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Research article

Identification and validation of a novel 17 coagulation-related genes signature for predicting prognostic risk in colorectal cancer

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ABSTRACT

Background: Patients with colorectal cancer commonly experience disturbances in coagulation homeostasis. Activation of the coagulation system contributes to cancer-associated thrombosis as the second risk factor for death in cancer patients. This study intended to discover coagulation-related genes and construct a risk model for colorectal cancer patients' prognosis.

Methods: Coagulation-related genes were identified by searching coagulation-related pathways in the Molecular Signatures Database. Transcriptomic data and clinical data were downloaded from the Cancer Genome Atlas and Gene Expression Omnibus datasets. Univariate Cox and backward stepwise regression were utilized to identify prognosis-related genes and construct a predictive risk model for the training cohort. Next, survival analysis determines the risk model's predictive power, correlation with clinicopathological characteristics, and nomogram. Additionally, we characterized the variances in immune cell infiltration, somatic mutations, immune checkpoint molecules, biological functions, and drug sensitivity between the high- and low-score patients.

Result: Eight hundred forty-five genes were obtained by searching the theme term "coagulation" after de-duplication. After univariate regression analysis, 69 genes correlated with prognosis were obtained from the Cancer Genome Atlas dataset. A signature consisting of 17 coagulation-related genes was established through backward stepwise regression. The Kaplan-Meier curve indicated a worse prognosis for high-score patients. Time-dependent receiver operating characteristic curve analysis demonstrated high accuracy in predicting overall survival. Further, the results were validated by two independent datasets (GSE39582 and GSE17536). Combined with clinicopathological characteristics, the risk model was proven to be an independent prognostic factor to predict poor pathological status and worse prognosis. Furthermore, high-score patients had significantly higher stromal cell infiltration. Low-score patients were associated with high infiltration of resting memory $CD4^+$ T cells, activated $CD4^+$ T cells, and T follicular helper cells. The low-score patients exhibited increased expression of immune checkpoint genes, and this might be relevant to their better prognosis. High-score patients exhibited lower IC50 values of Paclitaxel, Rapamycin, Temozolomide, Cyclophosphamide, etc. The differential signaling pathways mainly involve the calcium signaling pathway and the neuroactive ligand-receptor interaction. Lastly, a nomogram was constructed and showed a good prediction.

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Conclusion: The prognostic signature of 17 coagulation-related genes had significant prognostic value for colorectal cancer patients. We expect to improve treatment modalities and benefit more patients through research on molecular features.

Abbreviations used in this paper

AIC	Akaike Information Criterion
AUC	Area Under Curve
BP	Biological process
CC	Cellular component
CMS	Consensus molecular subtypes
CRC	Colorectal cancer
DEGs	Differentially expressed genes
EMT	Epithelial-mesenchymal transition
GEO	Gene Expression Omnibus
GO	Gene Ontology
ICI	Immune checkpoint inhibitor
KEGG	Kyoto Encyclopedia of Genes and Genomes
K-M	Kaplan-Meier
MF	Molecular function
MSI	Microsatellite instability
ROC	Receiver operating characteristic
TCGA	The Cancer Genome Atlas
TF	Tissue factor
Tfh	T cells follicular helper
TME	Tumor Microenvironment
TMB	Tumor mutational burden
VEGF	Vascular endothelial growth factor
VTE	Venous thromboembolism

1. Introduction

Colorectal cancer (CRC) ranks as the third most common cause of cancer-related death globally, with over 1.85 million cases diagnosed and 0.85 million deaths each year [1,2]. As developing nations advance, the global prevalence of CRC will rise to 2.5 million by 2035 [3]. While the occurrence appears stable and diminishing in developed countries, a worrying increase in patients with early onset (under 50) has been observed [4]. The early-onset patients are more likely to be in advanced stages and have poorer prognosis [5]. At the time of diagnosis, 20 % of CRC patients have metastatic lesions, and nearly 25 % of patients will develop metastases after treatment of local lesions [1]. Early diagnosis and surgical resection are the cornerstones of treatment, and the quality of surgical treatment is optimized [6]. Preoperative chemotherapy, downstaging preoperative radiotherapy, and systemic therapy allow surgery in patients with focal progression [7]. Systemic treatment is widely used in patients with advanced tumors, which includes adjuvant chemotherapy, targeted therapy, biologics agents, and immunotherapy [8]. Molecular profiling can guide specific patient subgroups to obtain precision medicine, but the prognosis of the remaining patients lacks assessment. Therefore, exploring new genetic biomarkers will help predict CRC patient-specific survival, reduce the no-response rate, and improve clinical outcomes through the genomic and transcriptome sequences.

Serum concentrations of carcinoembryonic antigen, carbohydrate antigen 24-2, and carbohydrate antigen 19-9 are relevant for diagnosis. Still, they have been found to have no significant correlation with prognostic-related clinical factors, such as TNM stage, depth of invasion, and histological type [9]. Microsatellite instability (MSI) was a biomarker used to predict response to immune checkpoint inhibitors (ICIs). Metastatic colorectal cancer with MSI-H might enhance disease control and prolong progression-free survival after PD-1 inhibitors treatment, but the clinical response rate was still less than 50 % [10]. Tumor mutational burden (TMB) refers to the aggregate count of somatic mutations detected per million bases. TMB was found to be a stronger predictor of the efficacy of ICIs and better than MSI-H [11].

Additionally, considering the complex mutational landscape and biological specificity of CRC, consensus molecular subtypes (CMS) were produced by the Colorectal Cancer Subtyping Consortium [12]. Immune cell invasion characterized CMS1, while CMS4 is characterized by stromal infiltration [13]; both subtypes showed poor survival [14,15]. With the continuous understanding of the complexity of tumor pathogenesis, it becomes imperative to develop a novel, sensitive, and generally applicable prognostic model for CRC patients.

The process of hemostasis involves complex and dynamic mechanisms that regulate the balance between clot formation and bleeding [16]. Tissue factor (TF) is widespread in various tissues and is central to the cancer-related coagulation process [17]. Activation of oncogenes and suppression of cancer-suppressing genes will cause the up-regulation of TF, which activates the external coagulation system [18]. Persistent hypoxia, due to a rapid proliferation of tumor cells, upregulates hypoxia-inducible factor 1α (HIF-1α). TF and plasminogen activator inhibitor-1 [19] expression increases the occurrence of venous thromboembolism (VTE). Antitumor therapy increases hypercoagulation risk in addition to tumor-induced coagulation system. Vascular endothelial growth factor (VEGF) inhibits platelet aggregation and promotes vascular regeneration [20,21]. VEGF inhibitors inhibit regenerating blood vessels to provide blood supply to tumors, so it used widely in targeted therapy for tumors. In a clinical trial, arterial occlusion due to VEGF inhibitors resulted in a 5-year cumulative disease rate of 31 % [22]. With advances in immune checkpoint inhibitor therapy in cancer and limited response rates, ICIs treatment increased thrombotic complications in patients of CRC [23,24]. A prospective cohort study involving 9754 patients established that cancer-related thrombosis is confirmed to be correlated with patients' poor survival [25]. No models of colorectal tumor prognosis have been established based on coagulation-related genes, even though this is the second risk factor for death in cancer patients [26].

With the advances in sequencing technology, sufficient expression profiling data is available in an open source, and the analysis and validation of the dataset provide fresh perspectives into the occurrence, development, and treatment of diseases. In this study, we systematically evaluated the association between the expression profile of coagulation-related genes and prognosis through The Cancer Genome Atlas (TCGA) dataset. A risk model based on coagulation-related genes was established and verified through the Gene Expression Omnibus (GEO) dataset, revealing its predictive value for individual CRC patients' outcomes. The association of RiskScore with clinicopathological features, immune cell infiltration, immunotherapy, drug sensitivity, somatic mutations, and biological function was investigated. At last, a stable nomogram was established.

