASSO analyses, using “survival” and “glmnt’ packages implemented in R, to reveal survival immune-related genes. Next, we verified the prognostic signature of these genes, and further confirmed its survival value using Kaplan-Meier analysis and ROC time dependent curves. We also evaluated the signature’s immune cell component and the results of immunotherapy.

Results

Analysis of 526 tumor samples revealed a total of 770 immune-related genes, after four steps of primary screening. Cox and LASSO analyses revealed 11 hub immune-related survival genes, namely ADRB2, AGER, BIRC5, CDNF, CXCL2, ESR2, F2RL1, IFITM1, PTX3, SEMA3G, and TCF7L2. Kaplan-Meier curves revealed prognostic signature of the 11 DEGs, with the high-risk score group in ccRcc patients exhibiting a worse survival rate ($P < 0.001$ in both the training and test sets, respectively). ROC analysis in the training set revealed an AUC value of 0.71 for both 1- and 3-year survival, and 0.65 for 5 years). Furthermore compared with low-risk score group, we found that the high-risk score group had a higher percentage of 10 cells in the immune component (Exhausted cells, Tr1 cells, nTreg cells, Th1 cells, Tfh cells, Effect memory cells, MAIT cells, Macrophage cells, CD8 T cells, and iTreg cells), a low percentage of 6 cells (CD4 native cells, CD8 native cells, Th17 cells, central memory cells, Neutrophil cells, and Gamma delta cells).

Conclusion

Our results revealed 11 survival immune-related genes (ADRB2, AGER, BIRC5, CDNF, CXCL2, ESR2, F2RL1, IFITM1, PTX3, SEMA3G, TCF7L2) in ccRcc patients. We used these genes to develop a signature that can act as an independent prognostic predictor for overall survival and response of ccRcc patients to immunotherapy.

Introduction

Renal clear cell carcinoma (ccRCC) is the most common type of renal carcinoma(1). Many ccRCC patients benefit from early tumor detection, because this allows diagnosis of renal cancer at the initial stage. Surgery represents the first-choice treatment therapy for this group of patients(2). Previous evidences have indicated that complete surgical excision of the tumor lengthens survival time for these patients, although about 20–40% of patients with localized ccRCC are likely to develop metastatic recurrence following operation(3). In recent years, immune checkpoint inhibitors have become the new treatment therapy for patients with metastatic ccRCC, with a series of clinical trials affirming their efficacy(4). However, some of the metastatic ccRCC patients did not benefit from this treatment therapy, possibly due to effect of individualized tumor microenvironment(5, 6). Particularly, the different responses are due to action of tumor-infiltrating immune cells, such as T cells sub-populations (CD8 + T, CD4 + T), which form a key anti-tumor immune component(7). Cell component analysis has been previously used to reveal different immune cell components among ccRCC patients with different prognosis(8). However, nothing is known regarding key genes that regulate the changes in cellular components. In the present study, we performed analysis of RNA sequence data and identified 11 immune-related genes of prognostic value in ccRCC patients. After a series of analyses, we developed a novel gene signature which is expected to improve the prognostication of the ccRCC.

Methods

Screening for immune-related genes in ccRcc

We first downloaded the latest RNAseq, clinical and following-up data results for a kidney clear cell carcinoma cohort from the UCSC website (https://xenabrowser.net/datapages/)(9). Then, we downloaded a list of immune-related genes from ImmPort (https://www.immport.org/home/)(10), which contains 2483 immune-related genes. Selection criteria for the immune-related genes were as follows: 1). All samples had clinical information; 2). Data was from tumor samples, while normal samples were removed; 3). All genes in all samples had a FPKM >1; and 4). All genes were immune-related. We randomly divided the enrolled samples into two groups (training and test sets), and found no statistical significances in clinical information between them.

Survival analysis of immune-related genes in the training set

We performed a series of analyses to identify immune-related genes associated with survival of ccRcc patients in the training set. Firstly, we employed a random forest analysis using the ‘glmnt’ package implemented in R and identified genes with $P<0.05$. Secondly, we used the same package to perform a LASSO analysis, three times, on the reserved genes then selected only those with $P<0.05$. Finally, we performed univariate analysis using the ‘survival’ package in R to identify immune-related genes associated with survival of ccRcc patients ($P<0.05$).

Construction and validation of a prognostic prediction signature

We used univariate Cox regression analysis to construct an immune related prognostic signature, then calculated the risk score for all the samples. Risk scores in the training set were calculated using the Kaplan-Meier (KM) and ROC analysis whereas those in the test set were calculated using KM analysis. We correlated the resulting risk scores with different clinical data, namely tumors, lymph nodes, degree of metastasis, and grades. The KM analysis, ROC analysis and the correlation were counted using Gradpad prism 8.0.

