

Developing a predictive model for evaluating the efficacy of disulfidptosis-related biomarkers in colon adenocarcinoma

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Abstract: Background: This study investigated colon adenocarcinoma (COAD), one of the most common types of cancers worldwide. In recent years, a novel cell death pathway, hydrogen sulfide poisoning, has been identified and targeting disulfide reductase has emerged as a new strategy for cancer treatment. However, the predictive potential of disulfidptosis-related genes (DRGs) in COAD and their characteristics in the tumor immune microenvironment (TIME) remain to be further elucidated.

Methods: This study obtained DRGs transcriptome and mutation data of COAD samples were obtained from the Tissue Cancer Genome Atlas (TCGA) database. Pearson and differential expression correlation analysis were used to identify COAD-related DRGs and a risk prognosis model for DRGs was constructed using the univariate least absolute shrinkage and selection operator (LASSO) and Cox regression analysis. Enrichment analysis was then conducted to explore the potential biological functions and signal transduction of differentially expressed genes associated with the model. The reliability of the model was validated through various statistical analyses such as survival analysis, receiver operating characteristic (ROC) curves, calibration plots, and bar graphs. The relationship between the prognostic model, immune microenvironment, and drug sensitivity was examined.

Results: We successfully established a risk prognostic model containing 6 DRGs (RPA2, TIMP1, WDR1, POLR3K, KTI12, and RTKN). This model performed well in predicting the overall survival of patients with COAD. Validation of this model through Cox analysis and clinical indicators showed considerable potential for predicting the prognosis of patients with COAD. Furthermore, there was a significant correlation between the DRGs prognostic model and tumor microenvironment (TME), immune infiltrating cells, and drug sensitivity ($p < 0.05$). HPA and experimental results verified that the expression levels of RPA2, TIMP1, POLR3K, KTI12, and RTKN in COAD tumors were higher than those in normal tissues, while the expression levels of WDR1 were opposite ($p < 0.01$).

Conclusion: This study constructed a risk model and identified 6 DRGs as potential molecular therapeutic targets for COAD. The prognosis and immune therapeutic response of patients with COAD are related to DRGs, and targeted therapy for DRGs may provide a new research direction for the diagnosis and

treatment of COAD.

Keywords: Disulfidptosis, Colon Adenocarcinoma, Molecular subtypes, Prognostic Markers, Tumor microenvironment

1. Introduction

Cancer is a multifactorial, multigenic disease, with its occurrence and progression involving various biological mechanisms(1–3)which can guide treatment decisions. However, there is still a lack of consensus regarding the optimal molecular markers or tests to use in clinical practice. Integrating gene expression data with clinical and histopathologic parameters enhances our understanding of disease processes, facilitates the identification of precise prognostic predictors, and supports the development of effective therapeutic strategies. The purpose of this study is to apply an integrated approach that combines morphological, clinical and bioinformatic data to reveal effective regulators of proliferation. Whole slide images generated from hematoxylin and eosin-stained sections of The Cancer Genome Atlas (TCGA. COAD arises from the colon epithelium and is the third most common cancer globally, causing a significant number of deaths every year and posing a serious challenge to global health(4). The incidence of COAD is continuously increasing worldwide, especially in the Asian region. According to the World Health Organization's prediction, the global number of COAD cases is expected to rise to 1.5 million by 2030(5). Due to the difficulty in detecting early symptoms, most patients are already in the advanced stage of the disease by the time of diagnosis, missing the opportunity for curative surgery(6). The current treatment options for COAD mainly include surgery, radiotherapy, and immunotherapy. Neoadjuvant chemotherapy combined with surgical resection and adjuvant chemotherapy after surgery are the primary treatment modalities for COAD(7)and a prognostic model was created based on 6 DRLs (AP003555.1, AL683813.1, SNHG7, ZEB1-AS1, AC074212.1, RPL37A-DT. Despite advances in COAD treatment in recent years, improving patient survival rates, the complexity, and instability of the genome have a significant impact on clinical treatment, resulting in low survival rates for metastatic and recurrent COAD patients, posing significant challenges to treatment. Currently, the TNM staging system is widely used to evaluate patient prognosis; however, even within the same stage, there are significant differences in patient survival rates, indicating limitations in predicting survival time with this assessment system. Therefore, in-depth research into the pathogenesis of COAD and a better understanding of its origin and progression is crucial for formulating more effective treatment strategies.

