



Development of a Platelet-Related Prognostic Model for Colorectal Cancer

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Colorectal cancer (CRC) represents one of the most common malignancies with high morbidity worldwide. Growing evidence has suggested that platelets are a fundamental component of the tumor microenvironment and play crucial roles in driving tumor biological behavior. The construction of a platelet-related prognostic model that can reliably predict CRC prognosis is of great clinical significance. The 1427 CRC-specific platelet-related genes were collected and mainly enriched in the ribosome and immune-related pathways. Based on platelet-related genes, three subtypes of TCGA CRC samples were identified by consensus clustering and characterized by differences in angiogenesis, epithelial–mesenchymal transition, immune infiltration, and prognosis. A total of 100 prognostic platelet-related genes were identified by univariate Cox regression. LASSO Cox regression further shrank those genes and constructed a 10-gene prognostic model. The patients with higher risk scores had significantly worse disease-specific survival than those with lower scores in both TCGA and validation cohorts. The risk score demonstrated good predictive performance for prognosis by receiver operating characteristic (ROC) curves. Furthermore, multivariate Cox regression analysis showed that the risk score was independent of TNM stage, sex, and age, and a graphic nomogram based on the risk score and clinical factors was developed to predict survival probability of CRC patients. Patients from the high-risk group were characterized by higher infiltration of immunosuppressive cells such as MDSC and Treg and higher expression of checkpoints *CTLA4*, *CD86*, and *PDCD1LG2*. Taken together, we identified three platelet-related subtypes and specifically constructed a promising 10-gene prognostic model in CRC. Our results highlighted the potential survival effects of platelet-related genes and provided evidence about their roles in regulating tumor immunity.

Keywords: colorectal cancer, platelet, subtype, prognostic model, tumor immunity

INTRODUCTION

Colorectal cancer is the third most common cancer type and a leading cause of cancer-related mortality worldwide (Bray et al., 2018; Siegel et al., 2022). The 5-year survival rate of CRC patients with stage I and II is 91 and 82%, respectively, while stage IV is approximately 12% (Miller et al., 2019). The variation in survival of CRC patients due to tumor heterogeneity prompts an urgent need to develop new robust prognostic markers to improve predictive accuracy and complement the traditional TNM stage and tumor status. Moreover, a more effective risk stratification system is needed to aid in the management of CRC patients.

TABLE 1 | Clinical characteristics of patients in the TCGA cohort.

| Characteristics | Variables | Number of samples |
|--------------------------|-----------|-------------------|
| Age | Age <60 | 123 |
| Age | Age >= 60 | 201 |
| Lymphovascular invasion | Unknown | 35 |
| Lymphovascular. invasion | NO | 201 |
| Lymphovascular invasion | YES | 88 |
| Perineural invasion | Unknown | 128 |
| Perineural invasion | NO | 146 |
| Perineural invasion | YES | 50 |
| Sex | Female | 142 |
| Sex | Male | 182 |
| Stage | I/II | 172 |
| Stage | III/IV | 138 |
| Stage | Unknown | 14 |
| Tumor site | Unknown | 2 |
| Tumor site | Colon | 245 |
| Tumor site | Rectum | 77 |

Growing evidence had showed that blood is a rich source of tumor-related biomarkers (Bardelli and Pantel, 2017; Kilgour et al., 2020), of which platelets are involved in a variety of tumor biological processes (Best et al., 2018), which can be used for biomarkers and diagnosis of cancer types (Best et al., 2018; Liu et al., 2020; Wurdinger et al., 2020). Platelet aggregation and degranulation along with the consequent release of platelet-derived proangiogenic mediators could influence tumor growth (Klinger and Jelkmann, 2002). Platelet-derived transforming growth factor-beta (TGF- β) and direct platelet-tumor cell interaction synergistically induce the epithelial-mesenchymal transition (EMT) in tumor cells and promote metastasis (Labelle et al., 2011; Miyashita et al., 2015). Furthermore, platelets can induce an immunosuppressive tumor microenvironment or support tumor cells to avoid immune elimination by protecting tumor cells directly from recognition by cytotoxic lymphocytes such as natural killer cells (McAllister and Weinberg, 2014; Best et al., 2018; Schmied et al., 2021).

Experimental data suggest that lower platelet count may reduce tumor growth and metastasis and could predict longer disease-specific survival (DFS) in diverse tumors (Møller Pedersen and Milman, 1996; Taucher et al., 2003; Ishizuka et al., 2012; Stone et al., 2012; Tao et al., 2021). In addition, platelet-related gene expression could be used to construct a prognostic model for predicting the survival of lung adenocarcinoma (Zhou et al., 2021). A recent study showed that the potential role of antiplatelet agents to suppress tumor progression by reducing platelet count or platelet activation was intriguing and validated. The platelet aggregation inhibitors exerted an inhibitory effect on metastatic spread (Kuznetsov et al., 2012), indicating that stratification of tumor samples based on the platelet-related biomarkers and antiplatelet agents therapy might be promising. A systematic study of tumor-educated blood platelets (TEPs) suggested that identified platelet-related genes enable CRC diagnostics (Best et al., 2015). However, whether these platelet-related genes are correlated with the prognosis in CRC patients remains to be studied.

TABLE 2 | Clinical characteristics of patients in the GSE161158 cohort.

| Characteristics | Variables | Number of samples |
|-----------------|-----------|-------------------|
| Age | Age <60 | 63 |
| Age | Age >= 60 | 128 |
| Stage | I/II | 107 |
| Stage | III/IV | 84 |

Therefore, it is of great clinical significance to establish a platelet-related prognosis model to guide individual therapy. This study combined bioinformatics analysis and further validation on the basis of large biopsy samples to identify specific platelet-related subtypes and developed a reliable platelet-related prognostic risk scoring system in CRC.

MATERIALS AND METHODS

CRC Sample Collection

The TCGA colorectal cancer (CRC) expression profile with log₂ transformation was downloaded from the UCSC Xena browser (<https://xenabrowser.net/>), from which colon and rectal cancer samples were extracted. The clinical information of TCGA CRC samples was obtained from the cBioPortal website (<http://www.cbioportal.org/>) based on the R package “cgdsr”. The 324 CRC samples with available expression and disease-free survival (DFS) information were retained as the discovery cohort (**Supplementary Table S1**) and their corresponding characteristics were shown in **Table 1**.