Functional and pathway enrichment analysis

We performed gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses for all immune-related genes using the online tool created by David (https://david.ncifcrf.gov/)(11). Thereafter, we compared differential expression patterns of the genes, between the high- and low-risk score samples, in the training set using the 'limma' package in R ($P<0.01$). We also performed GESA analysis, using the 'gsea' package in R, on the differentially expressed genes(12).

Survival analysis based on immune-related genes

Firstly, we compared expression levels of immune-related genes associated with survival of ccRcc patients in the training set and normal tissues across the TCGA KIRC cohort, then screened the web of Ualcan (http://ualcan.path.uab.edu/)(13) to assess gene expression. Secondly, we used KM curves in web of Ualcan to analyze survival and reveal levels of DNA methylation.

The relationship between risk scores with immune cell components, expression of immune-related genes, and immunotherapy response

We explored immune cell component using the online analysis tool (ImmuCellAI: http://bioinfo.life.hust.edu.cn/ImmuCellAI/)(14), which can analyze 24 immune cell components, and found an association with expression of 8immune-related genes, namely *CTLA4*, *CD274*, *LAG3*, *SIGLEC15*, *PDCD1, PDCD1LG2*, *HAVCR2*, and *TIGIT*).

Results

Identification of immune-related genes

A four-step analysis of 526 tumor samples revealed a total of 770 immune-related genes(fig 1 A). All the 526 patients were randomly divided into training (n=263) and test (n=263) sets. A summary of patients' age, sex, and the TNM stage across the two sets is outlined in Table I. We found no statistical differences ($P > 0.05$) in all these variables. We used random forest analysis to select 38 genes. Moreover, LASSO analysis resulted in 13 genes(fig 1 B, C), of which 11, namely ADRB2, AGER, BIRC5, CDNF, CXCL2, ESR2, F2RL1, IFITM1, PTX3, SEMA3G, and TCF7L2, were confirmed to be immune-related genes associated with survival following univariate analysis (Table II).

Construction and validation of a prognostic prediction signature

Univariate Cox regression analysis allowed construction of an immune-related prognostic signature of the 11 hub genes. The formula used was as follows: Risk Score=−0.00926×ADRB2−0.00502× AGER− 0.00787×BIRC5−0.00552×CDNF−0.000911× CXCL2−0.00167×ESR2−0.0109×F2RL1−0.0255×IFITM1− 0.00840×PTX3−0.02760×SEMA3G −0.0433×TCF7L2. Thereafter, we used the Kaplan-Meier and ROC analyses to calculate risk scores in the training set. Results revealed significantly shorter overall survival time in the high-risk score group, after KM analysis (we divided 50% of the 263 patients with high-risk score into high-risk score group, P value <0.001, fig 2 A). ROC curves showed that the constructed signature had good accuracy, with AUC values of 0.71, 0.71 and 0.65 at 1-, 3- and 5-years, respectively (fig 2 B C). Moreover, the high-risk score group recorded a shorter survival time in the test set (P< 0.01, fig 2 D). We also analyzed the relationship between risk scores with different clinical information (tumor, lymph node, metastasis degrees, and grades) and found that the degree of metastasis was strongly correlated with risk scores(fig 2 E F). Furthermore, a combination of metastasis degrees and risk score generated a better cutoff value in ROC analysis(fig 2 G).

Functional and pathway enrichment analysis

GO analysis revealed enrichment of the following biology processes in ccRcc specimens; regulation of chemotaxis, regulation of symbiosis, and encompassing mutualism through parasitism(fig 3 A). On the other hand, KEGG analysis results showed that the 11 immune-related genes might be playing key roles in colorectal and breast cancers. Pathway analysis showed that hippo signaling and Kaposi sarcoma-associated herpesvirus infection were strongly associated with the 11 immune-related genes(fig 3 B). A further analysis of samples with different risk scores revealed an additional 4190 differentially expressed genes (P<0.01), while results of GSEA analysis revealed five pathways with different responses between two groups (P<0.01, fig 3 C).