Most cancer cells primarily absorb extracellular cystine, a form of cysteine, through the cystine/glutamate antiporter, which is composed of the catalytic subunit SLC7A11 and the accessory subunit SLC3A2. Subsequently, the cells reduce cystine to cysteine and provide it to the cells. Recent studies have shown that overexpression of SLC7A11 under glucose starvation conditions promotes

cell death. Mechanistically, glucose starvation leads to insufficient nicotinamide adenine dinucleotide phosphate (NADPH) supply, hindering the reduction of cystine to cysteine. This results in the formation of numerous disulfide bonds between actin molecules, triggering disulfide stress(8). Subsequently, disulfide stress activates the seven subunits of the Rac/WAVE regulatory complex (WRC), interfering with actin-related protein signaling pathways, causing disruption of the cellular cytoskeleton, and ultimately inducing disulfide-mediated cell death (9)and the use of bioactive compounds has emerged as a novel and safe approach to solve this problem. Here, we discuss the information available related to phlorotannins, a type of polyphenol present in brown seaweeds that reportedly functions as antioxidants/pro-oxidants and anti-inflammatory and anti-tumorigenic agents. Specifically, available evidence indicates that dieckol and phloroglucinol promote mitochondrial membrane depolarization and mitochondria-dependent apoptosis. Phlorotannins also reduce pro-tumorigenic, -inflammatory, and -angiogenic signaling mechanisms involving RAS/MAPK/ERK, PI3K/Akt/mTOR, NF- κ B, and VEGF. In doing so, they inhibit pathways that favor cancer development and progression. Unfortunately, these compounds are rather labile and, therefore, this review also summarizes approaches permitting the encapsulation of bioactive compounds, like phlorotannins, and their subsequent oral administration as novel and non-invasive therapeutic alternatives for cancer treatment.”,”container-title”:”Antioxidants”,”DOI”:”10.3390/antiox12091734”,”ISSN”:”2076-3921”,”issue”:”9”,”language”:”en”,”license”:”http://creativecommons.org/licenses/by/3.0/”,”note”:”number: 9\npublisher: Multidisciplinary Digital Publishing Institute”,”page”:”1734”,”source”:”www.mdpi.com”,”title”:”Phlorotannins: Novel Orally Administrated Bioactive Compounds That Induce Mitochondrial Dysfunction and Oxidative Stress in Cancer”,”title-short”:”Phlorotannins”,”volume”:”12”,”author”:[{“family”:”Simón”,”given”:”Layla”},{“family”:”Ara-zo-Rusindo”,”given”:”Migdalia”},{“family”:”Quest”,”given”:”Andrew F. G.”},{“family”:”Mariotti-Celis”,”given”:”María Salomé”}],”issued”:{“date-parts”:[[“2023”,9]]}],”schema”:”https://github.com/citation-style-language/schema/raw/master/csl-citation.json”} . Currently, research on disulfide-mediated cell death mainly focuses on cancer cell lines with high expression of SLC7A11 and under glucose starvation conditions (10,11). In the cytoplasm of cancer cells, the formation of harmful disulfide bonds constitutes the fundamental process underlying disulfide-induced cell death. Disulfide stress represents a crucial step in the induction of disulfide-mediated cell death.

Unintentional cell death occurs as an uncontrolled process under accidental injury stimuli, whereas regulated cell death is a controlled process governed by biological signaling pathways(12). Liu et al. (13) proposed a new form of cell death named disulfidptosis, which differs from the traditional cell death mechanisms such as apoptosis, necroptosis, ferroptosis, and pyroptosis. Their study revealed that cancer cells with high expression of SLC7A11 continuously accumulate disulfides during glucose starvation, triggering a cellular stress response

to disulfides, leading to an elevation in disulfide bond content within the actin cytoskeleton, promoting actin filament contraction, ultimately disrupting the cellular cytoskeletal structure and resulting in cell death. The latest research findings underscore the significant role of disulfide-driven cell death in the onset and progression of tumors and indicate its potential as a novel molecular target.

With the rapid development of bioinformatics and chip technology, DRG has emerged as a critical player in genetic and biological processes such as pre-transcriptional and post-transcriptional regulation and epigenetic control, making it a focal point in microscopic disease research(14)the prognostic significance of disulfidptosis-related genes (DRGs. Numerous studies indicate that the abnormal expression or function of DRG is associated with various human diseases. Differential expression of DRGs has been observed to be closely associated with the occurrence, development, recurrence, and metastasis of many tumors. Through an in-depth investigation of the mechanisms of action of DRGs, we can gain a deeper understanding of their role in the initiation and progression of cancer and other diseases(15,16)000 new cases annually. Disulfidptosis, a newly discovered programmed cell death process, is linked to the actin cytoskeleton, which plays a vital role in maintaining cell shape and survival. The role of disulfidptosis is poorly depicted in the clear cell histologic variant of RCC (ccRCC. DRGs have emerged as a new frontier in tumor research, with the identification of diagnostic markers and prognostic assessments of DRG in COAD being one of the current focal points.

The focus of this study is to explore personalized treatment methods for COAD using machine learning techniques. We constructed a prognostic model of COAD based on DRGs utilizing the latest data from The TCGA database. We assessed the expression patterns of DRGs, the infiltration of the TME, and the molecular mechanisms underlying COAD prognosis. The results of this study provide new insights into the pathogenesis of COAD and its implications for treatment.

2. Materials and methods

2.1. Experimental method

Fig.1 illustrates the experimental design workflow of this study.