For a validation cohort, the gene expression data and the corresponding clinical information of 191 CRC samples were retrieved from the Gene Expression Omnibus database (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) at accession number GSE161158. The corresponding characteristics of GSE161158 were detailed in **Table 2**.

Functional Enrichment Analysis

The enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed using the R package “clusterProfiler” with default parameters. We considered the *p*-value, adjusted by the BH method to control the FDR, less than 0.05 as the statistical significance and showed the top ten ones.

Identification of Platelet-Related Subtypes

According to the previous study (Best et al., 2015), we obtained 2396 platelet-related genes in CRC and then screened out 1427 coding genes with $|\log_2 \text{fold change}|$ (tumor patients vs. adjacent normal samples) greater than 1 for subsequent analysis (**Supplementary Table S2**). Based on the 1427 platelet-related genes, the R package “ConsensusClusterPlus” was applied to classify TCGA CRC samples into clusters (100 iterations, 80% resampling rate Pearson correlation). The best three-cluster separation was selected because the consensus clustering cumulative distribution function (CDF) curve in the range of 0.1–0.9 was near flat when the number of clusters $k = 3$.

Gene Set Variation Analysis

To compare functional differences between platelet-related subtypes, we collected 15 angiogenesis and 11 EMT-relevant pathways from published resources including MSigDB, GO-BP, cancer hallmarks, REACTOME, and KEGG. Using gene set variation analysis (GSVA) from the R package “GSVA”, we calculated the activity score of the above pathways. Then the variation among subtypes was evaluated by the Wilcoxon rank-sum test. ns: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Estimation of Immune and Stromal Cell Infiltration

ESTIMATE (Yoshihara et al., 2013) was employed to infer the infiltrating extent of stromal and immune cells in tumor samples through the R package “estimate”. ESTIMATE is a popular algorithm, which was extensively utilized in tumor studies (Liu et al., 2022a; Liu et al., 2022b). To further explore the differences between immune cell subtypes, the CIBERSORT algorithm was used to assess the proportions of 22 immune cell subtypes based on the expression file (Newman et al., 2015). Based on a signature matrix of 547 genes, the 22 subtypes of infiltrating immune cells inferred by CIBERSORT include B cells, T cells, natural killer cells, macrophages, dendritic cells, eosinophils, and neutrophils. In addition, the gene signatures of 28 tumor infiltrating lymphocytes (TILs) were obtained from a previous study (Charoentong et al., 2017), and their activities in each sample were quantified using ssGSEA from the R package “GSVA”. The 28 subpopulations of TILs includes major types related to adaptive immunity: activated T cells, central memory (T_{cm}), effector memory (T_{em}) CD4⁺ and CD8⁺ T cells, gamma delta T (T_{γδ}) cells, T helper 1 (Th1) cells, Th2 cells, Th17 cells, regulatory T cells (T_{reg}), follicular helper T cells (T_{fh}), activated, immature, and memory B cells, as well as cell types related to innate immunity, such as macrophages, monocytes, mast cells, eosinophils, neutrophils, activated, plasmacytoid, and immature dendritic cells (DCs), NK cells, natural killer T (NKT) cells, and MDSCs.

Survival Analysis and Prognostic Model Construction

The 1427 candidate platelet-related genes in the TCGA CRC cohort were screened using univariate Cox regression analysis. Thus, 100 genes were identified as candidate biomarkers that were significantly related to DFS. Based on the “glmnet” in the R package, an optimal prognostic signature for CRC samples was built by LASSO Cox regression analysis using these candidate biomarkers. According to the optimal lambda value, a prognostic gene list with coefficients was generated from the LASSO model. Through multiplying the expression level of a prognostic gene by its corresponding LASSO coefficient, the risk score for each patient was calculated using the following formula: risk score = $\sum_{i=1}^n \text{coef}_i * \text{expr}_i$, where coef_i is the LASSO coefficient of gene i , and expr_i is the expression level of the prognostic gene i . Then, the patients were separated into high- and low-risk groups based on the median value of the risk score. Kaplan–Meier survival curves with the log-rank tests were applied to compare differences in survival between the patient groups. And a

time-dependent ROC curve analysis was used to evaluate the predictive efficacy using the R package “timeROC”.

Statistical Analysis

The Wilcoxon rank-sum test was conducted to compare the continuous variables between the two groups, such as gene expression level, immune infiltration, pathway activity, and risk score. The univariate Cox regression analyses and LASSO regression analyses were applied to identify the most significant platelet-related genes. The multivariate Cox regression analyses were used to confirm the risk score as an independent prognostic factor after adjusting for clinical factors. Kaplan–Meier analysis and the log-rank test were employed to evaluate correlations between groups and DFS. ROC was used to study the prediction efficiency of the prognostic model. The prognostic value of the nomogram was evaluated by the C-index. In the present study, all tests of significance were two-sided, and $p < 0.05$ was considered statistically significant. ns: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