Analysis of hub survival immune related genes

A comparison in expression levels in genes between training set (n=263) and normal tissue samples (n=71) revealed upregulation of 4 genes (IFITM1, CXCL2, AGER, and BIRC5) and downregulation of 3 genes (PTX3, F2RL1, and SEMA3G) in the tumor tissue samples (P<0.001, fig 4 A B). Conversely, 4 genes, namely ADRB2, CDNF, ESR2, and TCF7L2, did not exhibit any significant differences between tumor and normal tissues (P>0.05). A search of the 11 genes in the TCGA KIRC cohort (normal sample=72, and the tumor sample =533) in the web of Ualcan further revealed upregulation of 4 genes (IFITM1, CXCL2, AGER, and BIRC5) and downregulation of 5 others (PTX3, F2RL1, SEMA3G, CDNF, and ESR2) in tumor tissues. KM curves from the Ualcan database revealed 10 genes (ADRB2, AGER, BIRC5, CXCL2, ESR2, F2RL1, IFITM1, PTX3, SEMA3G, TCF7L2) which had statistical differences (P<0.05, fig 4 C). Notably, patients with upregulated ADRB2, F2RL1, SEMA3G, and TCF7L2, or downregulated AGER, BIRC5, CXCL2, ESR2, IFITM1, and PTX3, exhibited better survival rates. Furthermore, Expression levels of 7 genes were significantly correlated with levels of DNA methylation. Among the genes, TCF7L2, F2RL1, BIRC5, and CDNF significantly upregulated, whereas CXCL2, ESR2, and SEMA3G downregulated DNA methylation levels in the primary tumor tissues (P<0.05, fig 4 D).

Relationship between immune cell components with expression of immune-related genes and immunotherapy response

Results of ImmuCellAI analysis revealed that the high-risk score group of the training set had a higher percentage of 10 immune cells, namely Exhausted cells, Tr1 cells, nTreg cells, Th1 cells, Tfh cells, Effect memory cells, MAIT cells, Macrophage cells, CD8 T cells, and iTreg cells (fig 5 A). Conversely, a lower percentage was observed in 6 immune cells, namely CD4 native cells, CD8 native cells, Th17 cells, central memory cells, Neutrophil cells, and Gamma delta cells (fig 5 A, B).

Discussion

Immunotherapy has been introduced as a new treatment strategy for metastatic ccRCC (MccRcc) patients, in recent years. In fact, series of clinical trials have showed efficacy of two main immunotherapies, namely a single-agent which comprises immunotherapeutic agents targeting programmed death-1/programmed death-ligand 1 axis and MoAbs, which involves administering a combination of anti-PD1/PDL1 and anti-CTLA-4) to MccRcc patients(15). A previous phase III checkmate-025 trial proved that nivolumab, an anti-PD-1 monoclonal antibody, could enhance overall survival times of patients (media time: 25 months, HR: 0.73, 98% CI: 0.57–0.93; p = 0.002)(16). Results of another phase II trial, KEYNOTE-427, also revealed efficacy of pembrolizumab, an anti-PD-1 monoclonal antibody, in MccRcc patients(17). Moreover, a Phase III CheckMate-214 trial, using MoAbs to treat MccRcc patients, also revealed encouraging results. Specifically, a combination of nivolumab with ipilimumab, which is an anti-CTLA4 antibody, resulted in longer OS times (HR: 0.63; p < 0.001)(18, 19).

Although immunotherapy has generated encouraging results with regards to its efficacy to treat MccRcc patients, reliable biomarkers for predicting this efficacy and stratification of specific patient subgroups that best respond to the therapy remain unclear. Although PD-L1 expression has been shown to be the biomarker of choice for selecting patients that respond to immunotherapy in several cancer types, its value in MccRCC is contradictory following several clinical trials(20, 21). In fact, researchers have focused on the individual immune microenvironment, which causes different response to immunotherapy. Previous studies have shown that patients with high tumor immune cell inflation experience tumor progression, from local ccRCC to MccRcc t, while increased cell component of CD8 + T cells has been associated with better survival times in patients treated with suntinib(22). On the other hand, several other immune cells have been identified, although the underlying mechanism of the complex immune cell components as well as how they affect response to immunotherapy remain unclear.

Results of the present study revealed presence of 11 immune-related genes associated with survival, namely ADRB2, AGER, BIRC5, CDNF, CXCL2, ESR2, F2RL1, IFITM1, PTX3, SEMA3G, and TCF7L2. Previous studies have shown that AGE's protein is encoded the AGER gene, which is a member of the immunoglobulin superfamily of cell surface receptors(23), while ADRB2 and F2RL1 genes encode key proteins of G protein coupled receptor superfamily(24, 25). On the other hand, CXCL2 protein is part of a

chemokine superfamily that encodes secreted proteins involved in immunoregulatory and inflammatory responses(26), while the BIRC5 gene is a member of the inhibitor of apoptosis gene family, which encodes negative regulatory protein that prevent apoptotic cell death(27). Moreover, the PTX3 protein is induced by inflammatory cytokines in response to inflammatory stimuli(28), while theTCF7L2 gene encodes a high mobility group box containing transcription factor that plays a key role in the Wnt signaling pathway(29). Our analyses further revealed that 10 out of the 11 genes had survival value.

