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A novel Anoikis and immune-related genes marked prognostic signature for colorectal cancer

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Abstract

Colorectal cancer (CRC) is second most commonly diagnosed cancer with high morbidity and mortality. The heterogeneity of CRC makes clinical treatment tremendously challenging. Here, we aimed to comprehensively analyze the prognosis of CRC patients based on ANOIKIS- and immune-related genes. ANOIKIS-related genes were identified by differentially analysis of high anoikis score group (ANOIKIS_high group) and low anoikis score group (ANOIKIS_low group) divided by the cutoff value of anoikis score. Immune-related genes were screened by differentially analysis of high immune score group (ImmuneScore_high group) and low immune score group (ImmuneScore_low group) classified by the cutoff value of ImmuneScore. Prognostic ANOIKIS- and immune-related genes were identified by univariate Cox regression analysis. Multivariate Cox regression analysis were used for prognostic model construction. Ferroptosis expression profiles, the infiltration of immune cells, and the somatic mutation status were analyzed and compared. Univariate and multivariate Cox-regression analyses were performed to identify independent prognostic factors for CRC patient. Nomogram that contained the independent prognostic factors was established to predict 1-, 3-, and 5-year OS probability of CRC patients. Three ANOIKIS- and immune-related signatures were applied to construct a prognostic model, which divided the CRC patients into high-risk and low-risk groups. The patients with high-risk scores had obviously shorter OSs than those with low-risk scores. The time dependent ROC curve indicated that the risk score model had a stable performance to predict survival rates. Notably, the age, pathologic T, and risk score could be used independent indicators for CRC prognosis prediction. A nomogram containing the independent prognostic factors showed that the nomogram accurately predicted 1-, 3-, and 5-year survival rates of CRC patients. In our research, a novel prognostic model was developed based on ANOIKIS- and immune-related genes in CRC, which could be used for prognostic prediction of CRC patients.

Abbreviations: CRC = colorectal cancer, DEGs = differentially expressed genes, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MSigDB = molecular signatures database, OS = overall survival, PTEN = phosphatase and tensin, ROC = receiver operating characteristic curve, TCGA = The Cancer Genome Atlas, VEGF = vascular endothelial growth factor.

Key words: ANOIKIS and immune-related genes, colorectal cancer, nomogram, prognostic model

1. Introduction

Colorectal cancer (CRC) is ranked as the third most common malignant cancer worldwide, with an increasing incidence year by year. In 2020, a statistical study revealed approximately 147,950 cases diagnosed with CRC and 53,200 deaths have died of CRC in United State, Unfortunately, the 5-year overall survival (OS) rate of advanced patients is less than 14%.[1] Effective prevention and control of CRC progression is in emergency. Effective prevention and control of CRC, which can greatly reduce the national and even global economic burden. At present, system treatments including surgical, radiotherapy and adjuvant chemotherapy have been applied to CRC. Comprehensive treatments for patients with CRC have improved the survival rate of some advanced patients.[2] One of the most possible reason for the poor prognosis is the lack of a potential early prognostic factor.[3–5] Thus, it is an urgent need to explore the potential prognostic biomarkers for clinical diagnosis and therapy of CRC.

Anoikis is a programmed cell death activated in the absence of attachment of cells to an appropriate matrix, which is often disturbed in cancer. Inhibition of anoikis is an essential mechanism for the formation of metastases in cancer progression.[6–8] Anoikis resistance is the inherent characteristics of tumor cell. Without anoikis resistance, cancer cells will not survive after they detached from their primary site.[7,9] Recent researches have

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

GEO, TCGA and MSigDB belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

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indicated that anoikis resistance is an important mechanism of CRC metastasis and progression. Wang et al[10] proposed that CPT1A-mediated fatty acid oxidation promoted the metastasis of CRC cells by inhibiting anoikis. Paoli et al[9] demonstrated that subpopulations of micropapillary, sieve and solid structures of CRC resistant to anoikis, which is characterized by the lack of ECM exposure and low apoptosis rate. Emerging studies have revealed that immune-related genes may act as potential prognostic indicators for CRC.[10,11] However, there are no relevant studies on the combination of Anoikis- and immune-related genes to predict the CRC prognosis.

Therefore, our study intended to develop a novel prognostic model with the Anoikis- and immune-related genes for CRC based on bioinformatics method.

2. Materials and methods

2.1. Data acquisition and processing

RNA-sequencing (RNA-seq) data and their related clinical information of CRC samples were obtained from The Cancer Genome Atlas (TCGA) official website (https://portal.gdc.cancer.gov/), of which 615 CRC patients with complete clinical information were used for our analysis (Table S1, Supplemental Digital Content, http://links.lww.com/MD/H668).[12] In addition, RNA-seq data and clinical data of GSE17536 data set for 177 CRC patients were extracted from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds).[13]

A total of 47 ANOIKIS genes annotated by Gene Ontology (GO) were extracted from the Molecular signatures database (MSigDB).

2.2. CRC subtype analysis

Based on the 47 ANOIKIS genes, Gene set variation analysis algorithm of R was conducted to evaluate the anoikis score of each CRC patient. The cutoff value of anoikis score was determined by using survminer package in R, which divided the CRC patients into high anoikis score group (ANOIKIS_high group) and low anoikis score group (ANOIKIS_low group).

Estimate package of R was utilized to calculate the proportion of stromal and immune cells of each CRC patient, and then obtained the infiltrating immune cell score (ImmuneScore). The cutoff value of ImmuneScore was determined by employing survminer package in R, which classified the CRC patients into high immune score group (ImmuneScore _high group) and low immune score group (ImmuneScore _low group).