RESULTS

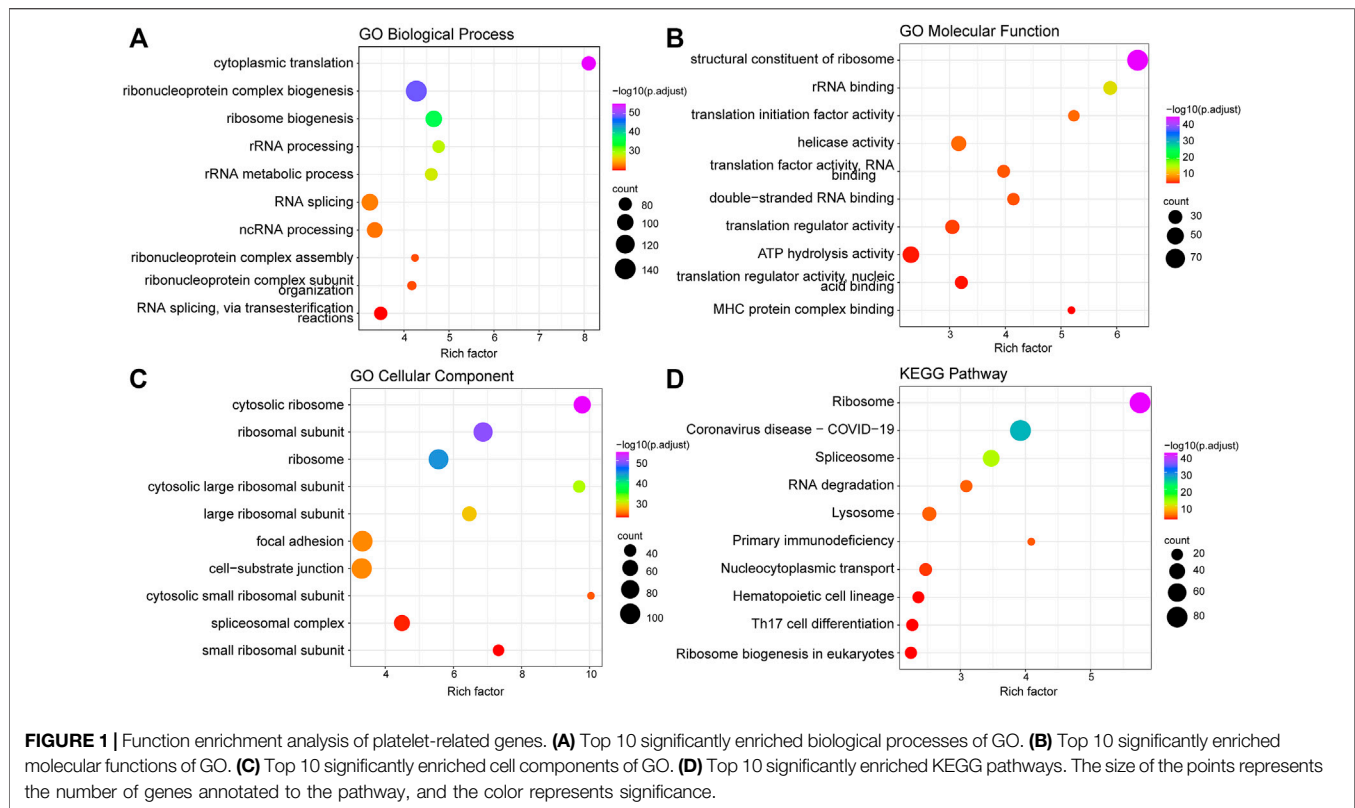
Function Enrichment Analysis of Platelet-Related Genes

After screening out 1427 candidate platelet-related genes, GO terms and KEGG pathway analyses were performed to further investigate their involvement in the biological process (for details, see Methods). GO analysis revealed that platelet-related genes were mainly enriched in cytoplasmic translation, ribosomal processing, and ribosomal metabolism biological processes (Figure 1A). This analysis additionally showed that these genes were chiefly involved in translation factors activity, ribosomal binding, and MHC protein complex binding under the molecular function category (Figure 1B). Furthermore, the enriched cellular components included cytosolic ribosome, cytosolic large ribosomal subunit, and cytosolic small ribosomal subunit (Figure 1C).

In regards to the KEGG pathway analysis, platelet-related genes were also significantly enriched in ribosome-related pathways (Figure 1D), which was consistent with the previous findings that protein synthesis continues in platelets through dynamic regulation of a ribosome rescue pathway, even though platelets lack nuclei to produce new mRNA and ribosomes (Ji et al., 2011; Mills et al., 2016). In addition, the major involvement in some immune-related pathways was also shown in KEGG results, such as primary immune deficiency, hematopoietic cell regulation, and Th17 cell differentiation (Figure 1D).

Identification and Characterization of Platelet-Related Subtypes in Colorectal Cancer

Based on the platelet-related genes, unsupervised consensus clustering distinguished the 324 TCGA CRC tumors into three distinct groups termed platelet-related subtype1 ($n = 130$), subtype2 ($n = 129$), and subtype3 ($n = 65$) respectively (details see Methods; Figure 2A). Further exploration revealed significant



differences in survival between three subtypes ($p = 0.0085$), among which the subtype3 had the worst DFS (**Figure 2B**). The mechanisms by which platelets contribute to tumor growth and metastasis include their potential to promote angiogenesis, EMT, and immune evasion (Best et al., 2018). Therefore, we further explored whether there are differences in those biological processes among the three platelet-related subtypes (details see Methods; **Supplementary Table S3**). The activity score of 15 angiogenesis and 11 EMT-related pathways were consistently significantly higher in subtype3 than in the other two subtypes, suggesting that subtype3 has the highest stromal activity and greatest ability to promote tumor progression (**Figures 2C,D**).

Since platelet-related genes were found to be involved in regulating immune-related pathways (**Figure 1D**), we further explored differences in immune microenvironment among distinct subtypes. To our surprise, the proportion of innate immune cell infiltration in subtype3 was also significantly higher than that in the other two subtypes, such as natural killer cells, macrophages, eosinophils, and mast cells (**Figure 2E**). Previous studies have shown that tumors with an immune exclusion phenotype also show the presence of large numbers of immune cells but remain in the stroma around the tumor cell nest rather than penetrating its parenchyma (Zhang et al., 2020). Additionally, we analyzed the oncogenic features of the three subtypes in CRC and found that subtype3 showed the highest oncogenic activities (**Supplementary Figure S1**), including cancer-associated fibroblasts (CAFs), angiogenesis, and tumor-associated

macrophages (TAM). Previous studies have shown that CAFs play a crucial role in the development of desmoplastic reactions and shape the tumor immune microenvironment in CRC (Koliaraki et al., 2017; Mochizuki et al., 2020). CAFs may, directly and indirectly, impact anti-tumor immune reaction through the recruitment of protumorigenic inflammatory cells, such as M2-like TAM (Zhang and Liu, 2013).

To validate the performance of subtypes, we conducted cluster analysis in the validation cohort (GSE161158, **Supplementary Figure S2A**). The results showed that there was a consistent phenomenon between the TCGA CRC cohort and the validation cohort. There were significant differences in patient survival among the three subtypes in the validation cohort (**Supplementary Figure S2B**). In addition, in line with the TCGA CRC cohort, the infiltration of 28 immune cells was obviously different among the three subtypes in the validation cohort (**Supplementary Figure S2C**). In general, platelet-related subtypes were characterized by differences in angiogenesis, epithelial-mesenchymal transition, immune infiltration, and prognosis, indicating the potential of platelet-related genes to predict prognosis in CRC.