We constructed a new prognostic signature comprising the 11 genes, and found that it had prognostic value following validation using a training set and test sets. ROC curves further indicated that the signature had remarkable value in 1-, 3- and 5-year survival rates of patients with ccRcc. A correlation between the signature and clinical data revealed that the signature's risk score was strongly associated with the degree of metastasis. Our results further showed that the signature, in combination with the degree of metastasis had a better prognostic value in ROC time dependent analysis. Analysis of cell components across high- and low-risk score groups, using ImmuCellAI, revealed an association between high-risk scores with higher percentage of 10 immune cells, namely Exhausted cells, Tr1 cells, nTreg cells, Th1 cells, Tfh cells, Effect memory cells, MAIT cells, Macrophage cells, CD8 T cells, and iTreg cells. Conversely, low-risk scores were predictors for higher percentage of 6 immune cells, namely CD4 native cells, CD8 native cells, Th17 cells, central memory cells, Neutrophil cells, and Gamma delta cells.

Conclusion

In summary, we identified 11 immune-related genes associated with survival of ccRcc patients. We incorporated these genes in a new signature that can act as an independent prognostic predictor for overall survival of patients and their response to immunotherapy.

Declarations

Author contributions

Shen junwen wrote the paper. Wang rongjiang and Guan liya edited the paper. Chen yu, Fang zhihao, Yao jianxiang and Ling yuhang analyzed the data. Tang jianer made the images out.

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Ethics approval and consent to participate

No application

disclosure

The authors have declared no conflicts of interest.

Availability of data and materials

This research was performed on the public datasets. We described the way how to get the information of the public datasets in the part of method.

Acknowledgements

No application

Declarations, Competing interests, and statement

no conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication. We want to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Supplement: this was not a clinical trial program and the research did not have a clinical trial registration number.

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Tables

Table I: the information of two sets

	The training set	The test set
Cases(n)	263	263
Age (>60 years old; <60 years old, n)	153, 110	126, 137
T stage (T1, T2, T3, T4, n)	121, 38, 100, 3	145, 31, 79, 8
N stage (N0, N1, Nx, n)	130, 10, 123	109, 6, 148
M stage (M0, M1, Mx, n)	207, 45, 11	213, 33, 17
Grade stage (I, II, III, IV, X, n)	6, 103, 109, 42, 3	7, 126, 96, 32, 2
Statue (alive, dead, n)	153, 110	202, 61

Table II: the 11 survival immune related genes in the training set

Genes	HR	CI95 Low	CI95 High	P value
ADRB2	0.9460	0.9195	0.9733	1.291836e-04
AGER	1.0419	1.0127	1.0719	4.698369e-03
BIRC5	1.0544	1.0269	1.0828	8.849143e-05
CDNF	0.9602	0.9220	0.9999	4.948343e-02
CXCL2	1.0326	1.0129	1.0526	1.078000e-03
ESR2	1.0251	1.0012	1.0496	3.925910e-02
F2RL1	0.9623	0.9419	0.9831	4.293688e-04
IFITM1	1.0469	1.0132	1.0817	6.060241e-03
PTX3	1.0374	1.0169	1.0583	3.047926e-04
SEMA3G	0.9529	0.9336	0.9727	4.111072e-06
TCF7L2	0.8484	0.7859	0.9159	2.573438e-05