2. Material and methods

2.1. Database download and processing

The "TCGAbiolinks" R package [27] was utilized to download transcriptome sequencing data and relevant clinical information for COAD and READ in the TCGA dataset as a training cohort (https://portal.gdc.cancer.gov/repository). GSE39582 and GSE17536 were downloaded from the GEO dataset as validation cohorts (https://www.ncbi.nlm.nih.gov/). Both datasets were sequenced by Affymetrix Human Genome U133 Plus 2.0 Array.

To exclude other confounding factors, we developed the inclusion criteria: (1) primary lesion of colorectal tumor; (2) patients with detailed information of age, gender, stage, and survival data. The exclusion criteria included (1) patients surviving for under 30 days; (2) patients sampled after neoadjuvant therapy; (3) pathologically confirmed as not colorectal tumors. With the inclusion and exclusion criteria, we finally obtained 555 colorectal cancer data in the TCGA dataset, 556 data from the GSE39582 dataset, and 175 data from the GSE17536 dataset as the validation cohorts. The GSE39582 and GSE17536 data are processed and annotated by the limma package, and the TCGA data are processed using the log (TPM+1) format.

2.2. Acquisition of coagulation-related genes

We searched the Molecular Signatures Database database for related signaling pathways (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp) with the theme term "coagulation" and obtained 845 genes as coagulation-related genes after de-duplication (Table S1).

2.3. Identification of coagulation molecules with prognostic significance in CRC

Univariate Cox regression analysis was applied to identify coagulation-related genes correlated with colorectal cancer survival in the TCGA dataset. $P < 0.05$ was regarded as the cutoff value. Further, the backward stepwise regression was selected to identify the best model and construct a coagulation-related score based on the Akaike Information Criterion (AIC).

$$\text{Risk score} = h_0(t)^{*}\exp(\beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \cdots \beta_n X_n)$$

2.4. Evaluation of model effectiveness

Patients were divided into two independent groups by the median RiskScore and defined as high-score and low-score groups. Survival analysis, time-dependent receiver operating characteristic (ROC) analysis, and risk factor linkage plots will analyze the predictive values of the risk model. Kaplan-Meier (K-M) curves were used to assess the prognosis between the two groups of patients. Further, the risk model was validated in two independent datasets separately to demonstrate its generalizability.

2.5. Analysis of the immune microenvironment composition

We calculated the proportions of 28 immune cells per tissue via the "CIBERSORT" R package [28]. In the tumor microenvironment, immune score, stromal score, and ESTIMATE score were calculated by the "ESTIMATE" R package to assess non-tumor components in tumor tissues [29].

2.6. Functional enrichment analysis of RiskScore groups

Differentially expressed genes (DEGs) in high-score and low-score groups were screened using the Mann-Whitney *U* test under a threshold of $|\log_2 \text{FoldChange}| > 1$, False Discovery Rate <0.05. The Volcano mapped by the "ggthemes" R package showed DEGs. Based on DEGs, the "clusterProfiler "R package provided classifications of biological terms and enrichment of gene clusters [30]. Gene Ontology (GO) analyzes the biological processes (BP), molecular functions (MF), and cellular components (CC) of differential genes. Gene-associated pathways were identified through the Kyoto Encyclopedia of Genes and Genomes (KEGG).

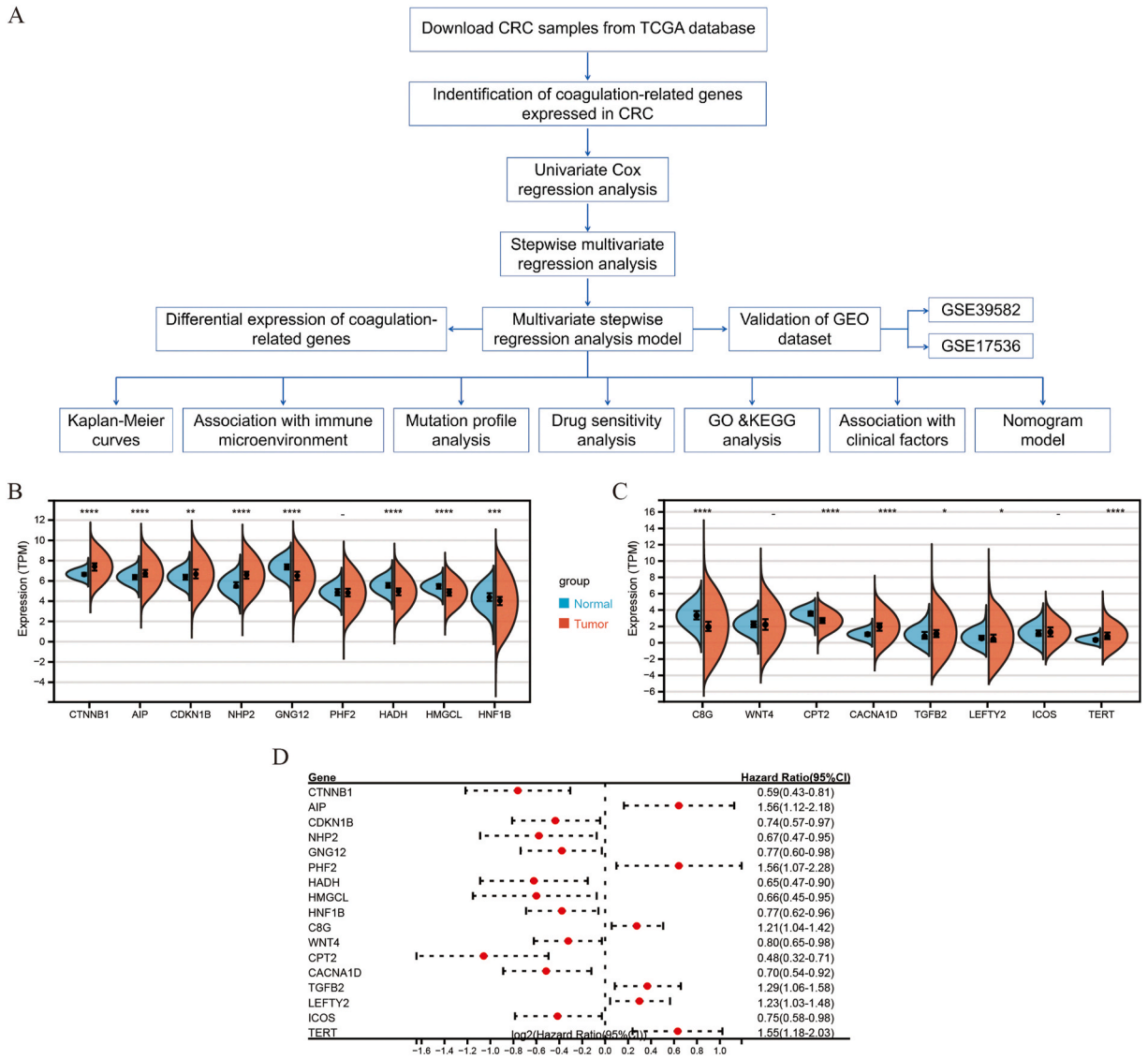

Fig. 1. (A) The flowchart of the study design. (B–C) The differential expression of 17 coagulation-related genes in tumor and normal tissues, including CTNNB1, AIP, CDKN1B, NHP2, GNG12, PHF2, HADH, HMGCL, HNF1B, C8G, WNT4, CPT2, CACNA1D, TGFB2, LEFTY2, ICOS, and TERT. (D) Univariate Cox regression analyses were performed on the candidate genes.

2.7. Comparison of significantly mutated genes

Genetic mutation was collected and compared between patients using single nucleotide polymorphism data from TCGA dataset. The somatic variation data for coagulation-related genes was analyzed through the "maftools" R package [31]. The "Rcircos" R package demonstrated the positioning of genes on chromosomes [32].

2.8. Drug sensitivity prediction

Immune checkpoint expression was extracted to compare variation between two Riskscore groups. IC50 of drugs were predicted through the "oncoPredict" R package [33], indicating a substance's effectiveness in inhibiting a specific biological process.