Identification of Prognostic Platelet-Related Genes and Construction of a Prognostic Model

To better investigate the prognostic role of platelet-related genes in CRC, univariate Cox regression analysis was applied

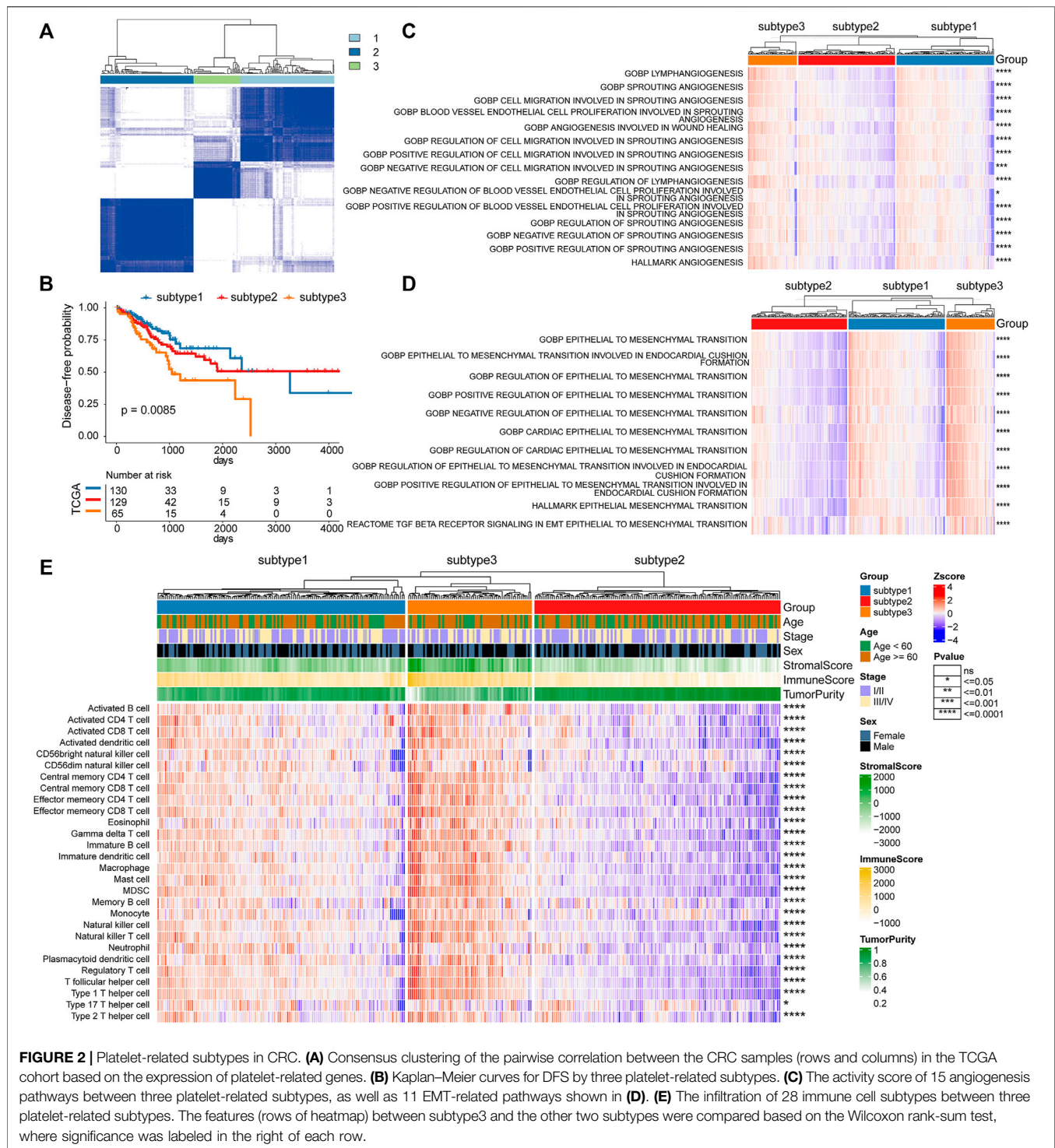


FIGURE 2 | Platelet-related subtypes in CRC. **(A)** Consensus clustering of the pairwise correlation between the CRC samples (rows and columns) in the TCGA cohort based on the expression of platelet-related genes. **(B)** Kaplan–Meier curves for DFS by three platelet-related subtypes. **(C)** The activity score of 15 angiogenesis pathways between three platelet-related subtypes, as well as 11 EMT-related pathways shown in **(D)**. **(E)** The infiltration of 28 immune cell subtypes between three platelet-related subtypes. The features (rows of heatmap) between subtype3 and the other two subtypes were compared based on the Wilcoxon rank-sum test, where significance was labeled in the right of each row.

in the TCGA cohort. The results showed that 100 genes were significantly associated with the DFS out of 1427 platelet-related genes (**Supplementary Table S4**). The top 20 prognostic platelet-related genes were shown in **Figure 3A**. Some of these genes had already been reported as prognostic biomarkers, such as *CLK1* ($p = 0.0245$) (Yang et al., 2021) and *SLC11A1* ($p = 0.014$) (Zhu et al., 2022) (**Figures 3B,C**).

To remove redundant prognostic factors, we performed a LASSO Cox analysis on 100 prognostic platelet-related genes. Ten predictors with the greatest influence on DFS in CRC patients were determined (**Supplementary Table S5**), of which 8 were risk genes (*TIAL1*, *C1orf198*, *CLK1*, *PTPRN*, *LSMEM1*, *ANKRD13D*, *ATP6AP1*, and *SKAP1*), and 2 were protective genes (*ERAP1* and *ANKRD17*) (**Figures 3D–F**). Previous studies