Figures

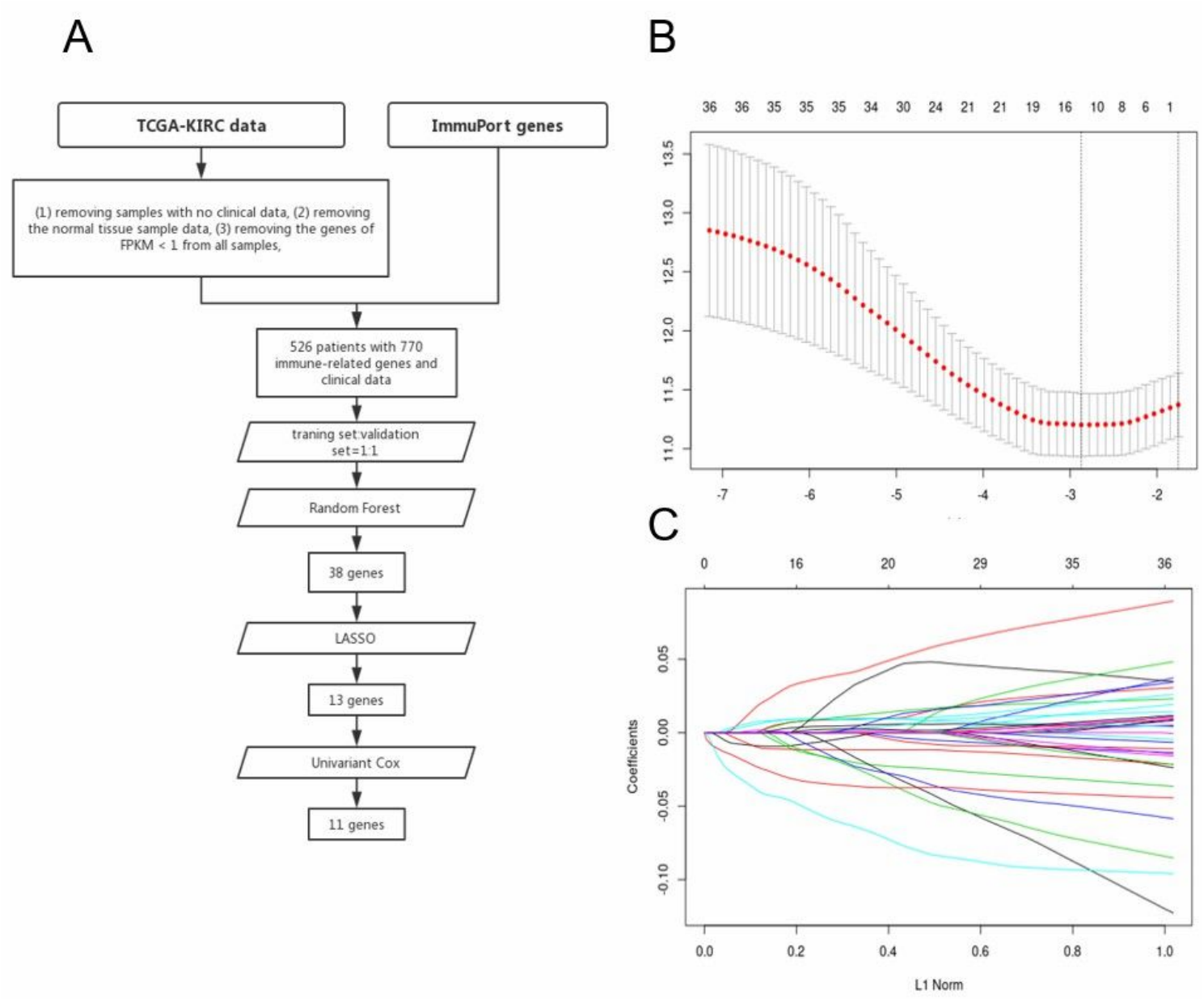

Figure 1

(A) The flow diagram of analysis. (B C) The images of COX analysis.