2.9. Correlation between riskscore and clinicopathological characteristics

Correlations were analyzed via plotting heatmap and comparing RiskScore between clinical characteristics, including age, gender, and pathological staging.

2.10. Establishment of a nomogram model

The "rms" R package was used to comprise coagulation-based risk scores and various other clinicopathological factors into the nomogram model. Univariate and multivariate analyses first screened each risk factor, and then the nomogram was constructed with these factors and validated through calibration curves.

2.11. Statistical analyses

All data calculations and statistical analyses in this study were conducted utilizing the R software (https://www.r-project.org/, version 4.1.2). In comparing two groups of independent variables, the two independent samples *t*-test was used to determine if the data conformed to a normal distribution. Data that deviated from normal distribution was examined using the Mann-Whitney *U* test, known as the Wilcoxon rank sum test. One-way analysis or the Kruskal-Wallis test was applied for comparison between multiple groups of samples. Pearson's chi-square test was applied to evaluate differences in the distribution of clinical factors. All statistical *P* values were double-sided, and differential genetic screening was regarded as statistically significant with a corrected $P < 0.05$. *P* values for the subsequent statistical tests were adhered to the descriptions provided in the text.

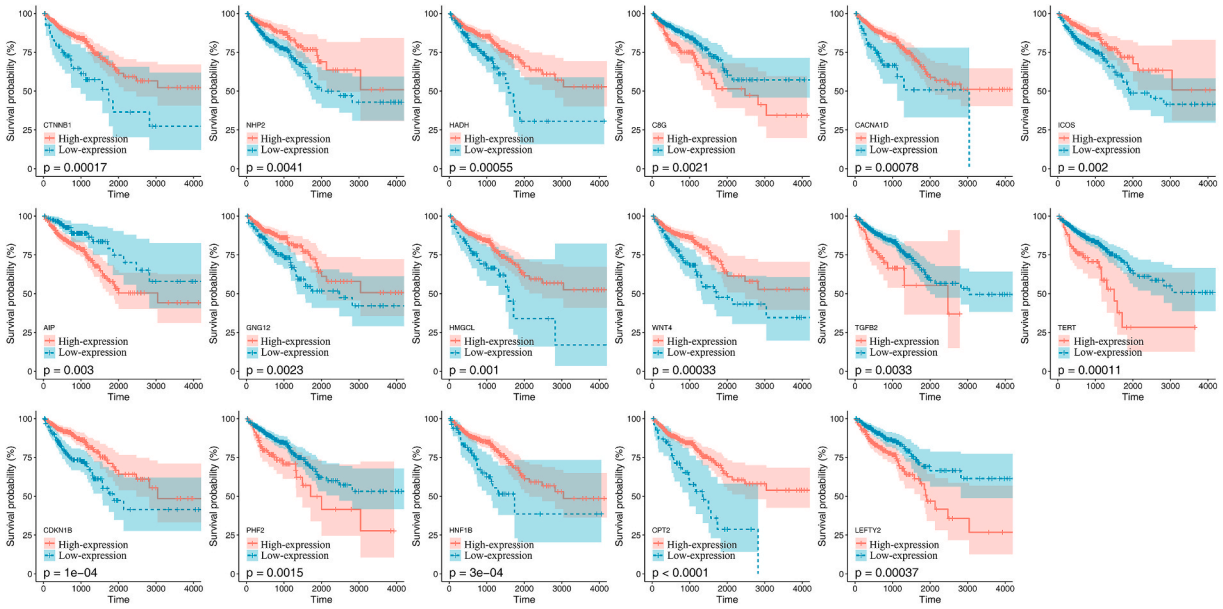

Fig. 2. The Kaplan-Meier curves of 17 coagulation-associated genes based on the TCGA dataset.

3. Results

3.1. Identification of coagulation-related genes with prognostic significance in CRC

Fig. 1A illustrates the flow chart of this study. As a training cohort, 845 coagulation-related genes were extracted from the TCGA database. Through univariate regression analysis, 69 prognosis-related genes were found significantly, with $P < 0.05$ (Table S2). A prognostic model was subsequently established by AIC-based backward stepwise regression, and 17 coagulation-related genes were selected. Further, we identified that the expression of genes varies between normal and tumor tissues. High expressions of these genes (CTNNB1, AIP, CDKN1B, NHP2, CACNA1D, TGFB2, LEFTY2, TERT) were found in tumor tissues. High expressions of these genes (GNG12, HADH, HMGCL, HNF1B, C8G, CPT2) were found in normal tissues (Fig. 1B and C). The results derived from univariate Cox regression analysis of candidate genes were demonstrated in the forest plot (Fig. 1D).

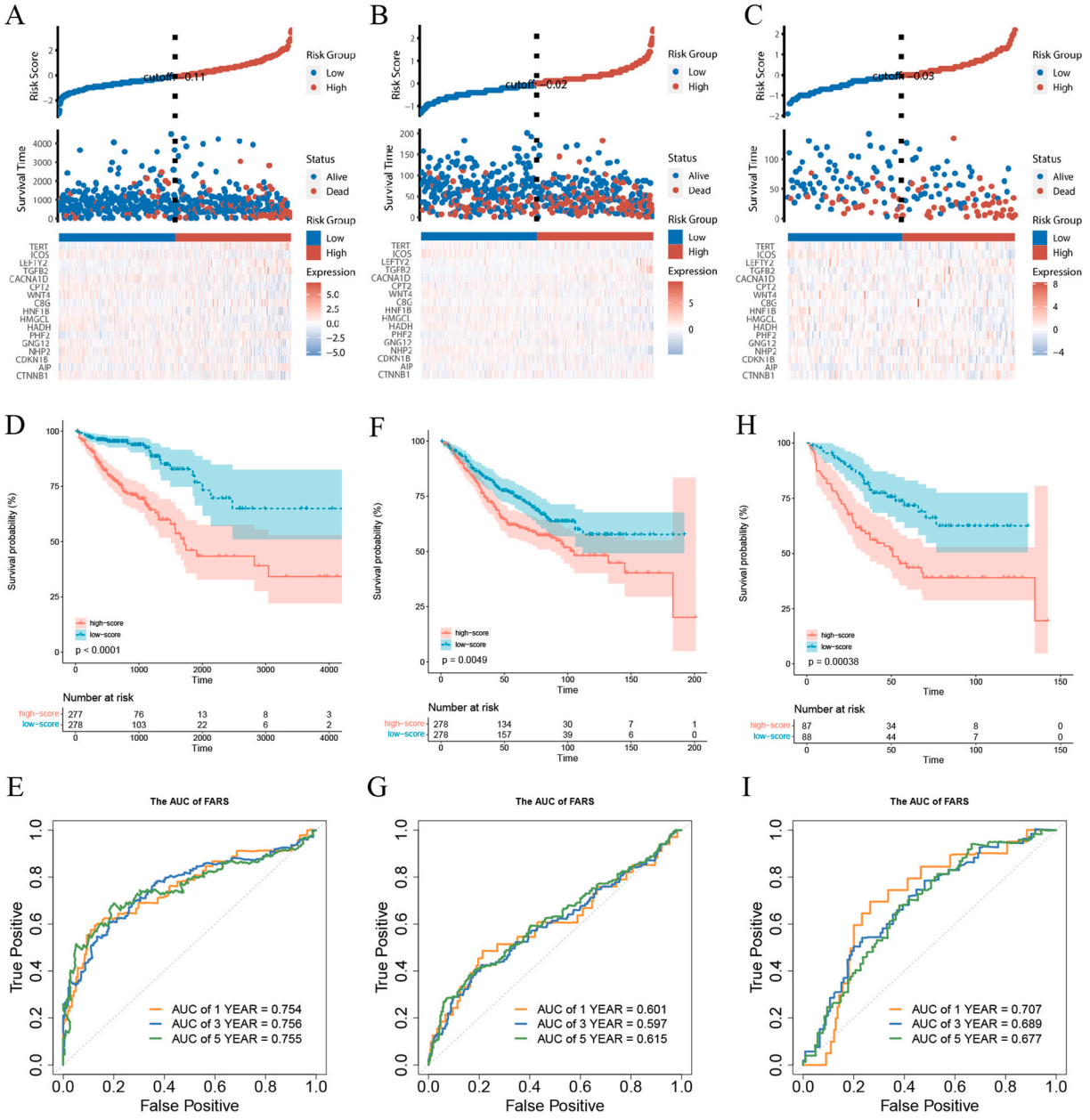

Fig. 3. Establishment and validation of the RiskScore model based on coagulation-related genes. The risk score correlation charts, Kaplan-Meier curves and time-dependent receiver operating characteristic curves for the TCGA dataset (A,D,E), GSE39582 dataset (B,F,G), and GSE17536 dataset (C,H,I).