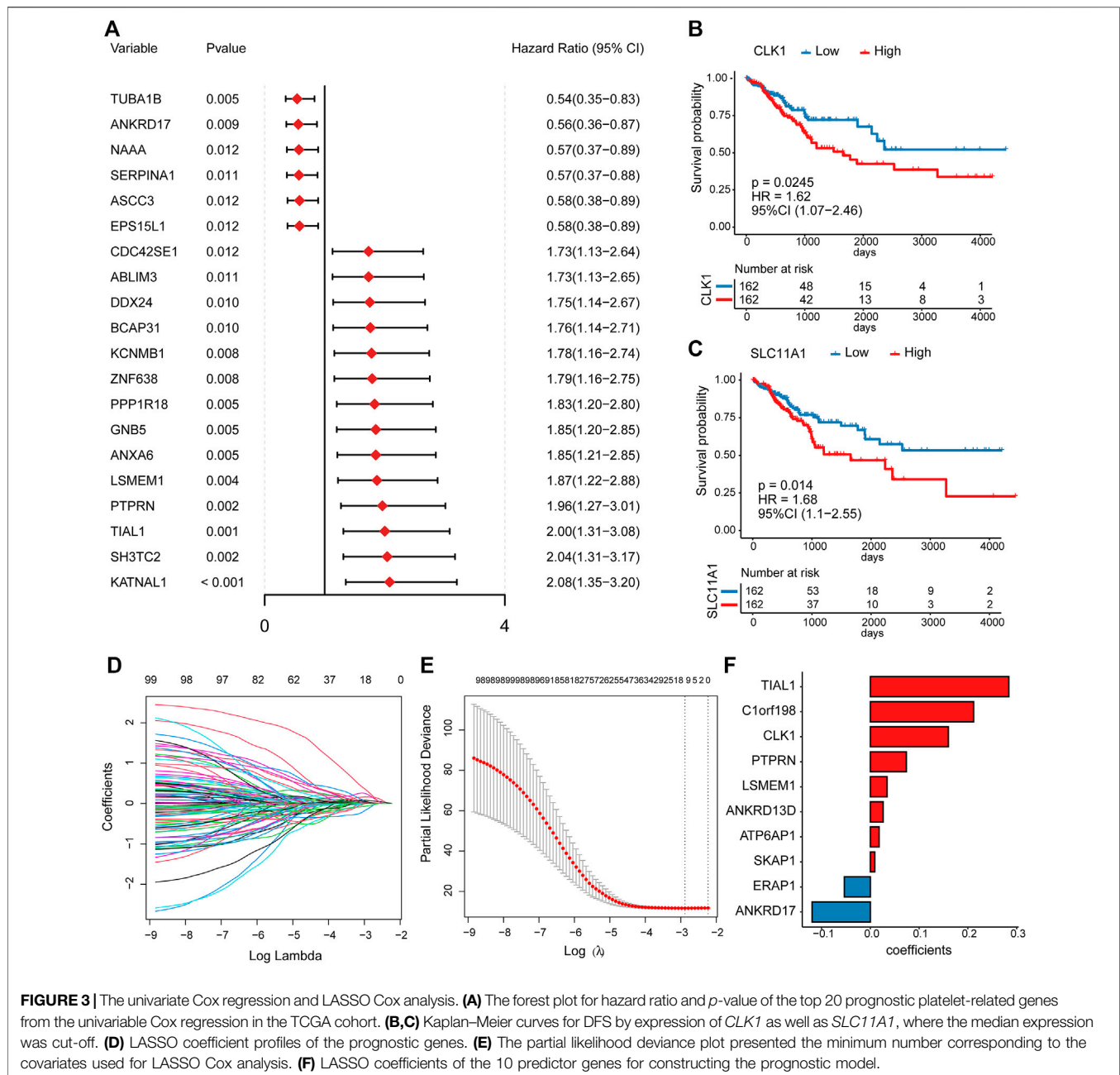


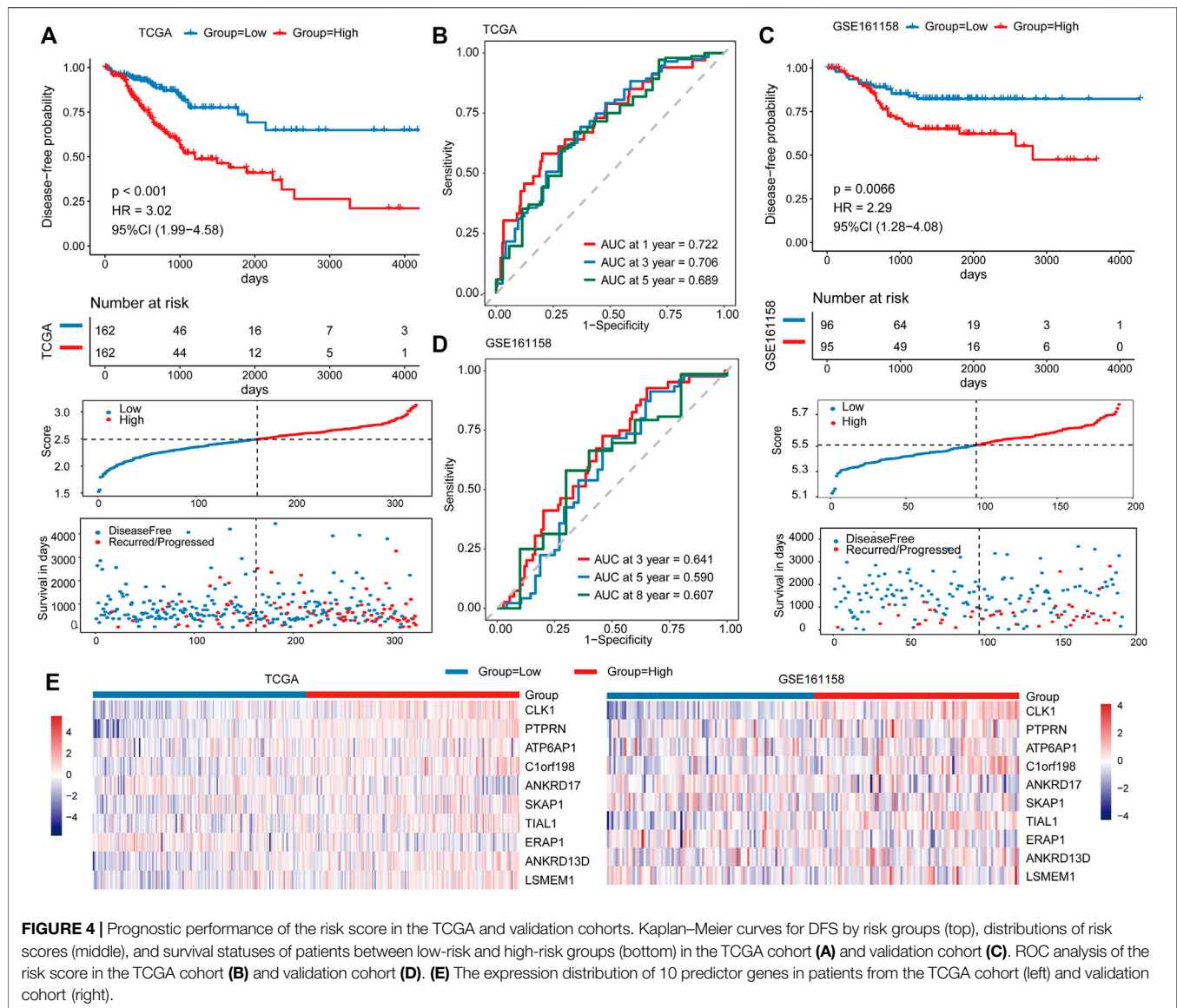
FIGURE 3 | The univariate Cox regression and LASSO Cox analysis. **(A)** The forest plot for hazard ratio and p -value of the top 20 prognostic platelet-related genes from the univariable Cox regression in the TCGA cohort. **(B,C)** Kaplan–Meier curves for DFS by expression of *CLK1* as well as *SLC11A1*, where the median expression was cut-off. **(D)** LASSO coefficient profiles of the prognostic genes. **(E)** The partial likelihood deviance plot presented the minimum number corresponding to the covariates used for LASSO Cox analysis. **(F)** LASSO coefficients of the 10 predictor genes for constructing the prognostic model.