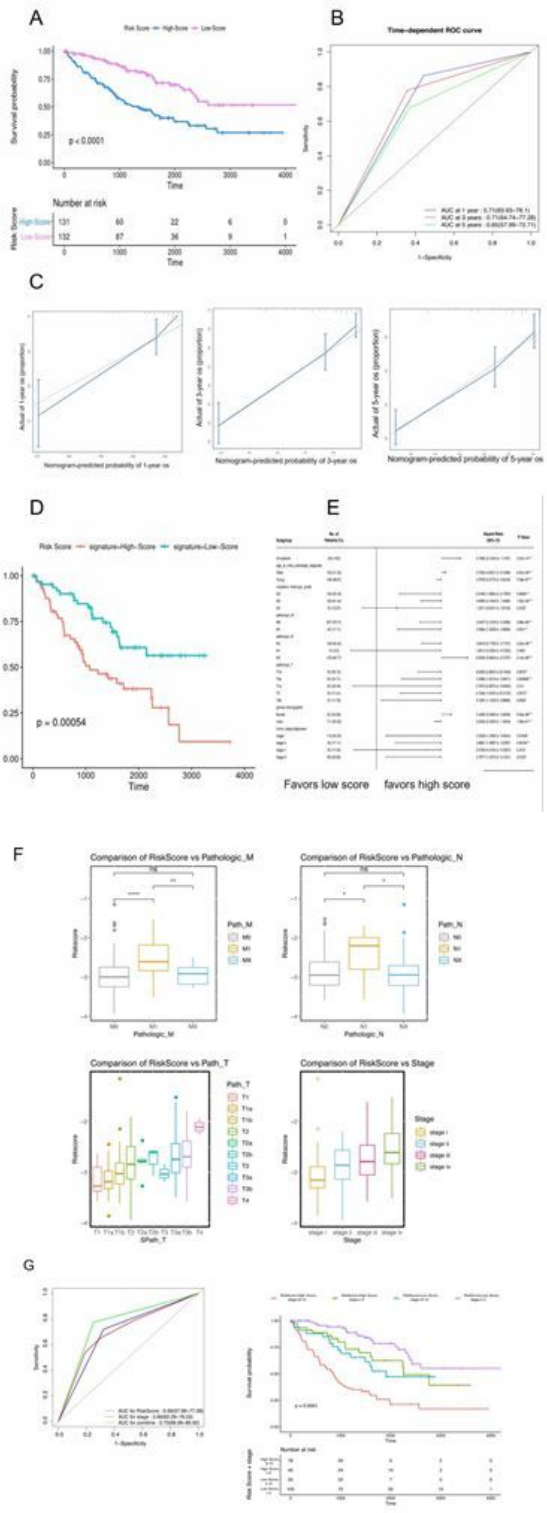

Figure 2

(A) The KM analysis in the training set. (B C) The ROC analysis in the training set. (D) The KM analysis in the test set. (E F) Relationship between risk scores with clinical information. (G) The combination ROC analysis and KM analysis.