K-M curves confirm the prognostic value of each gene ($P < 0.05$) (Fig. 2). Among the 17 coagulation-related genes, the high expression of 11 genes (CTNNB1, CDKN1B, NHP2, GNG12, HADH, HMGCL, HNF1B, WNT4, CPT2, CACNA1D, and ICOS) was correlated with a poor prognosis. The low expression of 6 genes (AIP, PHF2, C8G, TGFB2, LEFTY2, and TERT) was correlated with a poor prognosis (Fig. 2). However, in the K-M curves, the 95 % confidence interval between the high and low expression cohorts overlapped, indicating that a individual gene is unstable as a predictive marker.

3.2. Construction of a coagulation-related prognostic signature

Seventeen coagulation-related genes were obtained by stepwise regression screening, and the Cox regression risk model was established. Gene expression profile was multiplied using coefficients from the multivariate Cox proportional hazards, and the scores obtained were passed through the Predict function to calculate the risk scores. The specific formula is shown below:

$$\text{RiskScore} = h_0(t)*\exp[(-0.373529049*\text{CTNNB1}) + (0.832388344*\text{AIP}) + (-0.383917304*\text{CDKN1B}) + (-0.579357927*\text{NHP2}) + (0.539192767*\text{GNG12}) + (0.653687349*\text{PHF2}) + (0.361272641*\text{HADH}) + (-0.45893745*\text{HMGCL}) + (-0.232587546*\text{HNF1B}) + (0.209284587*\text{C8G}) + (-0.274509842*\text{WNT4}) + (-0.552855875*\text{CPT2}) + (-0.442143551*\text{CACNA1D}) + (0.341323229*\text{TGFB2}) + (0.187678125*\text{LEFTY2}) + (-0.520602913*\text{ICOS}) + (0.443639634*\text{TERT})]$$

Patients were categorized into groups with high- and low-score, depending on the median RiskScore value (Fig. 3A,B,C). K-M curves indicated a poorer prognosis for patients with high scores (Fig. 3D). The AUCs of the ROC curves were 0.754 at 1 year, 0.756 at 3 years, and 0.755 at 5 years, indicating that our prognostic prediction signature had moderate specificity and sensitivity (Fig. 3E).

We further validated this prognostic signature in two GEO datasets. Both GSE39582 and GSE17536 datasets demonstrated a poorer prognosis in high-score patients, demonstrating the stability of the signature (Fig. 3F–H). In GSE39582, the AUCs of the ROC curves

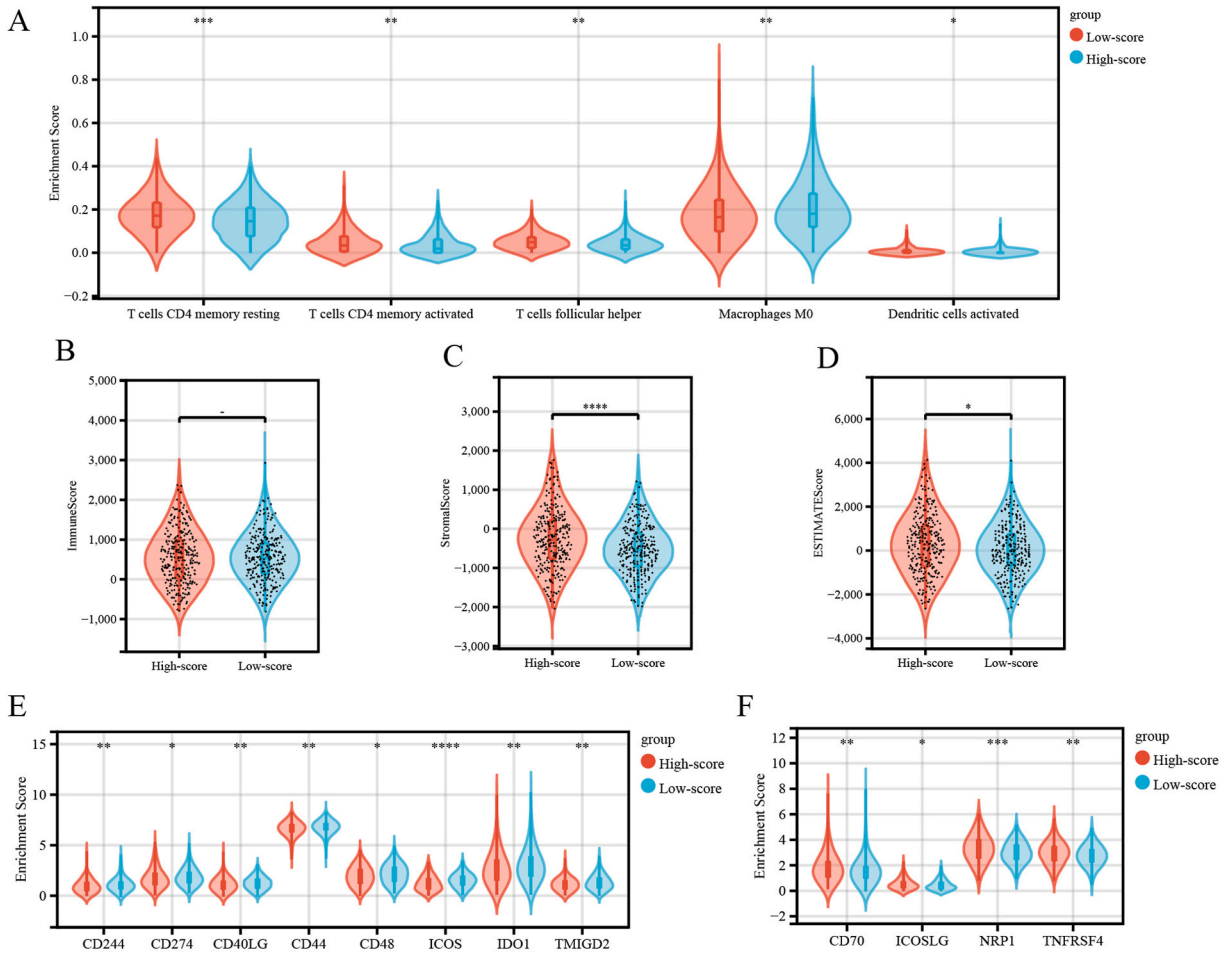

Fig. 4. Relationship of RiskScore to immune cell infiltration and expression of immune checkpoints. (A) Differential infiltration abundance of immune cells between the high- and low-score groups. Immune (B), stromal (C), and ESTIMATE (D) scores in two groups. High enrichment of immune checkpoint genes in high- (E) and low-score (F) groups. ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.001.

were 0.601 at 1 year, 0.597 at 3 years, and 0.615 at 5 years (Fig. 3G). In GSE17536, the AUCs of the ROC curves were 0.707 at 1 year, 0.689 at 3 years, and 0.677 at 5 years (Fig. 3I).