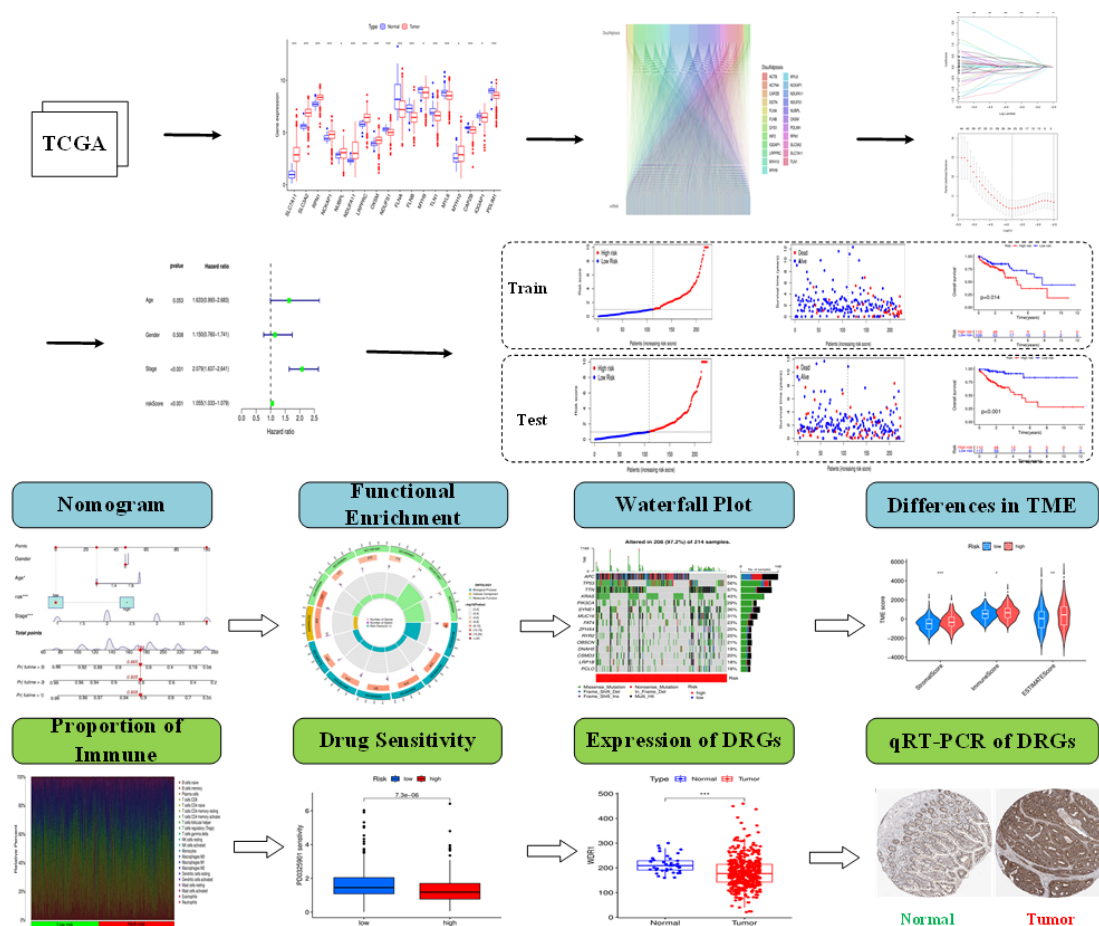


Figure 1. Experimental design

2.2. Data collection and processing

On January 3, 2025, we downloaded the transcriptome and somatic mutation data of COAD patients from the TCGA database (<https://portal.gdc.cancer.gov/repository>). We collected clinical information from 41 normal samples and 476 colorectal cancer tissue samples, including gender, age, TNM staging, tumor staging, etc.(17). Using the Perl programming language, we extracted the normalized RNA sequence data. We stored it in the format of fragments per kilobase of transcript per million mapped reads (18)the specific functions of lncRNAs in relation to OsRpp30-mediated disease resistance in rice remain elusive. We conducted a comprehensive analysis of lncRNAs, miRNAs, and mRNAs expression patterns in wild type (WT. Additionally, we employed the Strawberry Perl programming language to gather clinical sample pathology data (19)there has been no systematic study of disulfidptosis-related long noncoding RNAs (DRLs.

2.3. Construction and validation of a predictive model for DRGs

To screen DRGs associated with colorectal adenocarcinoma, we performed a Pearson correlation analysis between 23 DRGs and 5128 DRGs. During the

screening process, Pearson correlation coefficients with absolute values greater than 0.5 and p-values less than 0.001 were used as the thresholds. The patients participating in the study were randomly assigned to the experimental and control groups (Table 1), with no statistically significant differences in clinical characteristics between the two groups. Univariable Cox regression analysis was used in this study to assess DRGs associated with prognosis. To ensure data consistency, COAD patients in the TCGA-COAD dataset were randomly divided into training and testing cohorts at a ratio of 1:1 based on clinical data features. Compared with support vector machine (SVM), random forest, ridge regression, etc. LASSO can perform variable selection and regularization, thereby improving prediction accuracy(13). Genes identified through LASSO assist in calculating the risk score for each patient, and aggregation of gene expression levels with corresponding coefficients establishes the disulfidptosis prognostic signature, where $DRGs = \text{Sum}(\text{gene expression} \times \text{corresponding coefficient})$. We performed LASSO Cox regression analysis on overall survival (OS) and conducted 10-fold cross-validation to select DRGs related to CRC and with prognostic value. This method can compress coefficients of irrelevant variables in the model, thus enabling a more precise selection of DRGs associated with prognosis. Finally, we utilized the Cox regression analysis method to establish a prognostic model. We assessed their risk scores by calculating a statistical summation of the DRGs expression levels and their respective coefficients for each patient.