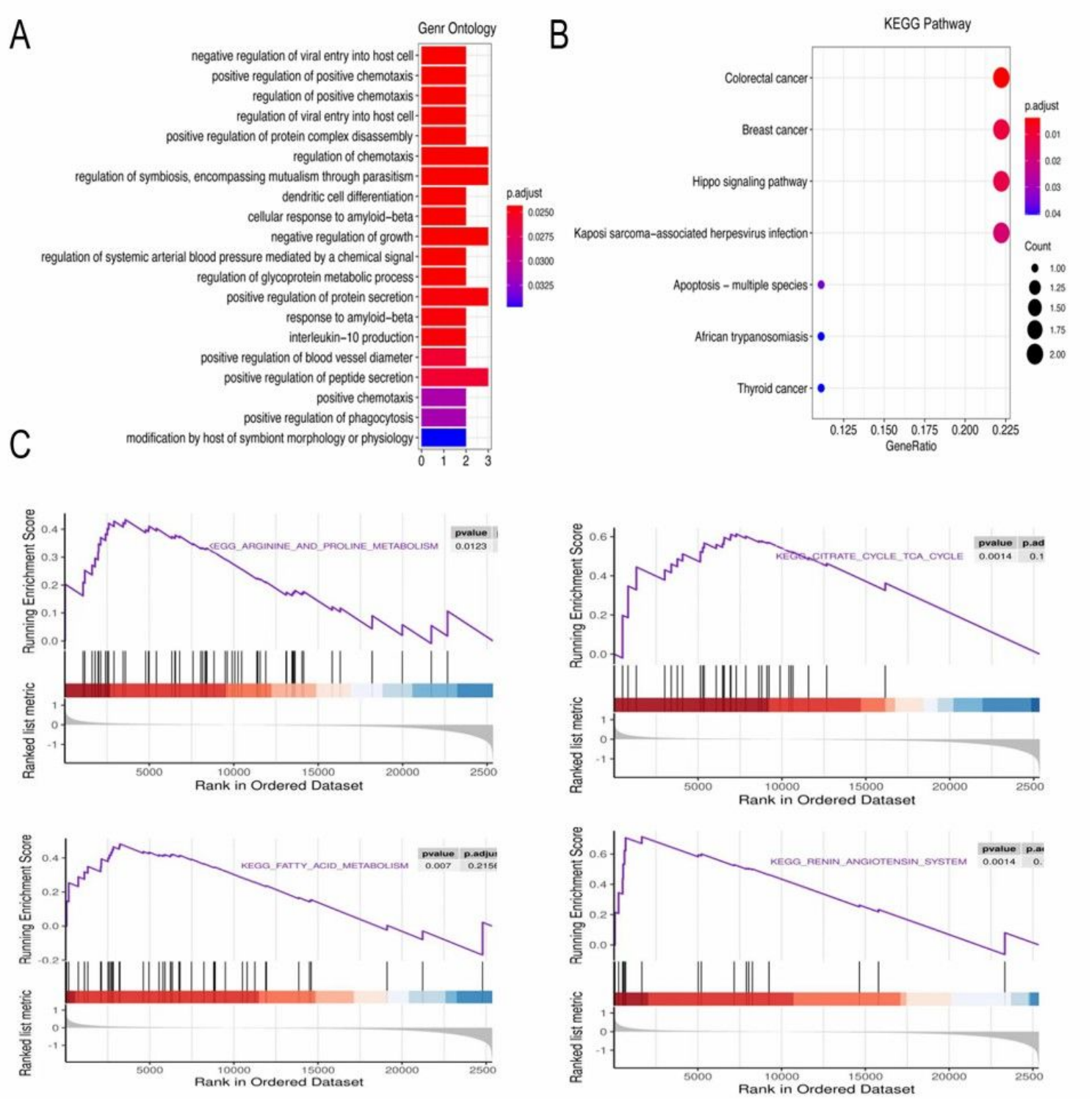

Figure 3

(A) The GO analysis of 11 hub genes. (B) The KEGG analysis. (C) Five positive pathways in the GSEA analysis.

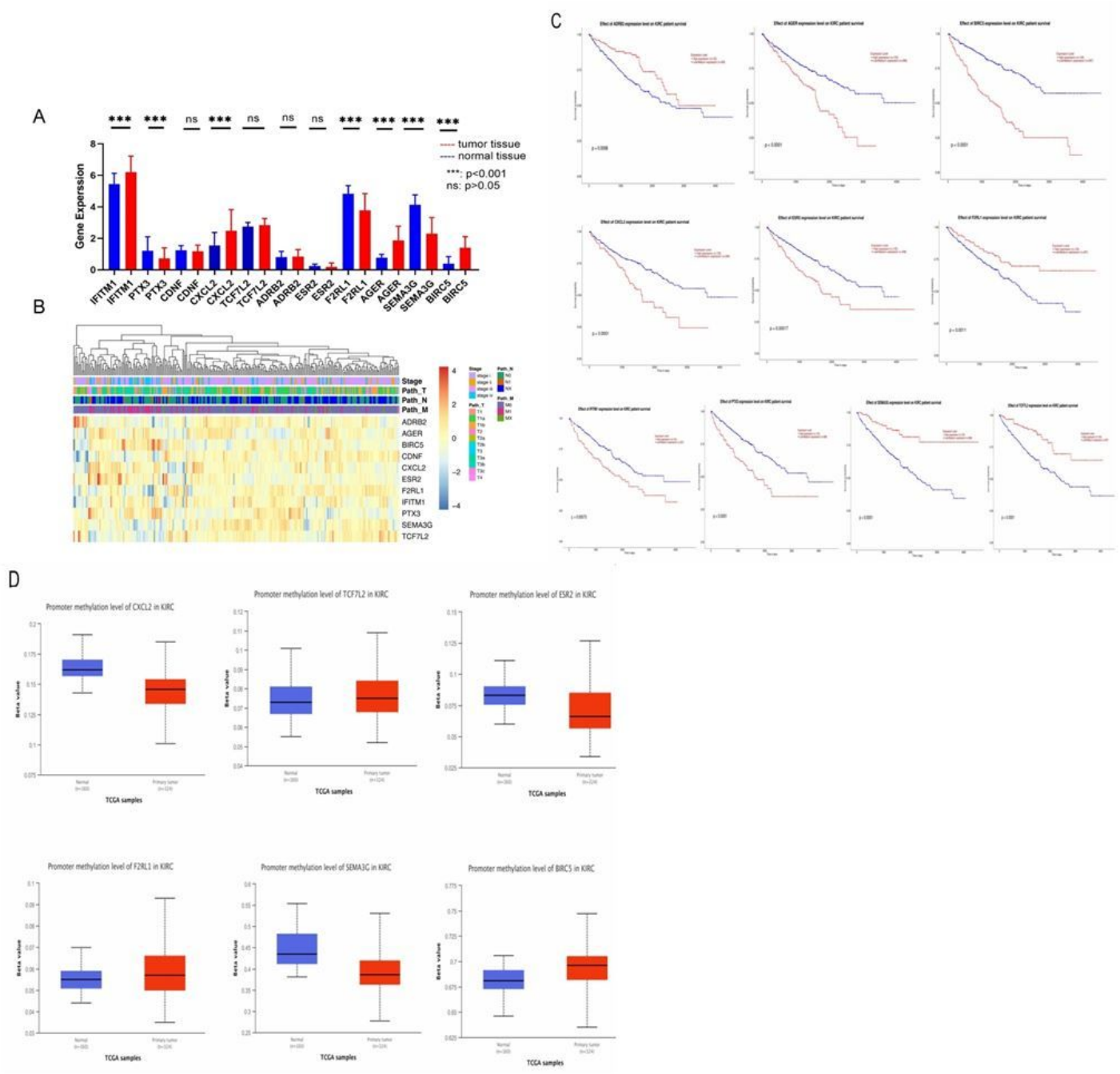

Figure 4

(A) The hub genes' expression in the training set. (B) The relationship between hub genes and clinical information. (C) The KM analysis of hub genes. (D) The DNA methylation level of the hub genes.