3.3. Relationship between RiskScore and immune cells infiltration

Infiltration of immune cells plays an integral role in tumor microenvironment (TME). Tumor-infiltrating immune cells have been shown to correlate with patient prognosis and impact the efficacy of immune checkpoint inhibitor therapy. The box plots showed that patients with low scores had a higher percentage of immune cells, including resting memory $CD4^+$ T cells, activated $CD4^+$ T cells, and T

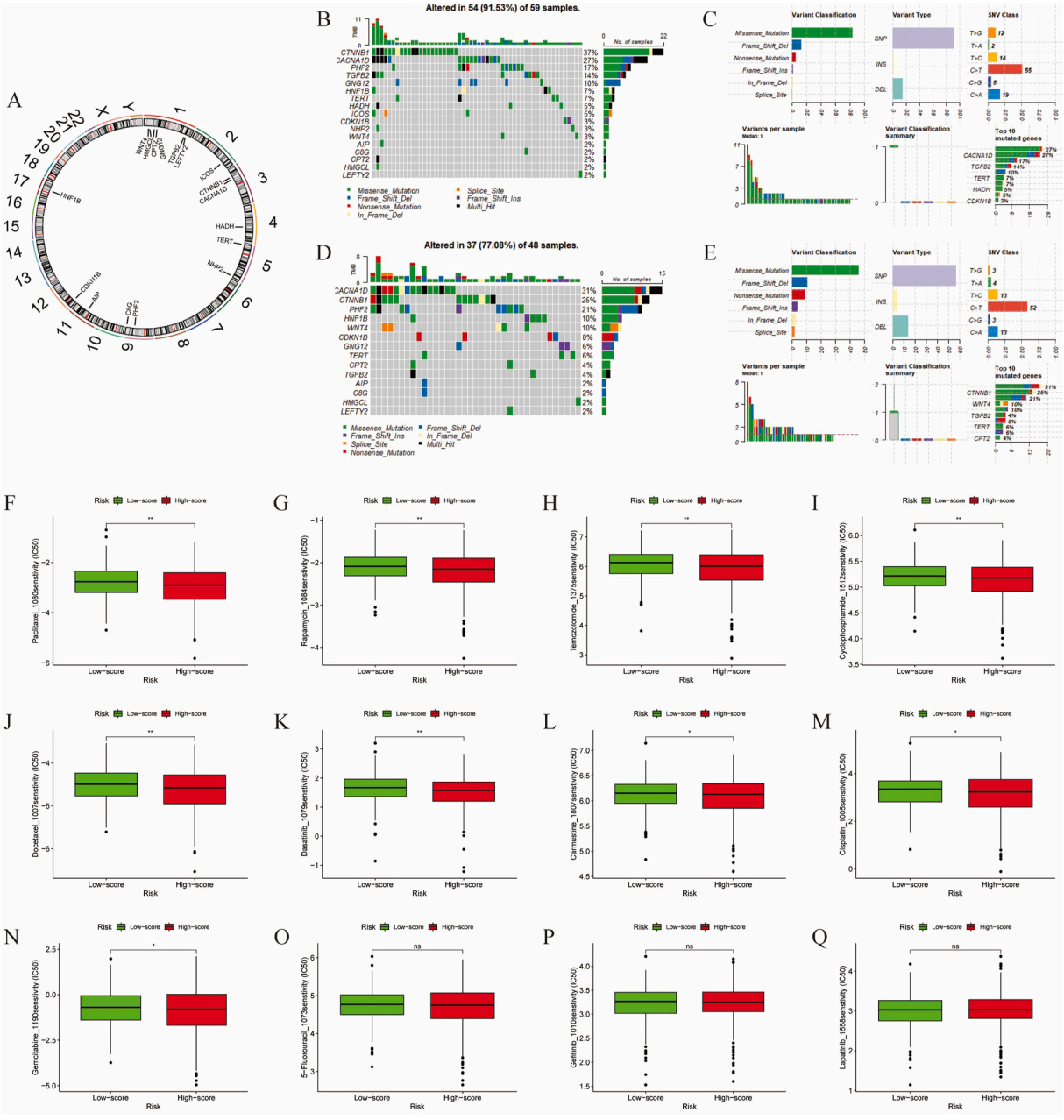

Fig. 5. Analysis of somatic mutations and drug sensitivity based on the RiskScore model. (A) Localization of RiskScore-related genes on chromosomes. MAF-summary map and oncoplots demonstrating differences in somatic mutations between high- (B–C) and low-score (D–E) groups in the TCGA dataset. Box plots showing the mean difference in estimated IC50 of representative drugs for colorectal cancer treatment, including Paclitaxel (F), Rapamycin (G), Temozolomide (H), Cyclophosphamide (I), Docetaxel (J), Dasatinib (K), Carmustine (L), Cisplatin (M), Gemcitabine (N), 5-Fluorouracil (O), Gefitinib (P), and Lapatinib (Q). ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$.

follicular helper (Tfh) cells (Fig. 4A). Meanwhile, the high-score group had a higher level of M0 macrophage infiltration. Activated dendritic cell infiltration was low in both groups, even though the results presented statistical differences. In the TME, no significant difference was found in immune scores between the two Riskscore groups (Fig. 4B), indicating no difference in the overall immune cell infiltration. Stromal scores (Fig. 4C) and ESTIMATE scores (Fig. 4D) were higher in high-score patients.

Further, we compared the expression of immune checkpoint genes in two RiskScore groups. Most of the genes were highly expressed in the low-score group, including CD244, CD274, CD40LG, CD44, CD48, ICOS, IDO1, and TMIGD2 (Fig. 4E). The expression of CD70, ICOSLG, NRP1, and TNFRSF4 was significantly higher in the high-score group (Fig. 4F). Higher expression of immune checkpoint genes means that patients may benefit from immune checkpoint inhibitor treatment potentially.

3.4. The association between RiskScore and genomic alterations

The Circos track map visualized the positioning of candidate genes on the genome (Fig. 5A). We analyzed the genomic mutations and copy number variants (CNVs) of 17 coagulation-related genes in two RiskScore groups in the TCGA cohort. Among the top five mutation frequencies, CTNNB1, CACNA1D, and PHF2 were common genes in both groups. In the high-score patients, the genes TGFB2 (14 % vs. 4 %) and GNG12 (10 % vs. 6 %) had a higher frequency of mutations, while gene WNT4 (10 % vs. 3 %) and CDKN1B (8 % vs. 3 %) were highly mutated in low-score patients (Fig. 5B–E).

3.5. Drug sensitivity in coagulation-related RiskScore model

To further explore whether there were differences in the antitumor treatments. We evaluated the IC50 of 198 antitumor drugs or inhibitors between two Riskscore groups. The bar chart showed the commonly used drugs for colorectal cancer (Fig. 5F-Q), finding that Paclitaxel, Rapamycin, Temozolomide, Cyclophosphamide, Docetaxel, Dasatinib, Carmustine, Cisplatin, Gemcitabine may be ideal drugs for treating high-score patients. 5-Fluorouracil as a first-line agent for colorectal cancer chemotherapy may not differ in efficacy between the two groups (Fig. 5O).

3.6. Discovery of biological functional and pathway enrichment

A total of 915 DEGs were screened under the threshold of False Discovery Rate <0.05 and $|\log_2 \text{FoldChange}| > 1$. The volcano map illustrated the DEGs between two RiskScore groups (Fig. 6A). Further, we explored the core biological functions enriched by these genes through GO and KEGG enrichment analysis (Supplementary Tables S3–6).