supported that *CLK1* and *ANKRD17* could be served as biomarkers or potential therapeutic targets for CRC (Ioana et al., 2010; Lian et al., 2020). Then, these 10 predictors were subjected to construct a platelet-related prognostic risk score using a linear combination of each gene expression level and its risk coefficient **Figure 3E** (details see Methods; **Supplementary Table S5**).

The Prognostic Capacity of the Risk Score

Risk scores were calculated based on a 10-gene prognostic model. Patients in the training dataset TCGA cohort were then stratified into high-risk group ($n = 162$) and low-risk group ($n = 162$) based

on the median risk score. The Kaplan–Meier survival analysis confirmed the high-risk group yielding reduced survival time ($p < 0.001$, **Figure 4A**). The predictive performance of the prognostic model was evaluated by the time-dependent ROC curves and the area under the curve (AUC) reached 0.722 at 1 year, 0.706 at 3 years, and 0.689 at 5 years, suggesting that this prognostic model exhibited good sensitivity and specificity (**Figure 4B**). In addition, we assessed the prognostic efficacy of risk score for all available patient information and found that risk score was associated with poor prognosis under different clinical factors (**Supplementary Figure S3**). Furthermore, we calculated the concordance index (C-index) of the model under the



conditions of risk score, clinical factors, and risk score and clinical factors, respectively. The results showed that the prognostic efficacy of combined status (C-index = 0.76) was better than that of risk score alone (C-index = 0.61) and clinical factors alone (C-index = 0.65, **Supplementary Figure S4A**).

We compared the prognosis performance of the risk score in this study with the genes that were associated with CRC prognosis (including *CLK1* and *SLC11A1*). Our results revealed that the risk score exceeded CRC prognosis both in C-index and AUC value at different time points, which indicated that the prognosis performance of our signature was better than CRC prognosis (**Supplementary Figures S5A,B**). To test the robustness of the model, the risk score was calculated for 190 patients as the independent validation cohort (GSE161158). Similarly, patients in the high-risk group exhibited markedly poorer survival than those in the low-risk group ($p < 0.001$, **Figure 4C**). The AUCs for 3, 5, and 8 years were 0.641, 0.590, and 0.607, respectively

(**Figure 4D**). In addition, the expression distributions of these 10 prognostic predictor genes in the TCGA and validation cohorts were consistent between the high- and low-risk groups (**Figure 4E**).

Clinicopathological Significance and Independence of the Risk Score

Subsequently, we further investigate the associations between risk scores and clinicopathological features. The risk scores of patients with the advanced stage was significantly higher than that of patients with early stage ($p = 1.4e-06$), and that of patients with lymphatic vascular invasion was higher than that of patients without invasion ($p = 6e-04$, Wilcoxon rank-sum test; **Figure 5A**). No other significant correlations were found between risk score and clinicopathological features, such as age, sex, perineural invasion, and tumor anatomic site.