We used the DRGs risk score to predict patients' prognosis risk and divided the samples into high- and low-risk groups based on the median risk score. Kaplan-Meier curve analysis for progression-free survival (PFS) and OS was performed using the R packages "survminer" and "survival" in the testing and training cohorts. These curves allowed for an intuitive representation of the survival differences between different risk groups, and the survival differences between high- and low-risk groups were also calculated. We employed the "ggplot2" and "Rtsne" R packages to generate scatter plots, risk curves, and heatmaps to visualize the COAD-associated mortality risk and the risk distribution among patients of different risk groups. In order to effectively reveal the differences between different risk groups, t-SNE visualization and principal component analysis (PCA) methods were employed. Furthermore, we evaluated the accuracy of the model in predicting 1-, 3-, and 5-year survival rates by plotting receiver operating characteristic (ROC) curves and calculating the corresponding AUC values.

2.4. Functional enrichment analysis in COAD

The gene set enrichment analysis (GSEA) method developed by Subramanian et al. was used to interpret the gene expression data(20)and novel biomarkers are urgently required to guide its prognosis. Material and methods: RNA-Seq data of COAD were downloaded through The Cancer Genome Atlas (TCGA. This method leverages shared biological functions, genomic focal points, chromosomal positions, or regulatory features to unveil the functionality and potential roles

of gene sets. We employed the R software package “ClusterProfiler” for functional enrichment (GO) analysis of six DRG. GSEA confirmed multiple significantly enriched pathways between the high- and low-risk groups.

2.5. Screening of tumor immune microenvironment and functional evaluation of immune cells

Cell composition analysis was performed using the Limma R package to compare cellular infiltration levels between high- and low-risk groups. Differences in immune cell, stromal cell, and ESTIMATES scores between the two groups were compared using the Wilcoxon rank-sum test. The CIBERSORT algorithm was employed to calculate the proportion of immune cell infiltration in COAD samples, allowing for an in-depth exploration of differences in TME between high- and low-risk groups and the correlation between immune cell and risk scores. Bubble plots were generated using algorithms such as Cibersortabs, TIMER, CIBERSORT, and EPIC. Furthermore, using a significance level of $p < 0.05$ as the selection criterion, immune cell infiltration was analyzed for survival using the R packages, with Kaplan-Meier curves being plotted. In addition, immune cell infiltration and immune-related functions were scored in COAD samples, and box plots were generated to analyze differences between low- and high-risk groups.

2.6. Statistical analysis

In this study, we first quantitatively analyze the selection of molecules and variables using methods such as LASSO regression and univariate and multivariate Cox regression. Subsequently, we employed the Kaplan-Meier method to plot survival curves and assessed intergroup differences using the log-rank test. Furthermore, we analyze differences between the high-risk and low-risk groups and the differences in clinical phenotype risk scores using the Wilcoxon rank-sum test. Finally, we used the chi-squared test to analyze differences in the distribution of clinical phenotypes between the high-risk and low-risk groups. It is noteworthy that all statistical analyses in this study were conducted using R software version 4.3.1, with $p < 0.01$ considered statistically significant.

3. Results

3.1. Disulldptosis-related DRGs Analysis

The DRGs expression levels in each COAD patient were determined by downloading and analyzing RNA sequencing data from 476 COAD and 41 normal tissues in the TCGA database. The patients were then randomly divided into training groups and test groups (Table 1). As show in Fig. 2A and B, the results showed that SLC7A11, RPN1, SLC3A2, NUBPL, NDUFA11, LRPPRC, NCKAP1, OXSM, MYH9, MYH10, and QGAP1 were upregulated in COAD, and NDUFS1, FLNA, FLNA, LN1, MYL6, CAPZB, and PDLIM1 were down-

regulated in COAD. Co-expression analysis was performed in the COAD cohort, and 5128 genes associated with disulfide death were identified through Pearson correlation analysis. We selected significant factors based on correlation coefficients $>|R|=0.5$ and $p<0.001$. Upon examination of **Figure 2C**, we observed a co-expression relationship between DRGs and DRGs. Based on the median risk score, we divided the entire cohort, training set, and test set into high- and low-risk groups. No significant differences in clinical characteristics were observed between the two groups. LASSO and Cox regression analysis was performed on COAD patients using the R software package “glmnet” to screen DRGs with the highest prognostic value (**Fig. 2D** and **E**). By introducing the multivariate Cox proportional hazard model, we identified six DRGs related to disulfides. Based on the expression levels of these six DRGs, we used the following formula to calculate the risk assessment for each sample: $RPA2 \times (1.88) + RTKN \times (1.28) + KTI12 \times (-1.48) + POLR3K \times (-1.06) + TIMP1 \times (0.62) + WDR1 \times (-1.41)$. Additionally, we created a heat map containing these six central DRGs (**Fig. 2F**). To further determine whether the risk score prognostic model is applicable to other cohorts of COAD patients, we used two external datasets (GSE33133 and GSE39582) to validate our research findings. As shown in Fig.3, the AUC value in the ROC curve analysis of all validation datasets is above 0.6. The external validation results indicate that these 6 genes can be used to evaluate the prognosis of COAD patients.