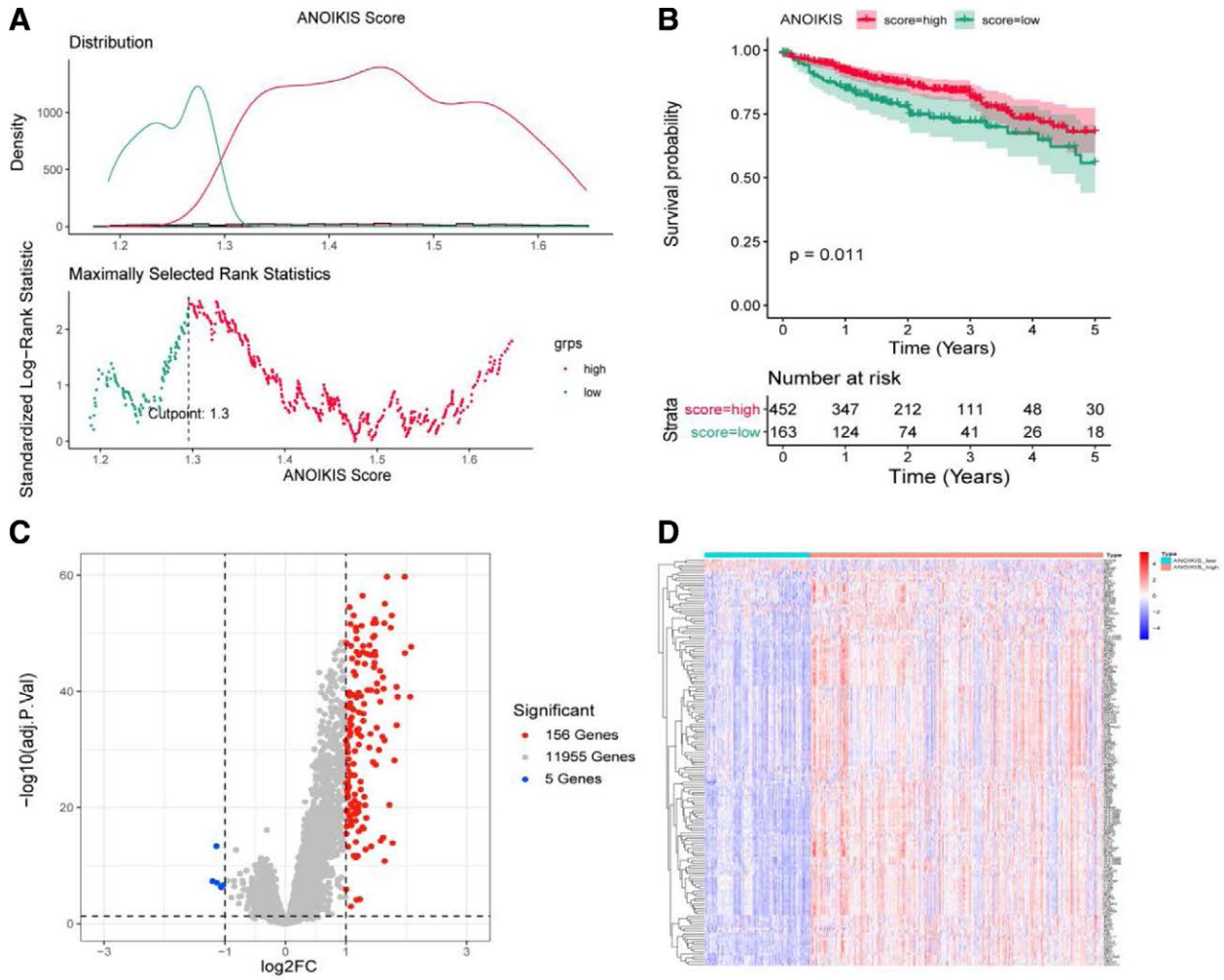

Figure 1. Identification of differentially expressed ANOIKIS-related genes based on CRC subtype analysis. (A) The anoikis score of each CRC patient by GSVA. The black dotted line is the optimal separation score. Green represents low anoikis score, red represents the high anoikis score. (B) the K-M analysis to compare the OS of the two subgroups. CRC patients with high anoikis score had an obviously better OS compared to low anoikis score group. $P < .05$ as significant. (C) Volcano plot showing the differentially expressed anoikis related genes in tumors versus normal tissue samples. Blue dots represent down-regulated anoikis related genes, and red dots represent up-regulated anoikis related genes. The gray dots represent that there are no significant differences between anoikis related genes. (D) Gene expression heat map of differentially expressed anoikis related genes in CRC. CRC = colorectal cancer, GSVA = gene set variation analysis.

2.3. Differentially expressed analysis

We employed the limma package to analyze the differentially expressed genes (DEGs) of the ANOIKIS_high and the ANOIKIS_low subtypes. Using the same methods, the DEGs between ImmuneScore_high and ImmuneScore_low groups were screened, which was shown in a heatmap generated by heatmap package. The screening criteria of DEGs is |log2(fold change)| > 0.5 and adjusted $P < .05$.

2.4. Functional enrichment analysis and protein-protein interaction (PPI) network of differentially expressed ANOIKIS- and immune-related genes

Differentially expressed ANOIKIS- and immune-related genes were acquired by Venn analysis. Then, GO and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differentially expressed ANOIKIS- and immune-related genes were performed by using the clusterProfiler R package to explore their potential functions in CRC progression. Adjusted $P < .05$ was considered as statistically significant. The PPI network of these ANOIKIS- and immune-related genes was constructed by Search Tool for Recurring Instances of Neighbouring Genes (STRING, https://string-db.org). The confidence degree was set to 0.4, and the PPI network was visualized by the Cytoscape software.

2.5. Development and validation of a prognostic model in CRC

Differentially expressed ANOIKIS- and immune-related genes were used to perform univariate Cox regression analysis in the TCGA database. The genes with $P < .05$ were subjected to a univariate Cox regression analysis. Subsequently, these genes were entered into a stepwise multivariate Cox regression analysis tested by Akaike Information Criterion to adjust the prognostic model. A risk score of each CRC patient was calculated by regression coefficient of univariate Cox regression analysis and the gene expression level. The CRC patients were divided into high- and low-risk groups based on the median risk score. The Kaplan–Meier (K-M) survival curves were generated to predict the differences of OS between high- and low-risk groups. The time-dependent receiver operating characteristic curve (ROC) analysis was performed by "timeROC" package in R to further predict the accuracy of this model for CRC prognosis. In our analysis, GSE17536 was used as an external validation set.