BP significantly enriched in GO enrichment analysis includes muscle system process, muscle contraction, modulation of chemical

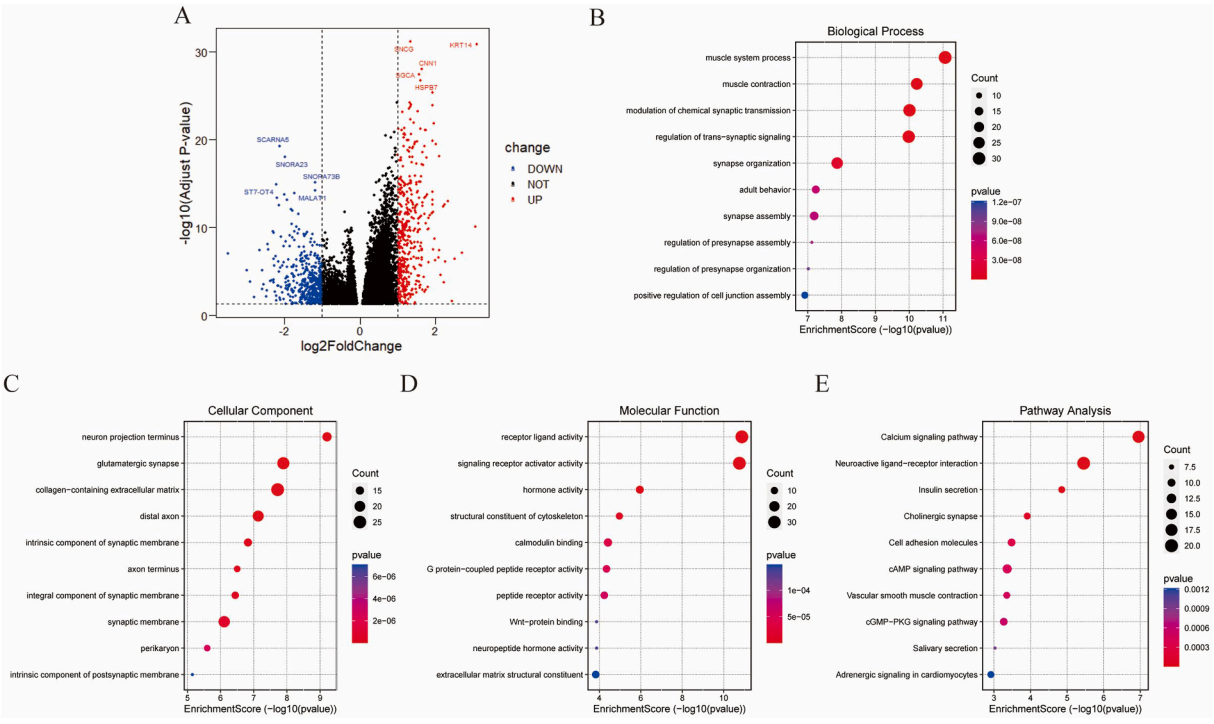

Fig. 6. Exploring the differential biological function of high- and low-score groups. (A) Volcano plot showing the differentially expressed genes in the high- and low-score groups. GO enrichment analysis (B–D) and KEGG enrichment analysis (E) based on differentially expressed genes.

synaptic transmission, and so on (Fig. 6B). The significantly enriched CC in GO enrichment analysis resulted in the following: neuron projection terminus, glutamatergic synapse, collagen-containing extracellular matrix, distal axon, and so on (Fig. 6C). The significantly enriched MF in GO enrichment analysis included receptor ligand activity, signaling receptor activator activity, hormone activity, and so on (Fig. 6D). In addition, KEGG enrichment analysis was conducted by differential expression genes to capture advanced biological functions. The differential pathways mainly involved the Calcium signaling pathway, the Neuroactive ligand-receptor interaction, Insulin secretion, and so on (Fig. 6E).

3.7. Associations between RiskScore and clinicopathological factors

The heatmap visualized the expression of 17 candidate genes and the distribution of different clinical and pathological factors in CRC patients in two Riskscore groups (Fig. 7A). More detailed clinical-based pathological information can be found in Table 1. Risk scores proved to be independent of age and gender (Fig. 7B and C). Meanwhile, higher risk scores indicated poorer prognosis (Fig. 7D) and high pathological stage (Fig. 7H). In subgroups analysis, we found that CRC patients with high distant metastasis (Fig. 7E), node metastasis (Fig. 7F), and pathological T stage (Fig. 7G) tended to have a higher score. These results suggest that our prognostic

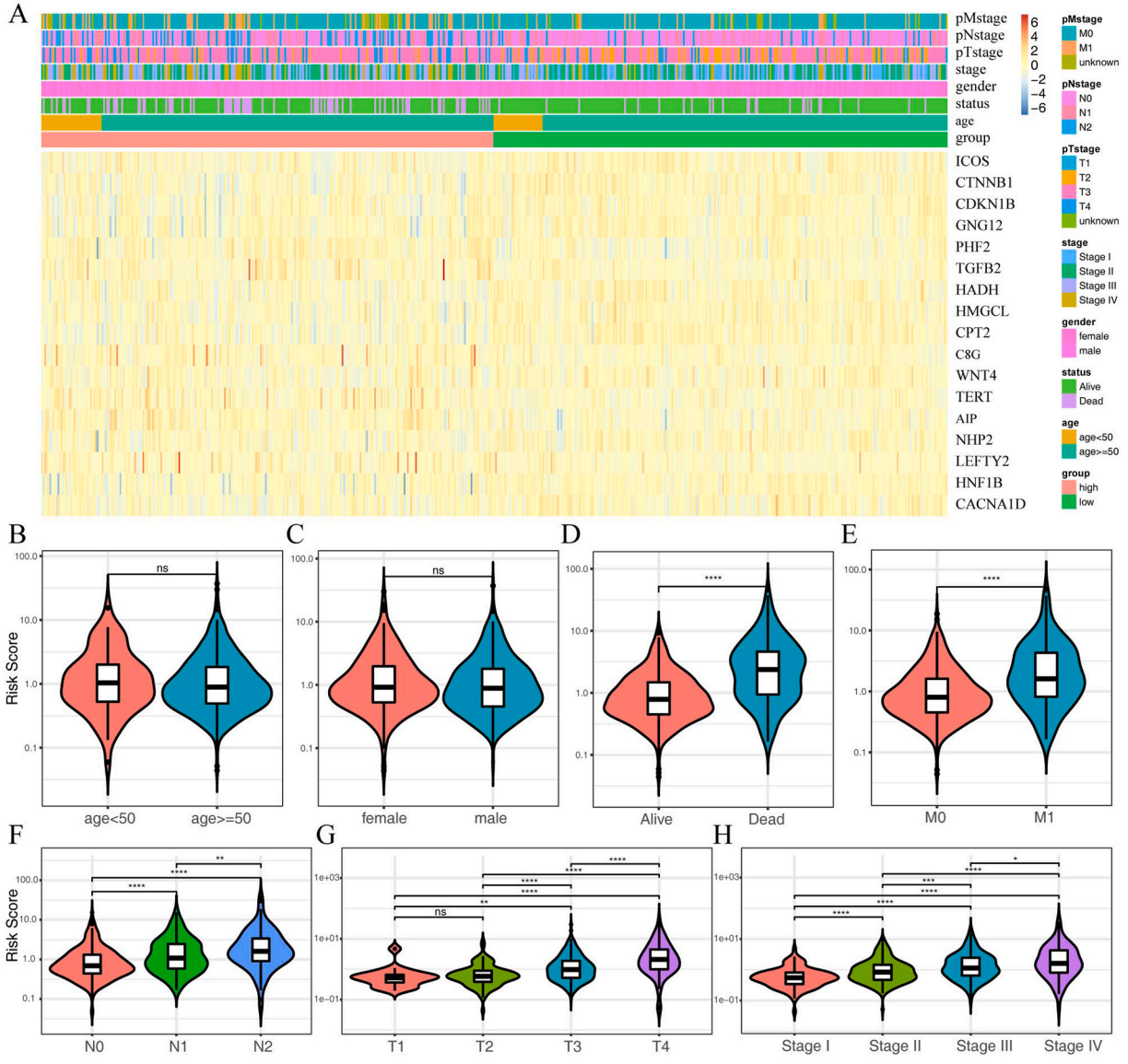

Fig. 7. The relationship between prognostic and clinicopathological factors of CRC patients. The heat map shows the expression of 17 coagulation-related genes and other clinicopathological parameters in the high-score and low-score groups (A). The violin plots showed the relationship between risk score and different clinical prognostic factors, including age (B), gender (C), survival state (D), metastasis (E), node metastasis (F), pathological T stage (G), and tumor stage (H). ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 1
Detailed clinical and pathological information of TCGA patients.