Table 1. Clinicopathological characteristics of COAD

Name	Type	Total N=448	Test n=244	Train n=244	P
Age	<=60	133(29.69%)	63(28.12%)	70(31.25%)	0.535
Age	>60	315(70.31%)	161(71.88%)	154(68.75%)	
Gender	FEMALE	214(47.77%)	99(44.2%)	115(51.34%)	0.156
Gender	MALE	234(52.23%)	125(55.8%)	109(48.66%)	
Stage	Stage I	75(16.74%)	32(14.29%)	43(19.2%)	0.3531
Stage	Stage II	176(39.29%)	89(39.73%)	87(38.84%)	
Stage	Stage III	124(27.68%)	63(28.12%)	61(27.23%)	
Stage	Stage IV	62(13.84%)	36(16.07%)	26(11.61%)	
Stage	unknown	11(2.46%)	4(1.79%)	7(3.12%)	0.5285
T	T1	10(2.23%)	3(1.34%)	7(3.12%)	
T	T2	76(16.96%)	36(16.07%)	40(17.86%)	
T	T3	305(68.08%)	155(69.2%)	150(66.96%)	
T	T4	56(12.5%)	30(13.39%)	26(11.61%)	
T	Tis	1(0.22%)	0(0%)	1(0.45%)	0.4052
M	M0	330(73.66%)	161(71.88%)	169(75.45%)	
M	M1	62(13.84%)	36(16.07%)	26(11.61%)	
M	MX	49(10.94%)	25(11.16%)	24(10.71%)	
M	unknown	7(1.56%)	2(0.89%)	5(2.23%)	0.7249
N	N0	266(59.38%)	129(57.59%)	137(61.16%)	
N	N1	102(22.77%)	54(24.11%)	48(21.43%)	
N	N2	80(17.86%)	41(18.3%)	39(17.41%)	