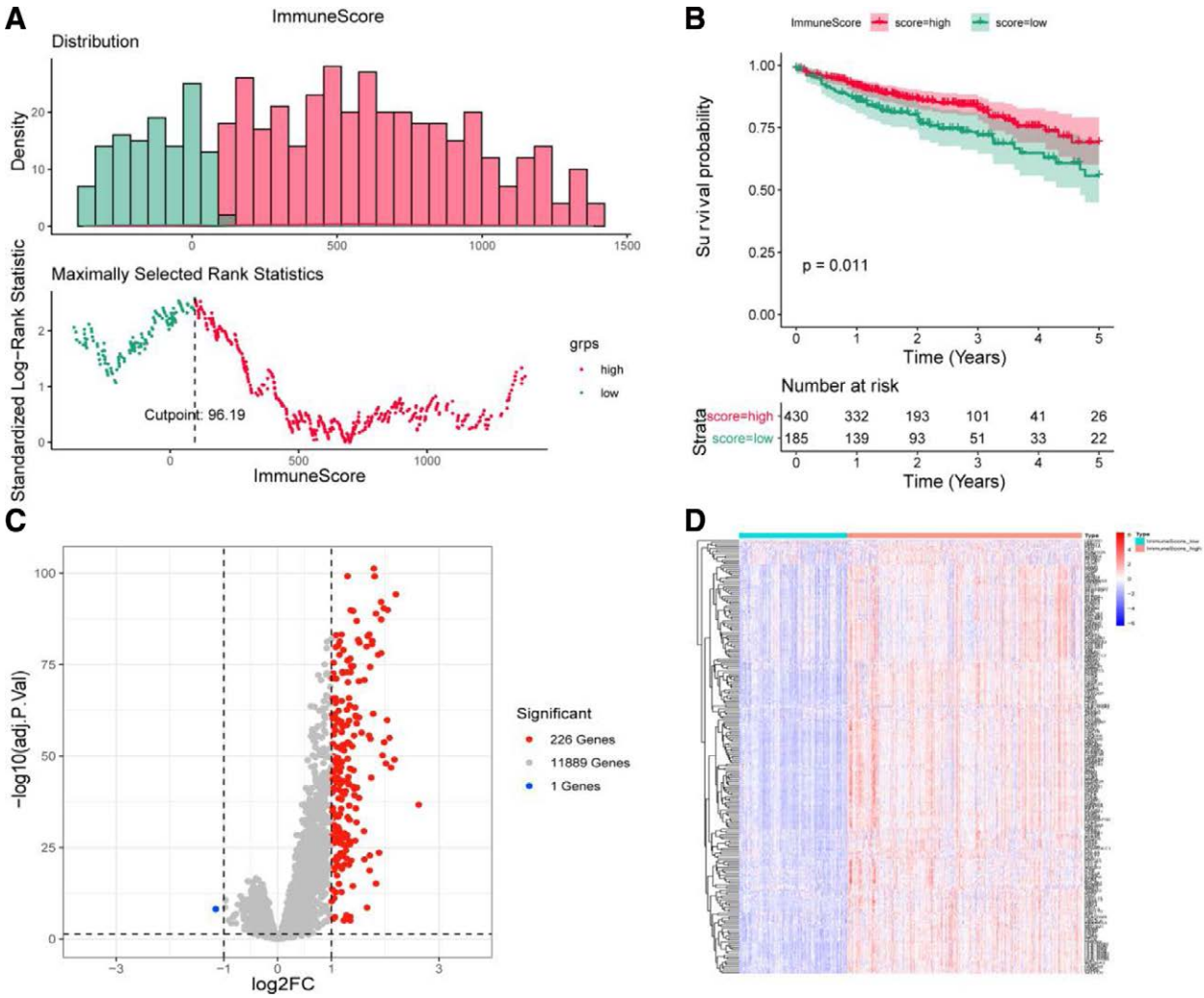

Figure 2. Identification of differentially expressed immune-related genes based on CRC subtype analysis. (A) The ImmuneScore of each CRC patient by R estimate. The black dotted line is the optimal separation score. Green represents low anoikis score, red represents the high anoikis score. (B) The K-M analysis to compare the OS of the two subgroups. CRC patients with high ImmuneScore had an obviously better OS compared to low ImmuneScore group. $P < .05$ as significant. (C) Volcano plot showing the differentially expressed immuno related genes in tumors vs normal tissue samples. Blue dots represent down-regulated immuno related genes, and red dots represent upregulated anoikis related genes. (D) Gene expression heat map of differentially expressed immune related genes in CRC. CRC = colorectal cancer, OS = overall survival.

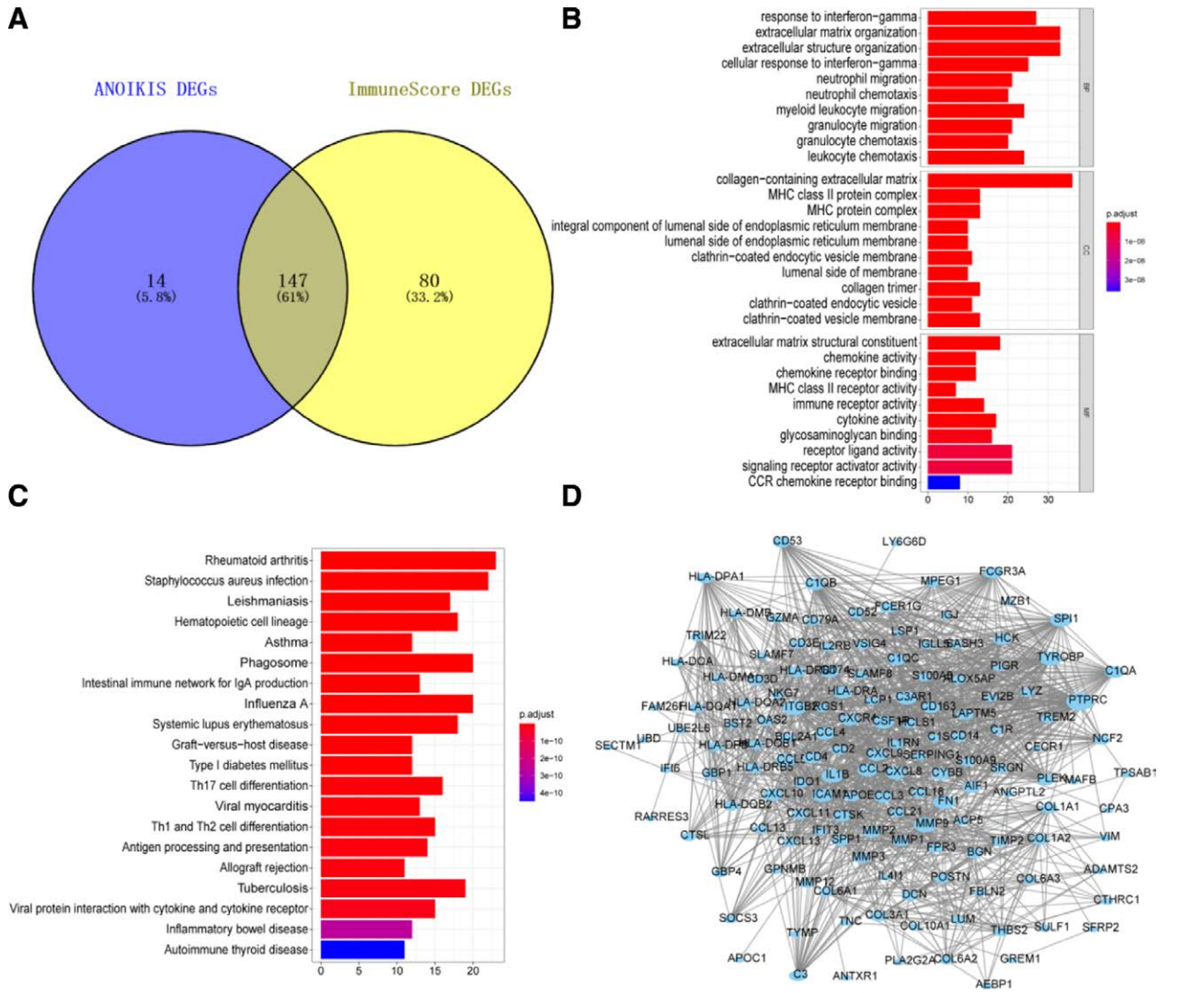

Figure 3. Identification of differentially expressed ANOIKIS- and immune-related genes. (A) The intersection of targeting ANOIKIS DEGs and ImmuneScore DEGs. (B) Results of the gene ontology (GO) term enrichment study. (C) Results of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment study. (D) 143 Hub genes of PPI network. The line represents the interaction between the genes. The darker the color, the bigger the degrees. DEGs = differentially expressed genes, PPI = protein-protein interaction.