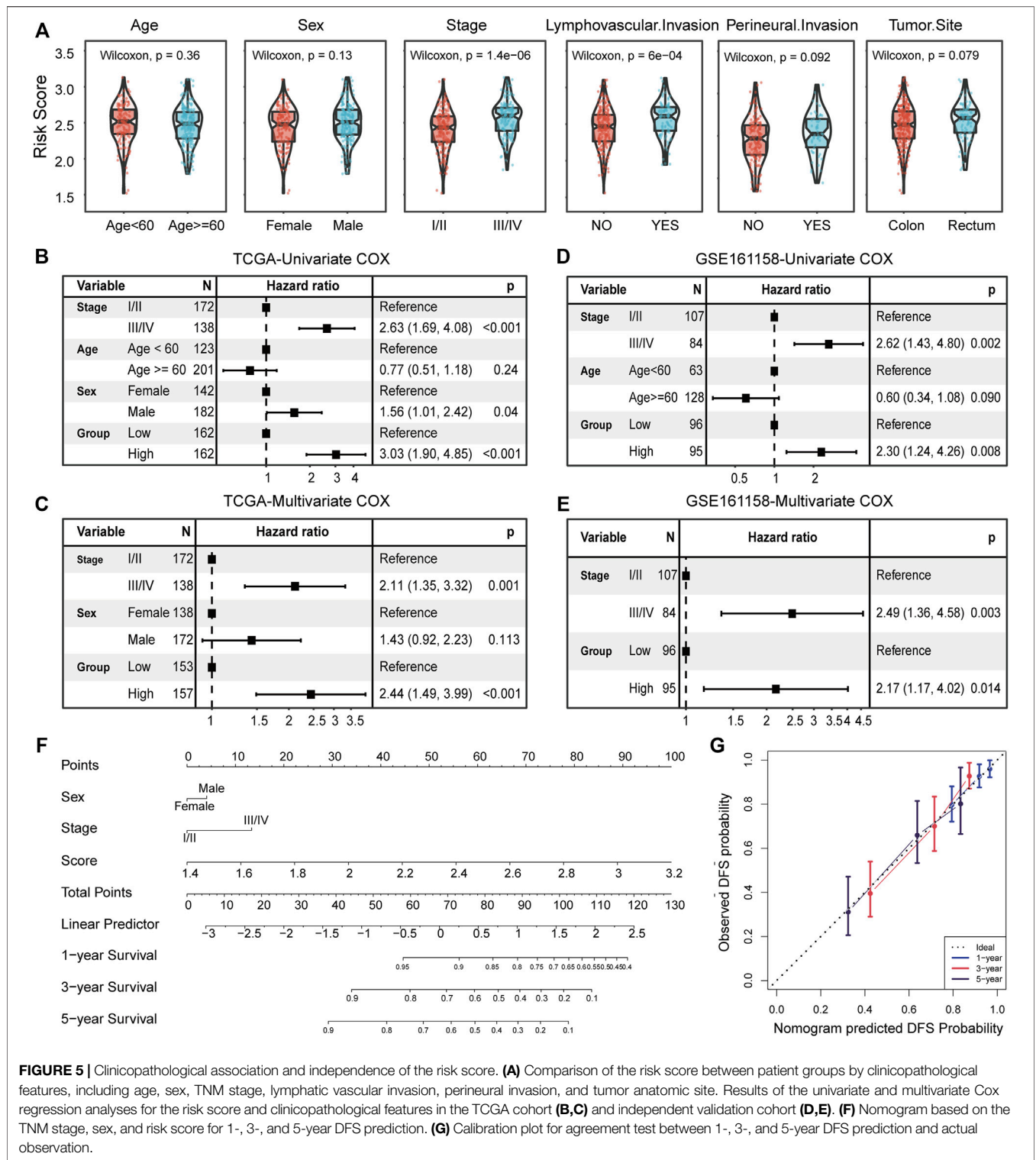
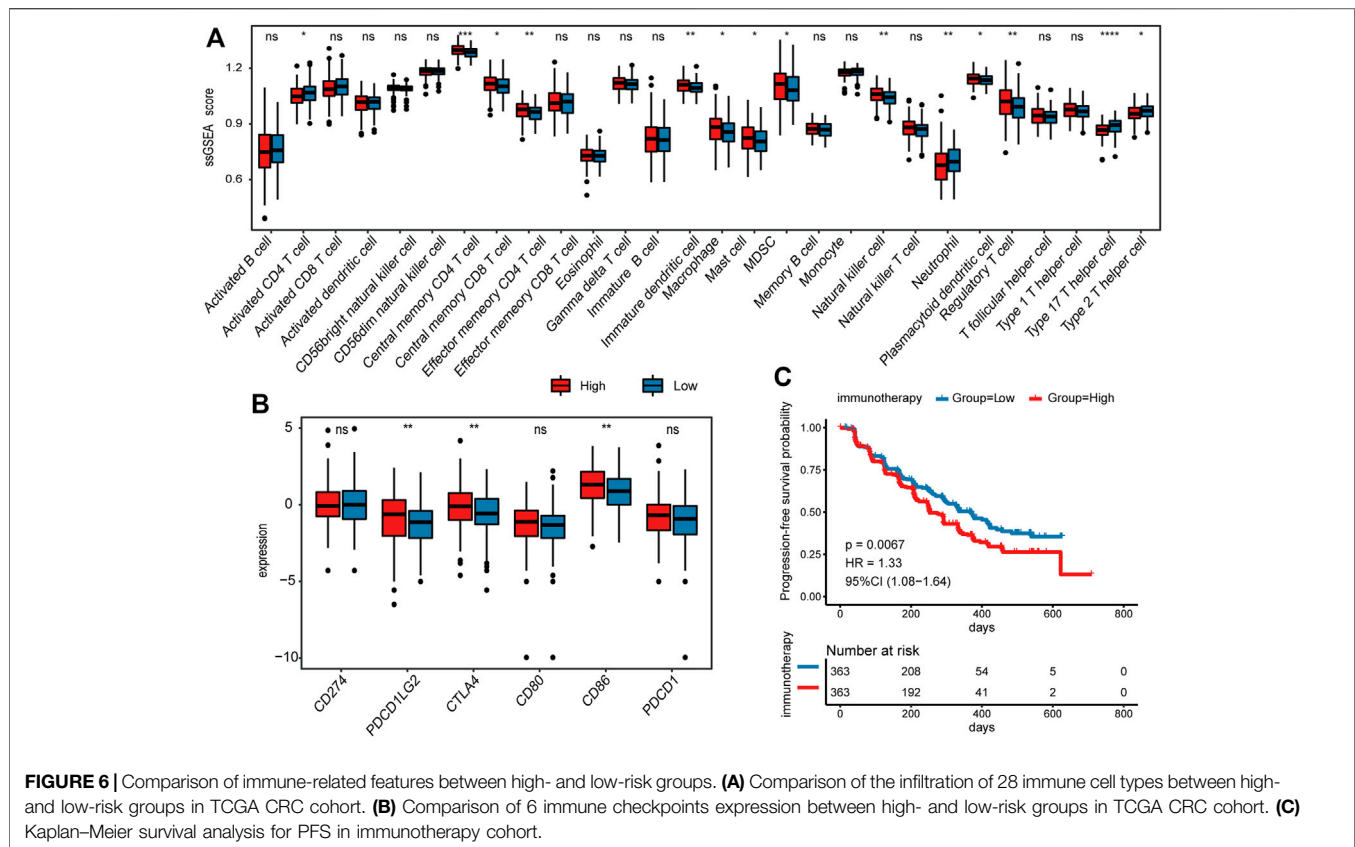


FIGURE 5 | Clinicopathological association and independence of the risk score. **(A)** Comparison of the risk score between patient groups by clinicopathological features, including age, sex, TNM stage, lymphatic vascular invasion, perineural invasion, and tumor anatomic site. Results of the univariate and multivariate Cox regression analyses for the risk score and clinicopathological features in the TCGA cohort **(B,C)** and independent validation cohort **(D,E)**. **(F)** Nomogram based on the TNM stage, sex, and risk score for 1-, 3-, and 5-year DFS prediction. **(G)** Calibration plot for agreement test between 1-, 3-, and 5-year DFS prediction and actual observation.

Univariate and multivariate Cox regression analyses were conducted to assess the independent predictive power of risk score. In the TCGA cohort, the univariate analysis indicated that TNM stage (I/II or III/IV), sex (female or male), and risk score (high or low) were significantly correlated with DFS ($p < 0.05$;

Figure 5B), especially for risk score (HR = 3.03, 95% CI 1.90–4.85, $p < 0.001$; **Figure 5B**). After adjusting for clinical factors, the risk score remained a significant independent prognostic factor (HR = 2.44, 95% CI 1.49–3.99, $p < 0.001$; **Figure 5C**). We extra reconsidered patient information



(including lymphovascular invasion, perineural invasion, venous invasion, and tumor site), and found that when we corrected these clinical factors, our risk score remained a significant independent prognostic factor (**Supplementary Figure S4B**). Correspondingly, the independent prognostic value of risk scores was also confirmed in the validation cohort (GSE161158) ($p = 0.014$; **Figures 5D,E**). In addition, a graphic nomogram based on the TNM stage, sex, and risk score was developed to predict the 1-, 3-, and 5-year survival probability of TCGA CRC patients (**Figure 5F**).