Variables	TCGA cohort (n = 555)	Risk score		*P*-value
		High score	Low score	
Type				0.302
COAD	408	209	199	
READ	147	68	79	
Gender				0.834
Female	252	127	125	
Male	303	150	153	
Age (mean ± SD, years)	66.16 ± 12.40	67.68 ± 12.32	64.64 ± 12.29	***0.004***
≤60 years	179	70(49.20)	93(51.52)	***0.034***
>60 years	376	207(73.93)	185(71.23)	
Pathology				0.254
Adenocarcinoma	483	243	240	
Mucinous adenocarcinoma	71	34	37	
NA	1	0	1	
Stage				0.257
I	101	47	54	
II	204	112	92	
III	167	78	89	
IV	83	40	43	
Pathological T stage				0.794
T1	18	9	9	
T2	98	44	54	
T3	379	193	186	
T4	59	31	28	
NA	1	0	1	
Pathological N stage				0.567
N0	315	161	154	
N1	139	69	70	
N2	101	47	54	
Pathological M stage				0.658
M0	423	215	208	
M1	81	39	42	
NA	51	23	28	

characteristics are strongly correlated with CRC clinical factors.

3.8. Independent prognostic factors and nomogram model of CRC

Investigating independent prognostic factors for CRC patients in the TCGA cohort, univariate and multivariate Cox regression analyses were performed on RiskScore and clinical parameters. Factors including predictive RiskScore, pathological stage, and age were identified as separate indicators predicting the prognosis in patients (Fig. 8A and B). Furthermore, based on the Cox analysis, a nomogram model for predicting prognosis was developed (Fig. 8C). The calibration curves showed good concordance between predicted and observed 1-, 3-, and 5-year OS values in the training cohort (Fig. 8D–F).

4. Discussion

CRC ranks as the third most prevalent tumor worldwide, representing one-tenth of all tumors and cancer-related deaths diagnosed worldwide each year [3]. Due to national screening and the widespread of colonoscopy, colorectal morbidity has remained stable, but 30–50 % of patients still experience recurrence after treatment [34]. With the overall review of genomics and transcriptomics, tumor-associated molecular pathways emerged as prognostic biological markers for early diagnosis and progression of tumors, which could guide the treatment of patients, including aging [35], stemness [36], immune [37], epithelial-mesenchymal transition (EMT) [38], and ferroptosis [39]. The coagulation system is involved in the proliferation, invasion, metastasis, and tumor-induced neoangiogenesis [40]. Coagulation pathway-related genes might be potential prognosis-related biomarkers.

The imbalance of the coagulation system is pathological and involves multiple clinical and biological factors. In the first aspect, colorectal tumors tend to occur in the advanced age, a population with a high prevalence of blood clotting due to advanced age [41], obesity [42], cardiovascular disease, respiratory disease [43], and other factors [44]. In pathogenesis, the balance of coagulation and bleeding was disrupted, and the hypercoagulable state eventually manifested complications, containing pulmonary embolism and deep vein thrombosis [45]. TF acts as the primary initiator of the blood coagulation cascade and promotes key malignant processes in tumors, including proliferation, migration, angiogenesis, and metastasis, through protease-activated receptor-2 signaling [46,47]. In a clinical study that included 170 patients undergoing curative colorectal cancer surgery, correlations were found between coagulation tests and factors such as tumor size, depth of invasion, and prognosis, including platelet, prothrombin time, platelet count, D-dimer, and fibrinogen degradation product [48]. Except for the cancer-mediated coagulation cascade and platelet activation, all antitumor

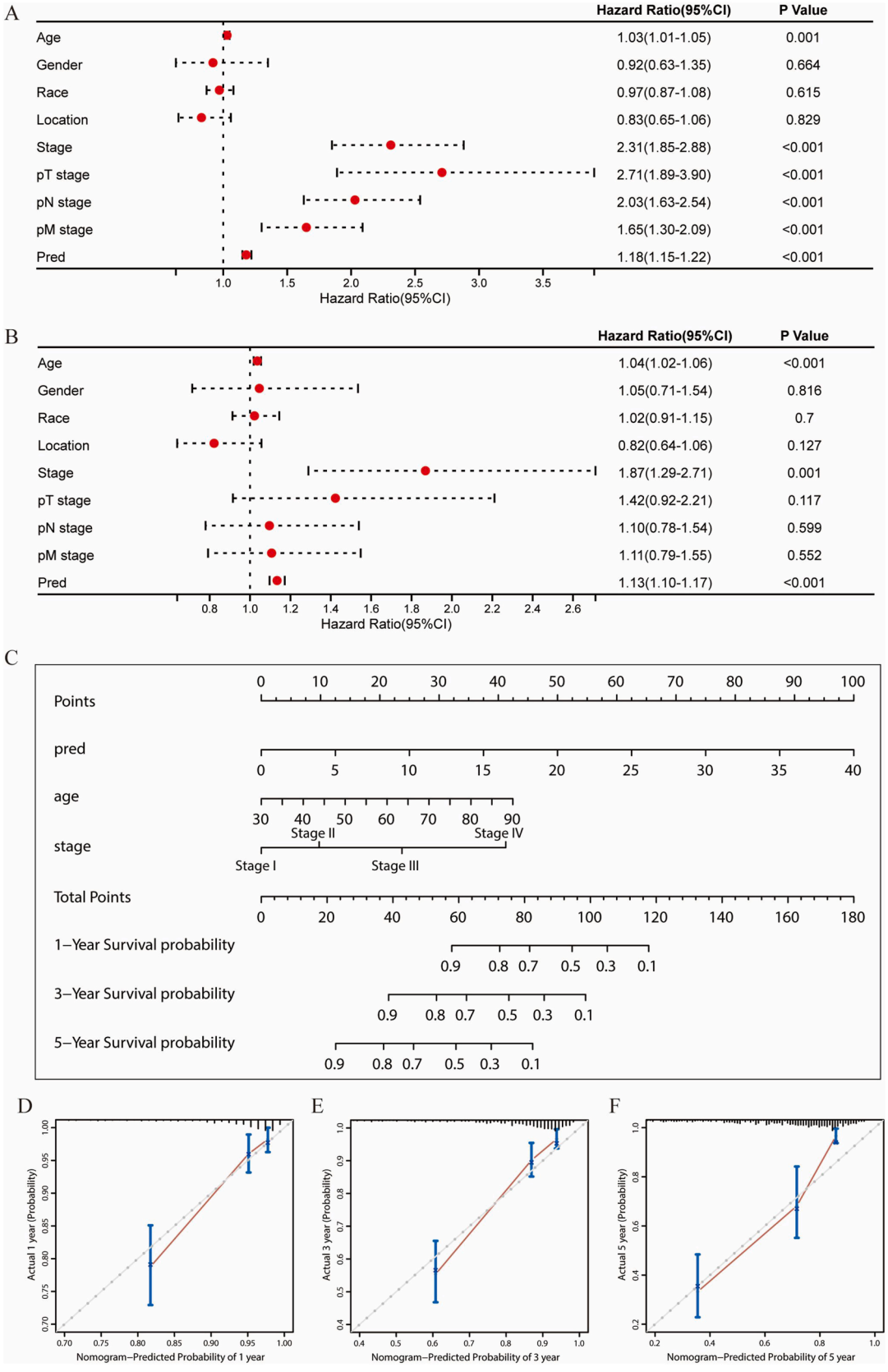

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Fig. 8. Construction of the nomogram model of CRC patients. Univariate (A) and multivariate (B) Cox regression analysis of overall survival for the prognostic signature and clinicopathological factors. (C) The nomogram model predicted the 1-, 3-, and 5-year OS in patients with CRC. The calibration curves for predicting patient OS at 1- (D), 3- (E), and 5-years (F) in the TCGA dataset.

treatments would increase the risk of venous thrombosis, with VTE rates rising from 3.9 % to 5.7 % in hospitalized patients receiving chemotherapy [49]. VEGF promotes vascular endothelial regeneration and suppresses platelet aggregation. VEGF inhibitors are commonly used in antitumor targeting treatments, and vascular embolism is a common side effect [18]. Over the past few years, direct oral anticoagulants have gradually proven as effective treatments for VTE as an alternative to lower molecular weight heparin [50,51].