2.6. The relationship of the risk score and clinic-pathological features of CRC patients

The association between the OS and clinic-pathological features including age, pathologic stage, pathologic T, pathologic M, pathologic N and risk score was investigated by univariate Cox analysis. The clinic-pathological features are associated with OS of CRC patients which was applied to conduct multivariate Cox regression analysis to screen the independent prognostic factors for CRC. A nomogram containing independent prognostic factors were generated using the R package, and the corresponding calibration plots was conducted to assess its performance.

2.7. Statistical analysis

All analyses in the present study were conducted with R software. The OS of the two subgroups were compared using the K-M analysis with a log-rank test. The significance of the differences in the risk score among different stage was assessed by the Wilcox on test. $P < .05$ was considered to be statistically significant (*$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$).

3. Results

3.1. Identification of differentially expressed ANOIKIS-related genes based on CRC subtype analysis

Based on the ANOIKIS genes, we evaluated the anoikis score of each CRC patient by gene set variation analysis, which adequately divided the CRC patients of the TCGA database into high anoikis score (ANOIKIS_high) and low anoikis score (ANOIKIS_low) subtypes (Fig. 1A). Then, the K-M analysis was applied to compare the OS of the two subgroups. The results showed that CRC patients with high anoikis score had an obviously better OS compared to low anoikis score group (Fig. 1B, $P = .011$). Moreover, we identified a total of 161 differentially expressed ANOIKIS-related genes between ANOIKIS_high and ANOIKIS_low subtypes based on the screening criteria of log2(fold change) > 0.5 and adjusted $P < .05$, of which 156 were

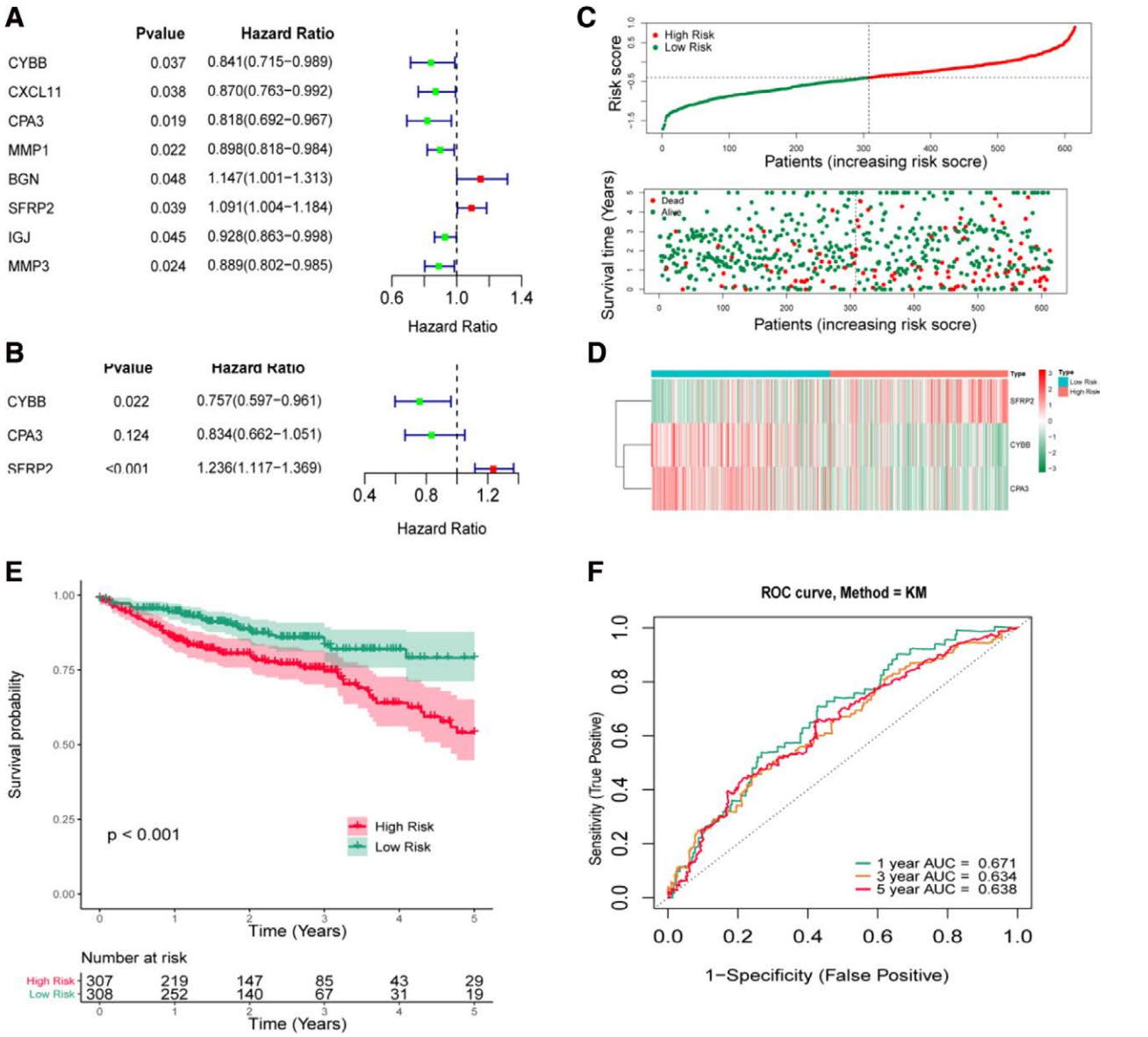

Figure 4. Construction of a prognostic model of ANOIKIS- and immune-related genes in CRC. (A) Univariate Cox regression analysis of ANOIKIS- and immune-related genes for CRC patients. (B) Multivariate Cox regression analysis of CYBB, CPAS, and SFRP2 genes for CRC patients. (C) The scatter plot of risk score distribution of CYBB, CPAS, and SFRP2 genes. One point refers to a sample, red point was sample with higher risk score, green point was sample with lower risk score, and the intersecting point represents the median of risk score. (D) The heat map of the mRNA expression of the three ANOIKIS- and immune-related genes. X-axis and Y-axis refer to genes and samples, respectively. (E) The Kaplan–Meier survival curve of three ANOIKIS- and immune-related genes. X-axis, time in days. Y-axis, survival probability. (F) Time-dependent (ROC) curve of the risk score in inferior prognosis prediction of three ANOIKIS- and immune-related genes for 1-, 3-, and 5-year overall survival. X-axis, false positive. Y-axis, true positive. CRC = colorectal cancer, ROC = receiver operating characteristic curve.

up-regulated and 5 were down-regulated genes (Fig. 1C and D, Table S2, Supplemental Digital Content, http://links.lww.com/MD/H669).