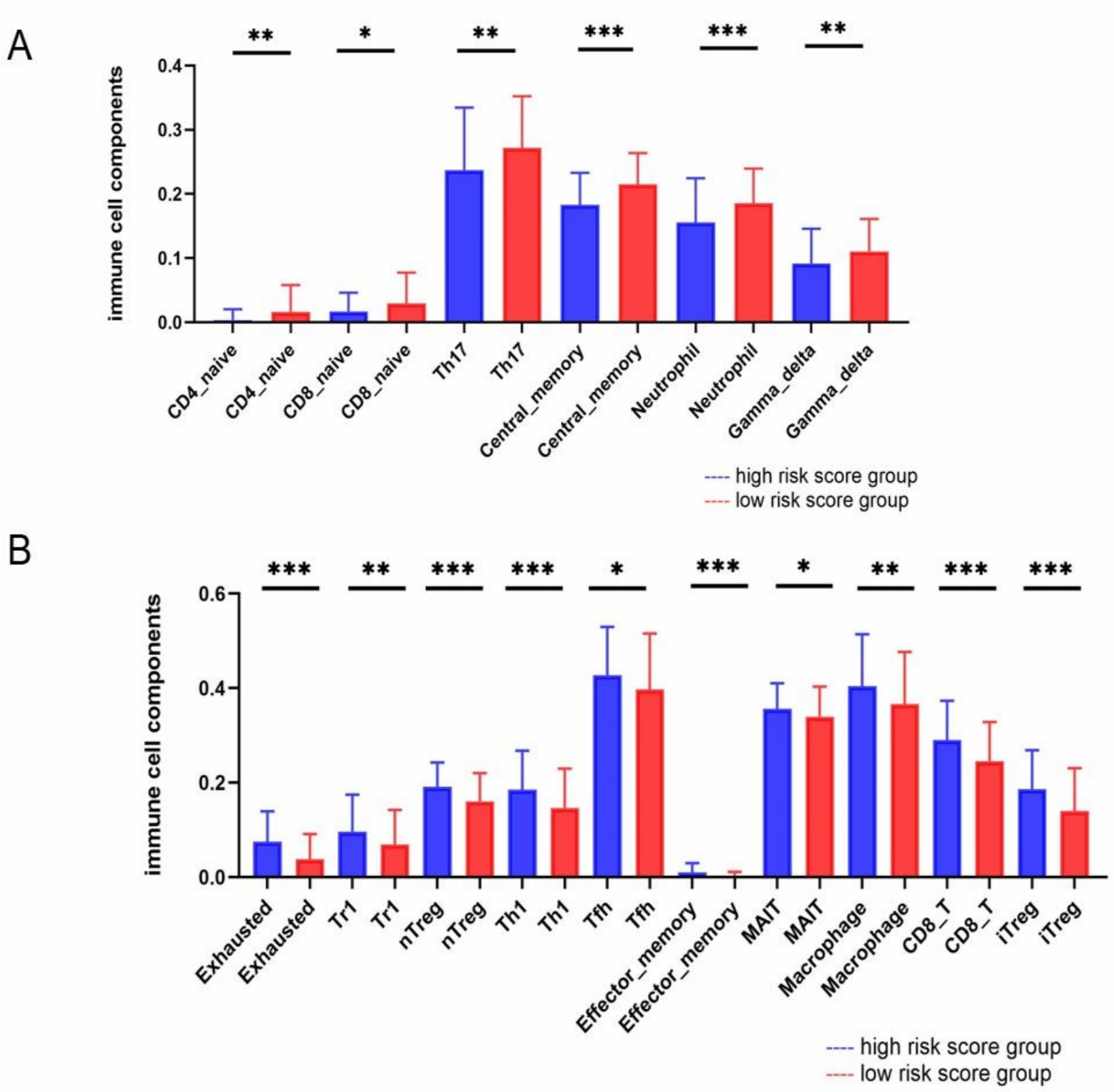

Figure 5

(A B) The percentage of immune cells between two groups.