This is the first study revealing a coagulation-related signature. First, 845 coagulation-related genes were acquired to explore potential prognostic biomarkers. We conducted univariate Cox regression and backward stepwise regression to identify 17 candidate genes (CTNNB1, AIP, CDKN1B, NHP2, GNG12, PHF2, HADH, HMGCL, HNF1B, C8G, WNT4, CPT2, CACNA1D, TGFB2, LEFTY2, ICOS, and TERT) closely related with the clinical prognosis of CRC patients. Among these genes, the main enriched pathways were "HALLMARK_COAGULATION", "HP_ABNORMAL_BLEEDING", and "HP_ABNORMALITY_OF_COAGULATION". Recent studies revealed that the transcriptional activity of CTNNB1 was reported to be associated with metastasis and invasion of colon tumors [51]. Aberrant CTNNB1 signaling stands as a fundamental process in colorectal cancer, and the promotion of CTNNB1 degradation would inhibit EMT and metastasis [52]. CDKN1B, as a substrate of S-phase kinase-associated protein 2, regulates cell proliferation and tumor progression by targeting the ubiquitination of several cell cycle regulators [53,54]. NHP2 acts as a fundamental element of the telomerase complex and is linked to poor overall survival in advanced-age patients [55]. HNF1B acted as an oncogene or proto-oncogene in different types of tumors, and its low expression correlated with malignant behavior and shortened disease-free survival in CRC patients [56]. WNT4 was found to initiate the WNT4/β-catenin pathway to initiate cancer-fibroblast formation and EMT [57]. After tumor resection, the expression level of WNT decreased, enabling it to become a potential biomarker for CRC [57]. The TGF-β signaling pathway has a major function in the evolution of CRC [58]. TGFB2 was also recognized to be associated with necrosis-related miRNAs employed in prognostic models of colon cancer [59]. TERT acts as the driving force behind the telomerase complex, and its activation facilitates tumor progression [60].

Further, we developed a multifactorial regression signature and divided the CRC patients into low- and high-score groups according to the median value of risk score. The K-M curve indicated that the patients with high scores obtained a poorer prognosis compared to the patients with low scores in the TCGA database. The ROC analysis revealed the excellent predictive power of our signature. Meanwhile, two independent GEO datasets were utilized to verify the model's stability. We further investigated the variances in the immunological microenvironment and somatic mutations within the high- and low-score groups. In contrast to the immune microenvironment of high-score patients, patients in the low-score group had a higher abundance of resting memory $CD4^+$ T cells, activated $CD4^+$ T cells, and Tfh cells. Tfh cells were found to perform antitumor immunity in a $CD8^+$-dependent manner and restored the efficacy of anti-PD1 therapy [61]. Previous research has consistently linked high immune cell infiltration to improved survival rates, including resting memory $CD4^+$ T cells [62]. CALGB/SWOG 80405, a phase III randomized trial, contained transcriptome data from 554 patients with primary malignancies. More infiltration of plasma cells and activated memory $CD4^+$ T cells were correlated with better overall survival [63]. In contrast, the high-score patients presented significantly higher infiltration of stromal scores. We hypothesized that in this coagulation-related gene signature, poor prognosis in high-score patients would be more relevant to stromal cells. Stromal cells in the tumor microenvironment influence multiple initiations of metastasis, including invasion, angiogenesis, and epithelial-mesenchymal transition [64,65]. Previous studies have identified subtypes of drug resistance and poor prognosis in CRC through stromal gene expression, which may be associated with tumor-associated fibroblasts [66]. Elevated immune checkpoint genes expressed in low-score patients, including CD244, CD274, CD40LG, CD44, CD48, ICOS, IDO1, and TMIGD2, suggesting these patients had more opportunities to benefit from ICIs treatment. In the CNVs analysis, the two groups showed different variation profiles, in which TGFB2, GNG12, WNT4, and CDKN1B were the primary differential genes. To explore whether the differential prognosis between the two Riskscore groups was related to differential biological functions. It was interesting to find multiple differential biological processes associated with neural signaling, including modulation of chemical synaptic transmission, signaling receptor activator activity, and neuroactive ligand-receptor interaction. Nerves, as a significant component of the tumor microenvironment, are closely correlated with the occurrence of metastatic lesions. Studies have identified that peripheral nerves interact with stromal cells to activate nerve-dependent tumor cell development. The increase in nerve density is consistent with the aggressive manifestation of the tumor. In vitro, experiments have also demonstrated that neurotransmitters can send signals directly to tumors and promote cell proliferation and migration. This shows that the unfavorable prognosis of patients with high scores may be related to the reactivation of developmental and regenerative pathways in the nerves. At the end of the study, univariate and multivariate Cox regression analyses identified predictive RiskScore, pathological stage, and age were identified as independent predictors of prognosis. The predictive nomogram was established and exhibited a perfect consistency between the 1-, 3-, and 5-year survival.

In this study, we performed a coagulation-related prognosis signature for CRC, which could stratify patients by their coagulation risk, provide guidance in treatment, and predict prognosis. Our study revealed the relationship between the clinical parameters and the expression of coagulation-related genes in CRC patients. Although we showed a correlation between poor prognosis and coagulation mechanisms and established a stable prognostic signature, our study still has some limitations. Although our study confirmed a specific prognostic signature, as a retrospective study, clinical cohort, and mechanistic studies are still required to test it. As the signature does not obtain particularly high sensitivity and specificity, whether it is predictable for the formation and occurrence of thrombosis needs to be further analyzed by including transcriptome data from patients with thrombosis at different stages. As coagulation dysfunction is associated with multiple pathological mechanisms, some candidate genes have only been identified to be correlated with poor

prognosis, but corroboration from previous studies is lacking.

5. Conclusion

We have presented a comprehensive study of coagulation-related genes in CRC patients for the first time through a series of bioinformatics analyses. We established a signature consisting of 17 genes to assess the prognosis of CRC patients, including CTNNB1, AIP, CDKN1B, NHP2, GNG12, PHF2, HADH, HMGCL, HNF1B, C8G, WNT4, CPT2, CACNA1D, TGFB2, LEFTY2, ICOS, and TERT. Based on our prognostic signature, a high-score score showed high associations with worse pathological staging and poor clinical outcomes. Moreover, more highly expressed immune checkpoint genes were found in the low-score group, indicating more opportunities for ICI treatments. Through clinical validation, we hope our signature will provide prognostic predictions and treatment guidance for CRC patients.

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Data availability statement

The analyzed data in this study were obtained from the TCGA dataset (http://portal.gdc.cancer.gov/repository) and the GEO dataset (https://www.ncbi.nlm.nih.gov/). The supporting data in this study were included in the article and supplementary materials.

Ethics approval statement

Review and/or approval by an ethics committee was not needed for this study because our research only utilized open-source data and did not involve the collection of human information data or biological samples.This study fulfils the terms of the exemption from ethical review.

Additional information

Additional material related to this article will be published and available online:

CRediT authorship contribution statement

Taojun Jin: Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jianmei Ji:** Software, Resources, Methodology, Investigation. **Xiaowen Xu:** Validation, Supervision, Funding acquisition. **Xinxing Li:** Writing – review & editing, Visualization, Conceptualization. **Biao Gong:** Writing – review & editing, Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32687.

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