3.2. Identification of differentially expressed immune-related genes based on CRC subtype analysis

By using the cutoff value calculated ImmuneScore, we classified the CRC patients into high immune score (ImmuneScore_high group) and low immune score subtypes (ImmuneScore_low group) (Fig. 2A). Subsequently, we compared the OS of the two subgroups by K-M analysis. Interestingly, the CRC patients with high immune score showed a better OS than that of low immune score group (Fig. 2B, $P = .011$). Based on the two subtypes, we obtained 227 differentially expressed immune-related genes, including 226 up-regulated and 1 down-regulated genes (Fig. 2C and D, Table S3, Supplemental Digital Content, http://links.lww.com/MD/H670).

3.3. Identification of differentially expressed ANOIKIS- and immune-related genes

As a result, 147 ANOIKIS- and immune-related genes were acquired by overlapping the identified DEGs (Fig. 3A). To further explore the biological functions of differentially expressed ANOIKIS- and immune-related genes in CRC, we performed GO and KEGG enrichment analysis by cluster Profiler R package. The differentially expressed ANOIKIS- and immune-related genes were significantly enriched in GO terms and KEGG pathways related to immune, such as response to interferon-gamma,

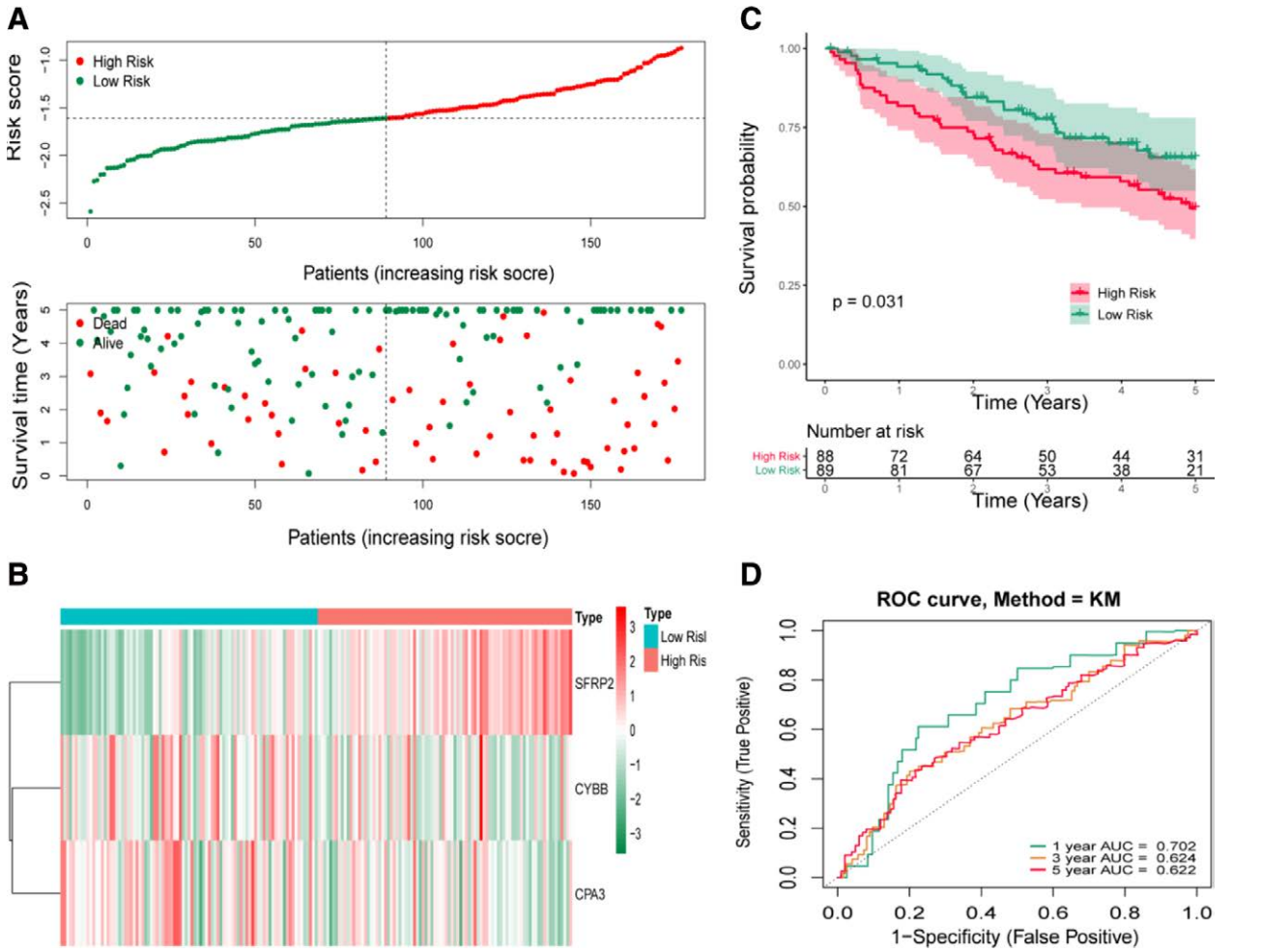

Figure 5. Validation of the prognostic model in CRC. (A) The scatter plot of risk score distribution of the samples and Distribution of the survival status, risk score and gene expression data of CRC patients in the training group. One point refers to a sample, red point was sample with higher risk score, green point was sample with lower risk score, and the intersecting point represents the median of risk score. (B) The heat map of the mRNA expression of the five IRGs. X-axis and Y-axis refer to genes and samples, respectively. (C) The Kaplan–Meier survival curve. X-axis, time in days. Y-axis, survival probability. (D) Time-dependent (ROC) curve of the risk score in inferior prognosis prediction for 1-, 3-, and 5-year overall survival. X-axis, false positive. Y-axis, true positive. CRC = colorectal cancer, ROC = receiver operating characteristic curve.

neutrophil migration, myeloid leukocyte migration, leukocyte chemotaxis (Fig. 3B and Table S4, Supplemental Digital Content, http://links.lww.com/MD/H671), and in Th17 cell differentiation, Th1 and Th2 cell differentiation, Intestinal immune network for IgA production, and Antigen processing and presentation (Fig. 3C and Table S5, Supplemental Digital Content, http://links.lww.com/MD/H672). A PPI network containing 143 nodes and 1527 edges revealed the complex interactions of these differentially expressed ANOIKIS- and immune-related genes in the development of CRC (Fig. 3D).

3.4. Construction of a prognostic model of ANOIKIS- and immune-related genes in CRC

To further demonstrate the significant prognostic relevance of ANOIKIS- and immune-related genes for CRC patients, we performed univariate Cox regression analysis, obtaining 8 genes with $P < .05$. As shown in (Fig. 4A), 6 of these genes (CYBB, CXCL11, CPA3, MMP1, IGJ, MMP3) were protective factors for better prognoses in CRC patients (HR < 1). BGN and SFRP2 acted as risk factors in CRC (HR > 1) Then, the 8 ANOIKIS- and immune-related genes was used to conduct multivariate Cox regression analysis. Ultimately, 3 ANOIKIS- and immune-related genes including CYBB, CPA3 and SFRP2 were identified to construct a prognostic prediction model (Fig. 4B). Accordingly, the risk score of each CRC patient was calculated as follows: Risk Score = (−0.277884556) * Express Value of CYBB + (−0.181284872) * Express Value of CPA3 + 0.212002305 * Express Value of SFRP2. The CRC patients were assigned into high-risk (n = 307) and low-risk (n = 308) subgroups based on the median value of risk score (Fig. 4C). The expression of three ANOIKIS- and immune-related genes were significantly different between high-risk and low-risk groups (Fig. 4D). K-M survival analysis illustrated patients with high-risk scores had obviously shorter OSs than those with low-risk scores (Fig. 4E, $P < .001$). The time dependent ROC curve showed that the AUC of the three ANOIKIS- and immune-related genes in the prognostic model was greater than 0.6 (0.671 at 1 year, 0.634 at 3 years, and 0.638 at 5 years), indicating that the risk score model had a stable performance to predict survival rates (Fig. 4F).