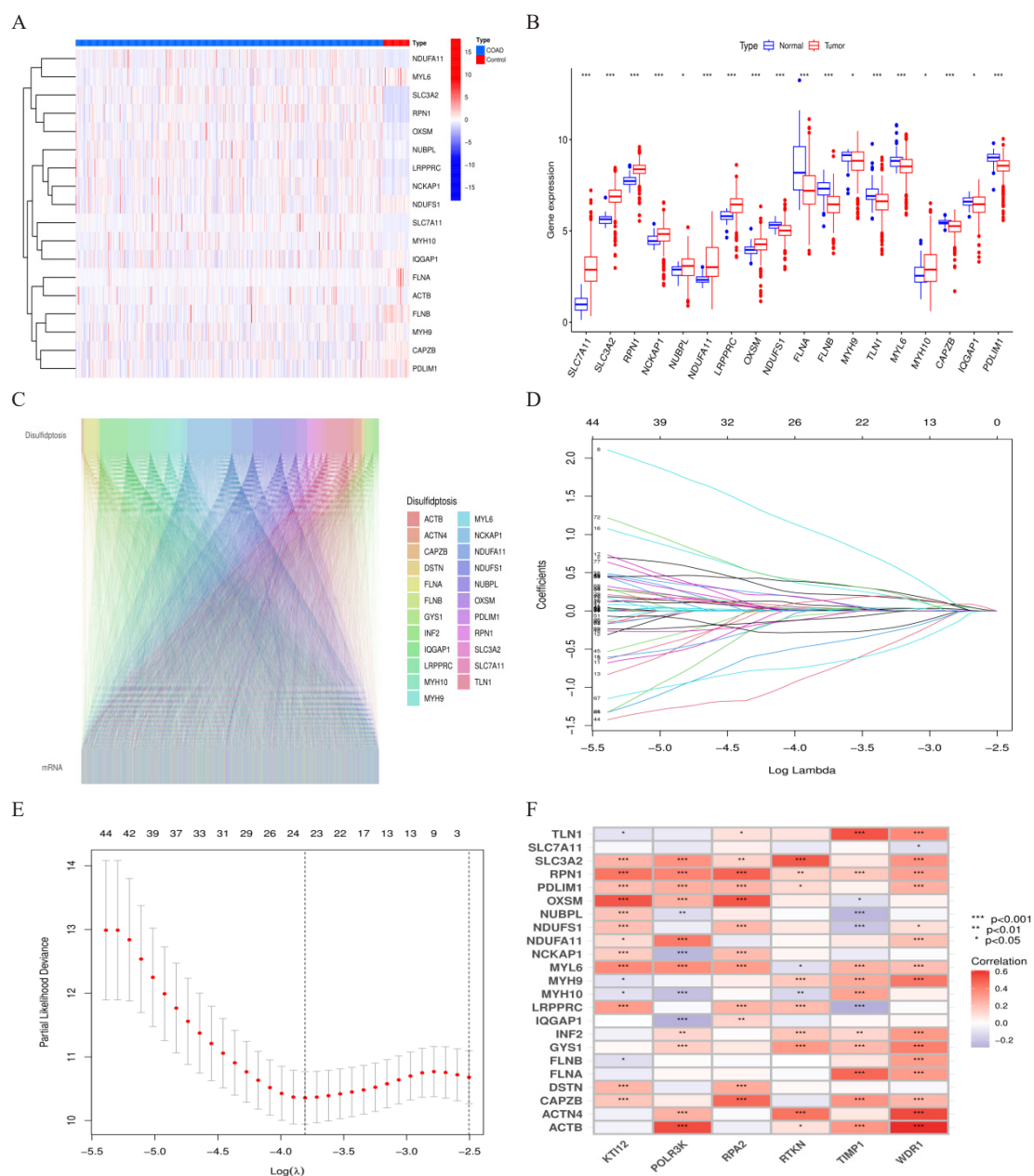


Figure 2. Construction of the DRGs prognostic signature in COAD. (A) 11 DRGs expression levels in the heatmap. (B) Different expression of 11 disulfide bond-related genes in COAD patients and normal people. (C) Sankey plot of disulfide bond associated DRGs. (D) Forest tree expression of six different B in low-risk and high-risk groups. (E) LASO regression screened DRGs at the minimum point of cross-validation. (F) Heat map between disulfide sag-related DRGs.

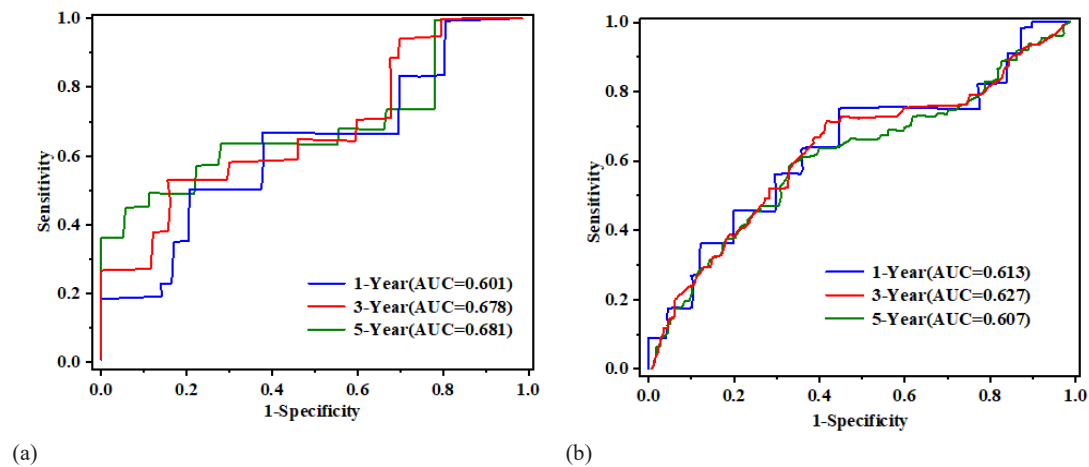


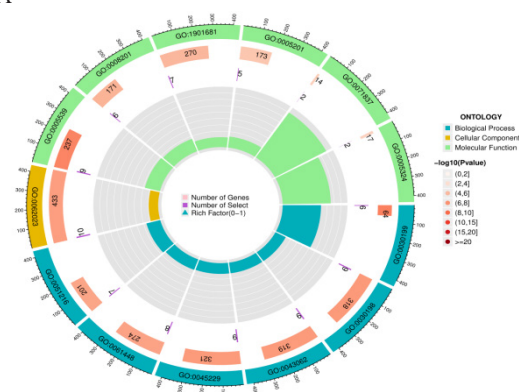
Figure 3. ROC results validated externally using two datasets. (a) GES33133 (b) GES39582.

3.2. Functional enrichment analysis

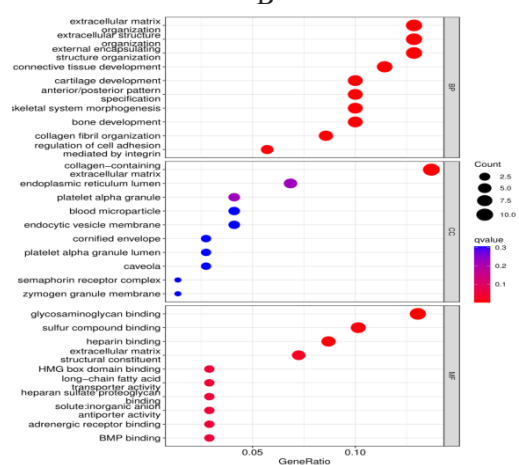
We conducted a thorough investigation into the molecular mechanisms of DEGs and their roles in the occurrence and development of cancer. The GO analysis revealed significant enrichment of these DEGs in cellular components and molecular functions (**Fig.4A**). We further explored the molecular mechanism of DRGs and their impact on COAD development. GO analysis results demonstrated that collagen-containing extracellular matrix was enriched with differentially expressed genes at the extracellular matrix component level and the molecular function level for glycosaminoglycan binding (**Fig.4B**). Additionally, the KEGG enrichment analysis results show enrichment of these genes in pathways-related to phagosome (**Fig.4C**).