The calibration plot showed an optimal agreement between the prediction by the nomogram and actual observations (**Figure 5G**), indicating the potential of the risk score to construct a combination marker. Overall, the risk score not only serves as a new independent and robust prognostic marker but also complements the traditional TNM stage.

Comparison of Immune-Related Features Between Different Risk Groups

To further explore the differences in immune status between high- and low-risk groups, we calculated the infiltration of 28 immune cell subtypes by ssGSEA (details see Methods). The infiltration of macrophage, MDSC, and Treg were significantly increased in the high-risk group of the TCGA CRC cohort (Wilcoxon rank-sum test, $p < 0.05$; **Figure 6A**), which was consistent with their role in facilitating tumor growth, angiogenesis, invasion, as well as

metastasis (Goswami et al., 2017; Hashemi Goradel et al., 2019; Olguín et al., 2020). Moreover, immature dendritic cells, memory CD8 T cell, and memory CD4 T-cell subtypes were also increased in the high-risk group. On the contrary, infiltrations of activated CD4 T cells, neutrophils, Th17 cells, and Type 2 T helper cells were significantly reduced (Wilcoxon rank-sum test, $p < 0.05$; **Figure 6A**). Subsequently, the immune infiltration assessed by the CIBERSORT algorithm supported the above results (**Supplementary Table S6**).

In addition to immune infiltration, we also investigated the immune checkpoint molecules expressed by immune cells that regulate anti-tumor immune responses. Among the 6 immune checkpoints, *CTLA4*, *CD86*, and *PDCD1* were highly expressed in the high-risk group (Wilcoxon rank-sum test, $p < 0.05$; **Figure 6B**), suggesting that these genes may serve as key targets for anti-tumor therapy in CRC. In addition, we applied the prognostic model to 726 patients who received immunotherapy (Killock, 2020), and divided them into low- and high-risk groups. Interestingly, the results showed that high-risk scores indicated worse progression-free survival (PFS) ($p = 0.0067$; **Figure 6C**), implying the potential of platelet-related genes as prognostic markers for immunotherapy.

DISCUSSION

CRC is characterized by a high socioeconomic burden, high morbidity, and mortality (Bray et al., 2018). However, effective

prognostic biomarkers in CRC are urgently needed. Accumulated evidence demonstrated that tumor-educated platelets played an important role in tumor progression (Joosse and Pantel, 2015; Best et al., 2018). Therefore, by means of the prominent effectiveness of transcriptome analysis on clinical issues (Cieslik and Chinnaiyan, 2018; Rodon et al., 2019; Sailer et al., 2019), we explored the value of platelet-related genes in the clinical application of CRC, with particular attention to prognosis. In this study, we identified three platelet-related subtypes and specifically constructed a 10-gene prognostic model to predict DFS in CRC. Among them, many genes were discovered to have the potential to predict prognosis in CRC. For example, *TIAL1* was top significant in both univariate and LASSO Cox regression analysis and was found to be a prognostic risk factor for breast cancer (Aritake et al., 2004; Suvanto et al., 2020). However, the relationship between *TIAL1* with CRC is rarely reported. These findings provide a new perspective on CRC prognosis based on platelet-related genes. Interestingly, the risk score calculated by our prognostic model was independent of the TNM stage in multivariate Cox analyses, although it was significantly higher in advanced tumors. Moreover, the risk score was demonstrated as a complement to the traditional TNM staging by the nomograms analysis. These results suggested that the risk score may serve as a transcriptomic predictor of CRC metastasis and aid in the development of liquid blood markers for CRC in the future.

We found that platelet-related subtype 3 had significantly higher activities of angiogenesis and EMT pathway, which have been confirmed as hallmarks of cancer (Hanahan and Weinberg, 2011). Importantly, subtype 3 did have the worst survival. In addition, both subtype3 and high-risk groups had significantly higher infiltration of macrophages and NK cells. Macrophages promoted tumor progression (Goswami et al., 2017) and platelets protected tumors from NK cells with killing capacity (Schlesinger, 2018), which was consistent with previous studies. Platelets are the major source of TGF- β in the tumor microenvironment by expression of the TGF- β -docking receptor GARP, which is highly expressed by activated Tregs (Wallace et al., 2018; Kopp et al., 2009). In this study, we found that the patients with subtype3 had significantly higher levels of TGF β activities (Z-Score) and Tregs infiltration than other patients (**Supplementary Figures S6A,B**). A previous study revealed that the GARP-TGF β complex together with platelet-secreted lactate inhibited T-cell immunity against both melanoma and colon cancer (Rachidi et al., 2017; Metelli et al., 2018), which supports our study (**Supplementary Figures S6A,B**).

Previous studies have shown that Th17 cells could promote cytotoxic T-cell activation in tumor immunity, and reduce tumor growth (Martin-Orozco et al., 2009; Canderan and Dellabona, 2010). In our study, Th17 infiltration was significantly higher in

the low-risk group of CRC patients with a good prognosis, which is in line with previous studies. The immune checkpoints *CTLA4*, *CD86*, and *PDCD1LG2* were found highly expressed in the high-risk group. Together, these findings suggested that platelet-related subtypes or risk groups have intricate mechanisms that contribute to tumor progression and thus affect survival in patients with CRC. In this study, some shortcomings and prospects should be addressed. This study was completely based on public databases, and some key genes or results need to be externally validated by further experiments. For example, the biological functions of the 10 predictor genes, the relationship between risk scores, and the clinical benefits of antiplatelet agents in CRC need to be verified in the future.

In conclusion, our study firstly revealed a significant association between platelet-related genes and prognosis in CRC. Three platelet-related subtypes were identified and a platelet-related prognostic model was constructed as an independent prognostic risk predictor in CRC. Validation of the external dataset further confirmed the independence and predictive performance of this model. This study deepens our understanding of platelet-related genes in CRC and provides new potential prognostic and therapeutic biomarkers.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

PW conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft. WZ and HC prepared figures and/or tables and approved the final draft.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fgene.2022.904168/full#supplementary-material>

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