3.5. Validation of the prognostic model in CRC

To further validate the robustness of our constructed prognostic model in an external cohort of CRC patients, a GEO cohort composed of 177 CRC patients was utilized to calculate the risk score using the same formula. Ultimately, these

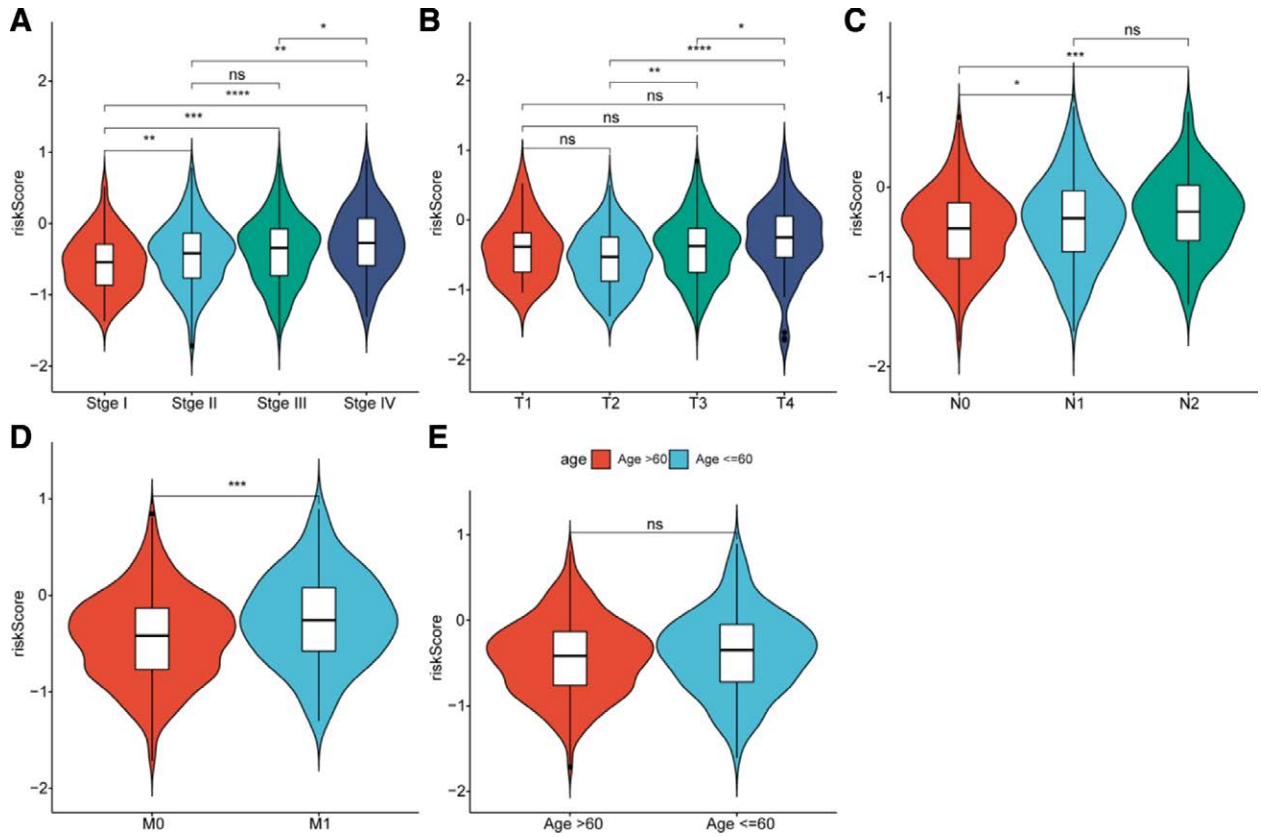

Figure 6. Correlation analysis between risk model and clinical factors. Violin represent the distribution of risk score in CRC samples stratified by different factors, including stage (A), pathologic T (B), pathologic N (C), pathologic M (D), age (E). $P < .05$ was considered to be statistically significant (*$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$). CRC = colorectal cancer.