We investigated in depth the molecular mechanism of DRGs associated with disulfide bonds, exploring their impact on the occurrence and progression of COAD. The GSEA analysis showed significant enrichment of pathways related to phagocytosis recognition and B cell receptor signaling pathway in the high-risk group (**Fig.4D**). Specifically, the high-risk group displayed significant enrichment for the ECM receptor interaction pathway (**Fig.4F**). In contrast, in the low-risk group, functional pathways related to Spliceosomal TRL snRNP complex assembly Y exhibited significant enhancement (**Fig.4E**). Additionally, pathways such as citrate cycle TCA cycle and oxidative phosphorylation also showed significant enrichment in the low-risk group (**Fig.4G**).

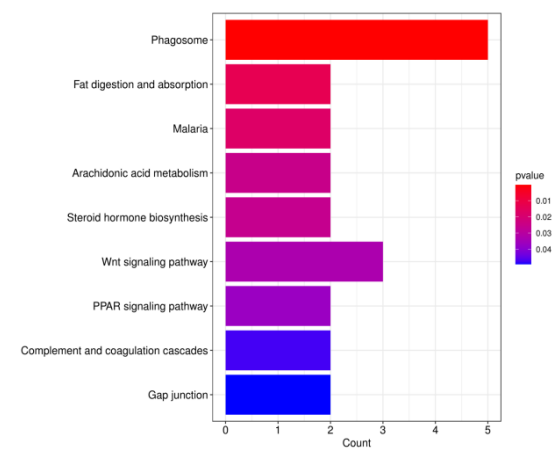
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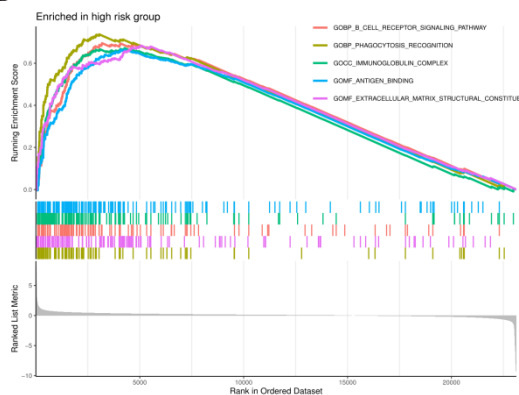
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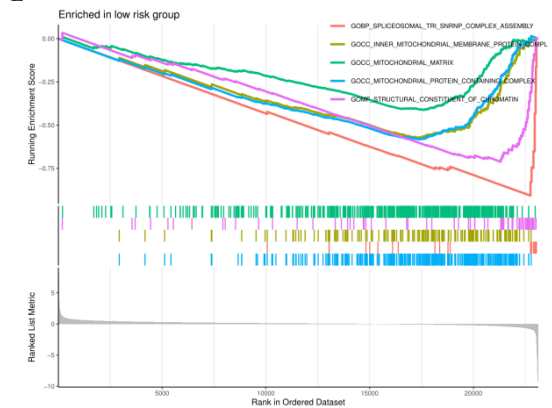
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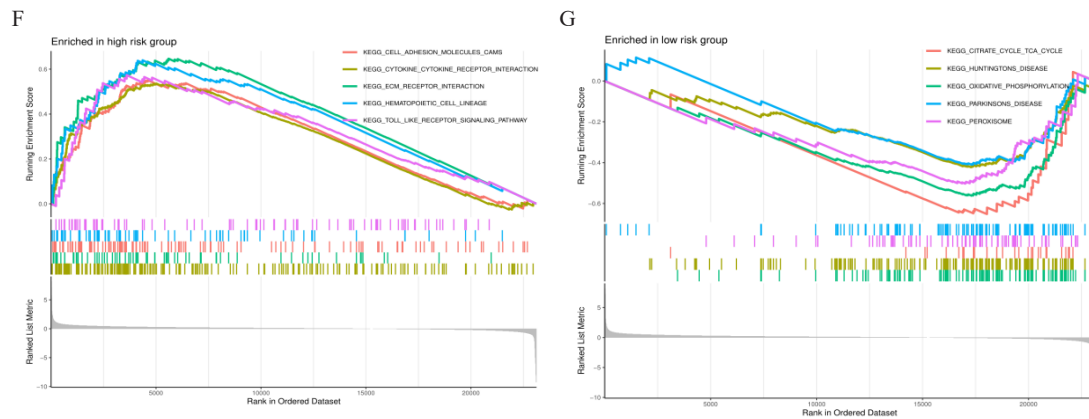


Figure 4. Functional enrichment analysis. (A) GO analysis for DEGs. (B) The pathways obtained by KEGG analysis were visualized in bubble plots. (C) KEGG pathway enrichment analysis. (D) GSEA functional analysis in high-risk patients. (E) GSEA functional analysis in low-risk patients. (F) GSEA pathway analysis in high-risk patients. (G) GSEA pathway analysis in low-risk patients.