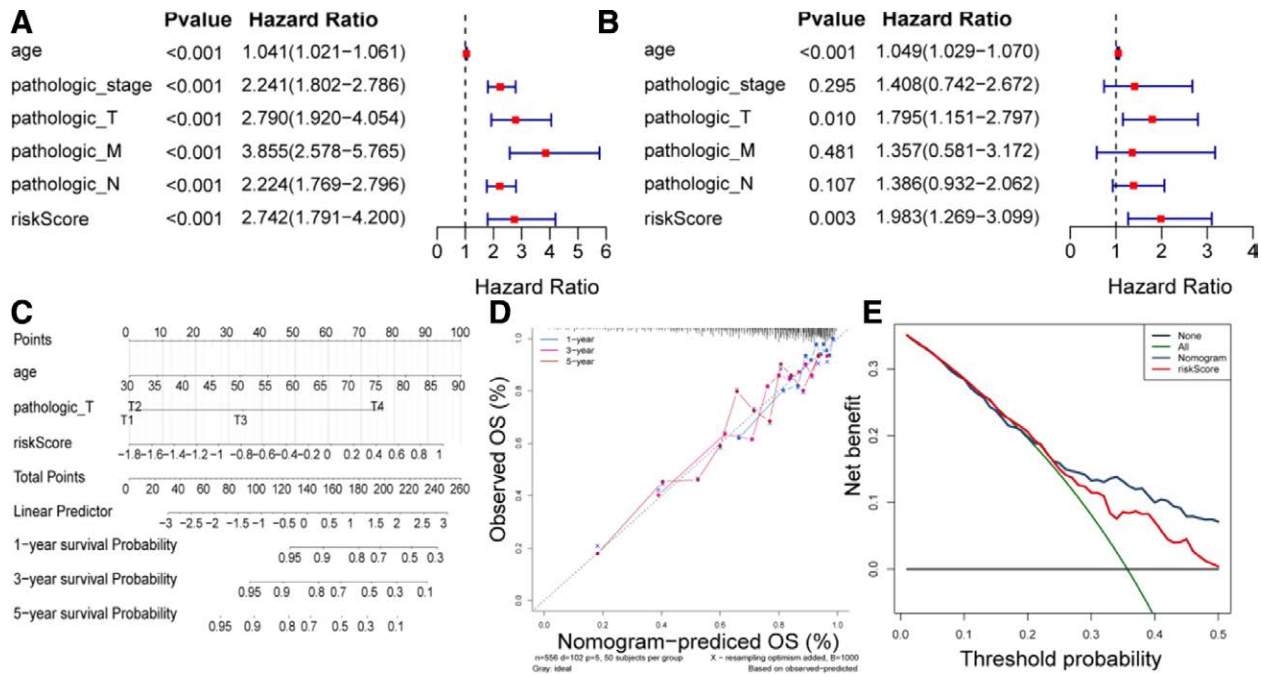

Figure 7. Independent prognostic value of risk models. (A) Univariate Cox regression analysis in CRC. (B) Multivariate Cox regression analysis in CRC. $P < .05$ is considered as statistically significant. (C) The nomogram using age, stage and risk score. For each patient, three lines are drawn upward to verify the points received from the three predictors of the nomogram. The sum of these points situates on the "Total Points" axis. Then a line is drawn downward to assess the 1-, 3-, and 5-year overall survival of CRC. (D) The calibration plot to evaluate the nomogram predicted 1-, 3-, and 5-year overall survival of CRC. (E) The DCA curves for the risk score and combined nomogram model in prognosis prediction of CRC. CRC = colorectal cancer, DCA = the decision curve analysis, OS = overall survival.

CRC patients in the GSE17536 cohort were categorized into the low-risk group (n = 89) and the high-risk group (n = 88) (Fig. 5A). Moreover, the expression of three prognostic biomarkers was shown in Figure 5B. Consistent with the results of the TCGA dataset, the CRC patients with low risk score had significantly longer OSs (Fig. 5C, $P = .031$). In addition, the AUCs for OS were 0.702 at 1 year, 0.624 at 3 years, and 0.622 at 5 years, indicating stable prognostic accuracy of our prognostic model constructed by the 3 ANOIKIS- and immune-related genes (Fig. 5D).

3.6. Risk score is an independent prognostic factor for CRC patients

CRC patients in the TCGA dataset were classified by their age, Stage, pathologic T, pathologic M, pathologic N, respectively. The results indicated that the there was significantly differences of risk scores of CRC patients among different stage (Fig. 6A–D). However, we did not observed significantly different between different age (Fig. 6E).

Besides, multivariate Cox regression was performed to evaluate whether these clinico-pathological features (including age, Stage, pathologic T, pathologic M, pathologic N) and the risk score were independent factors for CRC prognosis (Fig. 7A and B). The results demonstrated that the age, pathologic T, and risk score could be used independent indicators for prognosis prediction of CRC patients. The independent prognostic factors included age, pathologic T, and risk score (Fig. 7C). The results illustrated that the nomogram accurately predicted 1-, 3-, and 5-year survival rates of CRC patients (Fig. 7D). The decision curve analysis for risk score and nomogram model indicated that the reliability of the nomogram model containing clinical factors in prognosis prediction of CRC (Fig. 7E).

4. Discussion

CRC remains the leading cause of mortality in the worldwide. The incidence of CRC is increasing worldwide year by year. CRC has more high mortality and poor prognosis when diagnosed at advanced stages. A molecular marker (biomarker) is defined as a biological molecule which can be tested in blood, tissues and other fluids. It is alse can be iedentified in a special pathological or physiological process for some diseases. What is more, Biomarkers serve as valuable strategies for cancer detection, diagnosis, prognosis prediction and treatment choice. In clinical practice, biomarker is one of the best way to monitor the treatment response and to help the doctor made the next treatment decision.[14] In order to improve the prognosis, many molecular biomarkers for CRC have been comprehensively explored to predict the prognosis in the past 20 years. Adenomatous polyposis coli is highly expressed and predicted as a prognostic biomarker for CRC.[15] Vascular endothelial growth factor (VEGF) has been reported to be as one of the angiogenic factors in CRC. It is expressed in approximately 50% of CRCs, but it is hardly expressed in normal colonic tissues. So, VEGF-1 expression is considered as one of valuable prognostic biomarker in CRC.[16] PTEN (Phosphatase and tensin) homolog protein acts as a tumor suppressor gene in the regulation of the cell cycle, cell proliferation, differentiation and apoptosis.[17,18] A meta-analysis of five small clinical studies have indicated that PTEN loss in tumors is associated with poor prognosis in patients with local advance stage or metastatic CRC treated with cetuximab based therapy based on.[19] Until now, Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are the most widely used biomarkers in clinical practices. Because of the poor sensitivity and specificity, the widely application of the two biomarker are limited.[20] Instead of single biomarker, combination of different biomarkers will help to predict the prognosis and avoid over treatment for CRC patients.

In our research, 6 of 147 ANOIKIS- and immune-related genes (CYBB, CXCL11, CPA3, MMP1, BGN, SFRP2, IGJ, and MMP3) were identified to be associated with the prognosis of CRC, of which CYBB (cytochrome b-245 beta chain gene), CPA3 (Carboxypeptidase A3), and SFRP2 (secreted frizzled-related protein) were used to construct a prognostic prediction model for CRC. The risk score of this prognostic model that divided the CRC patients into high-risk and low-risk groups was an independent factors for CRC prognosis.

Among the three prognosis genes signatures, the human SFRP2 gene is the member of secreted frizzled-related protein (SFRP) family that located on chromosome 4q31.3 and encoded a 295-aa protein.[21] Many studies have reported that SFRP2 was involved in of the pathogenesis of various of cancers. Co-hypermethylation of SFRP2 was also considered as independent prognostic predictors for postoperative CRC patients.[22] Also, methylated SFRP2 in plasma was critical for the prognosis and early detection of gastric cancer.[23,24] Li et al[25] found that SFRP2 modulated the apoptosis and metastasis of non-small cell lung cancer A549 cells by regulating mitochondrial fission via Wnt pathways. Recent study revealed that high expression of SFRP2was associated with primary tumor size, TNM stage, and lymph node metastases of breast cancer and lead to poor prognosis.[26] CYBB gene is located on the X-chromosome, and the mutation in this gene account for about 70% of Chronic Granulomatous Disease(CGD) cases.[27,28] CPA3 belongs to the metallocarboxypeptidase family, which contains a 16-residue signal peptide sequence, a 95-residue NH2-terminal activation segment, and a 310-residue CP enzyme domain. It regulates the function of peptide hormones, which plays an important role in the growth and/or differentiation of prostate epithelial cells.[29] However, the effect of CYBB and CPA3 on CRC has not clear.

Currently, several researchers have reported that there is a potential relationship between anoikis and immunity. Extracellular (Granzyme B) GrB contribute to pathogenesis in cases of immune dysregulation. It also cleaves extracellular matrix components,[30–33] and induces anoikis in susceptible cells in vitro.[34] Moreover, there was a specific association between NK cells infiltration and Anoikis in CRC patients.[35]

To the best of our knowledge, this is the first study to pay attention on the combination of Anoikis- and immune-related genes as prognostic signature for CRC. However, our study has some deficiency. On the one hand, it is necessary to further verify the results by clinical samples and experimental data. On the other hand, the biological function and mechanism of this 3 anoikis and immune-related genes in CRC progression are need to be further explored.

In conclusion, we identified a novel prognosis prediction model constructed by 3 anoikis and immune-related genes for CRC, providing new insights into clinical prognosis value for CRC.

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Author contributions

Conceptualization: Zhiqiang Cai.
Data curation: Zhiqiang Cai.
Methodology: Zhiqiang Cai.
Project administration: Fuxiang Zhou.
Software: Zhiqiang Cai.
Supervision: Fuxiang Zhou.
Validation: Fuxiang Zhou.
Writing – original draft: Zhiqiang Cai.
Writing – review & editing: Fuxiang Zhou.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7–30.
[2] Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. Lancet. 2019;394:1467–80.
[3] Shen C, Xuan B, Yan T, et al. m(6)A-dependent glycolysis enhances colorectal cancer progression. Mol Cancer. 2020;19:72.
[4] Zhang Y, Liu H, Liu X, et al. Identification of an exosomal long non-coding RNAs panel for predicting recurrence risk in patients with colorectal cancer. Aging (Albany NY). 2020;12:6067–88.
[5] Hu T, Liu H, Liang Z, et al. Tumor-intrinsic CD47 signal regulates glycolysis and promotes colorectal cancer cell growth and metastasis. Theranostics. 2020;10:4056–72.
[6] Gilmore AP. Anoikis. Cell Death Differ. 2005;12(Suppl. 2): S1473–7.
[7] Guadamillas MC, Cerezo A, Del Pozo MA. Overcoming anoikis-pathways to anchorage-independent growth in cancer. J Cell Sci. 2011;124:3189–97.
[8] Jinka R, Kapoor R, Sistla PG, et al. Alterations in cell-extracellular matrix interactions during progression of cancers. Int J Cell Biol. 2012;2012:219196.
[9] Paoli P, Giannoni E, Chiarugi P. Anoikis molecular pathways and its role in cancer progression. Biochim Biophys Acta. 2013;1833:3481–98.
[10] Wang YN, Zeng ZL, Lu J, et al. CPT1A-mediated fatty acid oxidation promotes colorectal cancer cell metastasis by inhibiting anoikis. Oncogene. 2018;37:6025–40.
[11] Li M, Wang H, Li W, et al. Identification and validation of an immune prognostic signature in colorectal cancer. Int Immunopharmacol. 2020;88:106868.
[12] Zhining W, Jensen MA, Claude Zenklusen J. A practical guide to the cancer genome atlas (TCGA). Methods Mol Biol. 2016;1418:111–41.
[13] Emily C, Tanya B. The gene expression omnibus database. Methods Mol Biol. 2016;1418:93–110.
[14] Sidransky D. Emerging molecular markers of cancer. Nat Rev Cancer. 2002;2:210–9.
[15] Chen TH, Chang SW, Huang CC, et al. The prognostic significance of APC gene mutation and miR-21 expression in advanced-stage colorectal cancer. Colorectal Dis. 2013;15:1367–74.
[16] Falchook GS, Kurzrock R. VEGF and dual-EGFR inhibition in colorectal cancer. Cell Cycle. 2015;14:1129–30.
[17] Luo HY, Xu RH. Predictive and prognostic biomarkers with therapeutic targets in advanced colorectal cancer. World J Gastroenterol. 2014;20:3858–74.
[18] Hobert JA, Eng C. PTEN hamartoma tumor syndrome: an overview. Genet Med. 2009;11:687–94.
[19] Shen Y, Yang J, Xu Z, et al. Phosphatase and tensin homolog expression related to cetuximab effects in colorectal cancer patients: a meta-analysis. World J Gastroenterol. 2012;18:2712–8.
[20] Knudsen AB, Zauber AG, Rutter CM, et al. Estimation of benefits, burden, and harms of colorectal cancer screening strategies: modeling study for the US preventive services task force. JAMA. 2016;315:2595–609.
[21] Katoh M, Katoh M. Comparative genomics on SFRP2 orthologs. Oncol Rep. 2005;14:783–7.
[22] Liu X, Fu J, Bi H, et al. DNA methylation of SFRP1, SFRP2, and WIF1 and prognosis of postoperative colorectal cancer patients. BMC Cancer. 2019;19:1212.
[23] Yan H, Chen W, Ge K, et al. Value of plasma methylated SFRP2 in prognosis of gastric cancer. Dig Dis Sci. 2021;66:3854–61.
[24] Miao J, Liu Y, Zhao G, et al. Feasibility of plasma-methylated SFRP2 for early detection of gastric cancer. Cancer Control. 2020;27:1073274820922559.
[25] Li P, Zhao S, Hu Y. SFRP2 modulates non-small cell lung cancer A549 cell apoptosis and metastasis by regulating mitochondrial fission via Wnt pathways. Mol Med Rep. 2019;20:1925–32.
[26] Huang C, Ye Z, Wan J, et al. Secreted frizzled-related protein 2 is associated with disease progression and poor prognosis in breast cancer. Dis Markers. 2019;2019:6149381.
[27] Roos D, Kuhns DB, Maddalena A, et al. Hematologically important mutations: X-linked chronic granulomatous disease (third update). Blood Cells Mol Dis. 2010;45:246–65.
[28] Stasia MJ, Li XJ. Genetics and immunopathology of chronic granulomatous disease. Semin Immunopathol. 2008;30:209–35.
[29] Huang H, Reed CP, Zhang JS, et al. Carboxypeptidase A3 (CPA3): a novel gene highly induced by histone deacetylase inhibitors during differentiation of prostate epithelial cancer cells. Cancer Res. 1999;59:2981–8.
[30] Froelich C, Zhang X, Turbov J, et al. Human granzyme B degrades aggrecan proteoglycan in matrix synthesized by chondrocytes. J Immunol. 1993;151:7161–71.
[31] Ronday HK, van der Laan WH, Tak PP, et al. Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the invasive front of the synovium in rheumatoid arthritis. Rheumatology. 2001;40:55–61.
[32] Buzza MS, Zamurs L, Sun J, et al. Extracellular matrix remodeling by human granzyme B via cleavage of vitronectin, fibronectin, and laminin. J Biol Chem. 2005;280:23549–58.
[33] Buzza MS, Dyson JM, Choi H, et al. Antihemostatic activity of human granzyme B mediated by cleavage of von Willebrand factor. J Biol Chem. 2008;283:22498–504.
[34] Choy JC, Hung VHY, Hunter AL, et al. Granzyme B induces smooth muscle cell apoptosis in the absence of perforin: involvement of extracellular matrix degradation. Arterioscler Thromb Vasc Biol. 2004;24:2245–50.
[35] González-Llorente L, Santacatterina F, García-Aguilar A, et al. Overexpression of mitochondrial IF1 prevents metastatic disease of colorectal cancer by enhancing anoikis and tumor infiltration of NK cells. Cancers (Basel). 2019;12:22.