3.3. Construction and validation of a risk model for COAD

In this study, a prognostic risk assessment model for colorectal cancer was constructed, and risk scores were calculated for 476 tumor samples, using a median risk value of 0.96 as the threshold to categorize patients into high- and low-risk groups. We generated risk score curves for six key DRGs, and heatmaps and scatter plots showing the risk scores, expression levels, and survival status of the key DRGs in the two patient groups are presented (Fig.5). The study demonstrated that, in the training, testing, and entire datasets, the survival rate of patients in the high-risk group continuously decreased as the disease progressed (Fig.5A, B and C). This indicates that the model has good predictive ability for the prognosis of patients with colorectal cancer.

Fig.5D, E, and F show that patients in the high-risk group had higher mortality rates compared with patients in the low-risk group. The heatmap displayed the expression levels of six DRGs (RPA2, TIMP1, WDR1, POLR3K, KT112, and RTKN) in the low-risk and high-risk groups (Fig.5G, H and I). The high expression levels of five genes (RPA2, TIMP1, WDR1, POLR3K, and RTKN) in the high-risk group suggest their potential as risk factors for colorectal cancer patients, closely associated with poor prognosis. On the contrary, the low expression level of the KT112 gene suggests its potential as a protective factor for colorectal cancer patients. The significant difference in gene expression between the high- and low-risk groups suggests that the accuracy of the prognostic assessment model was validated.

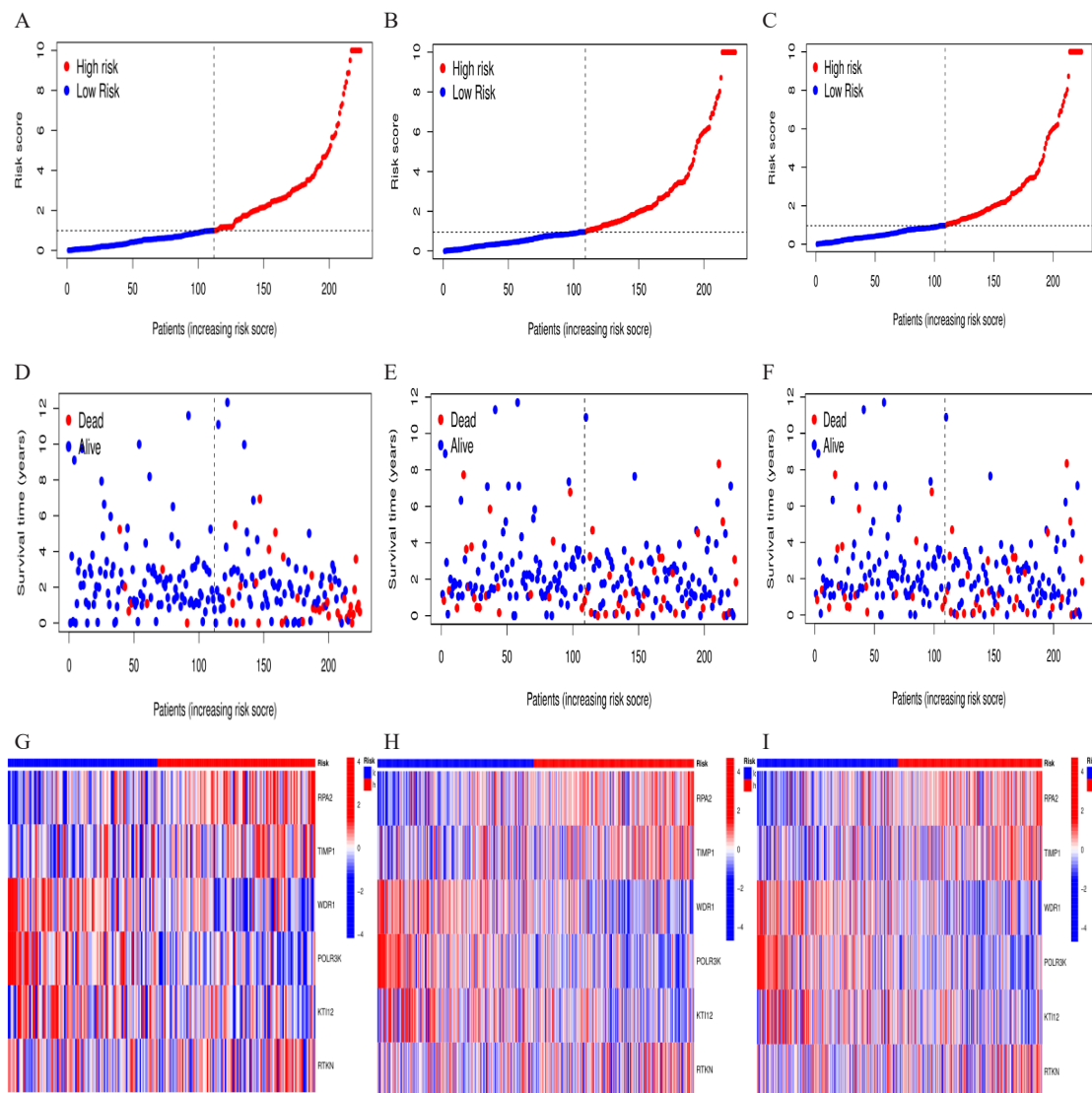


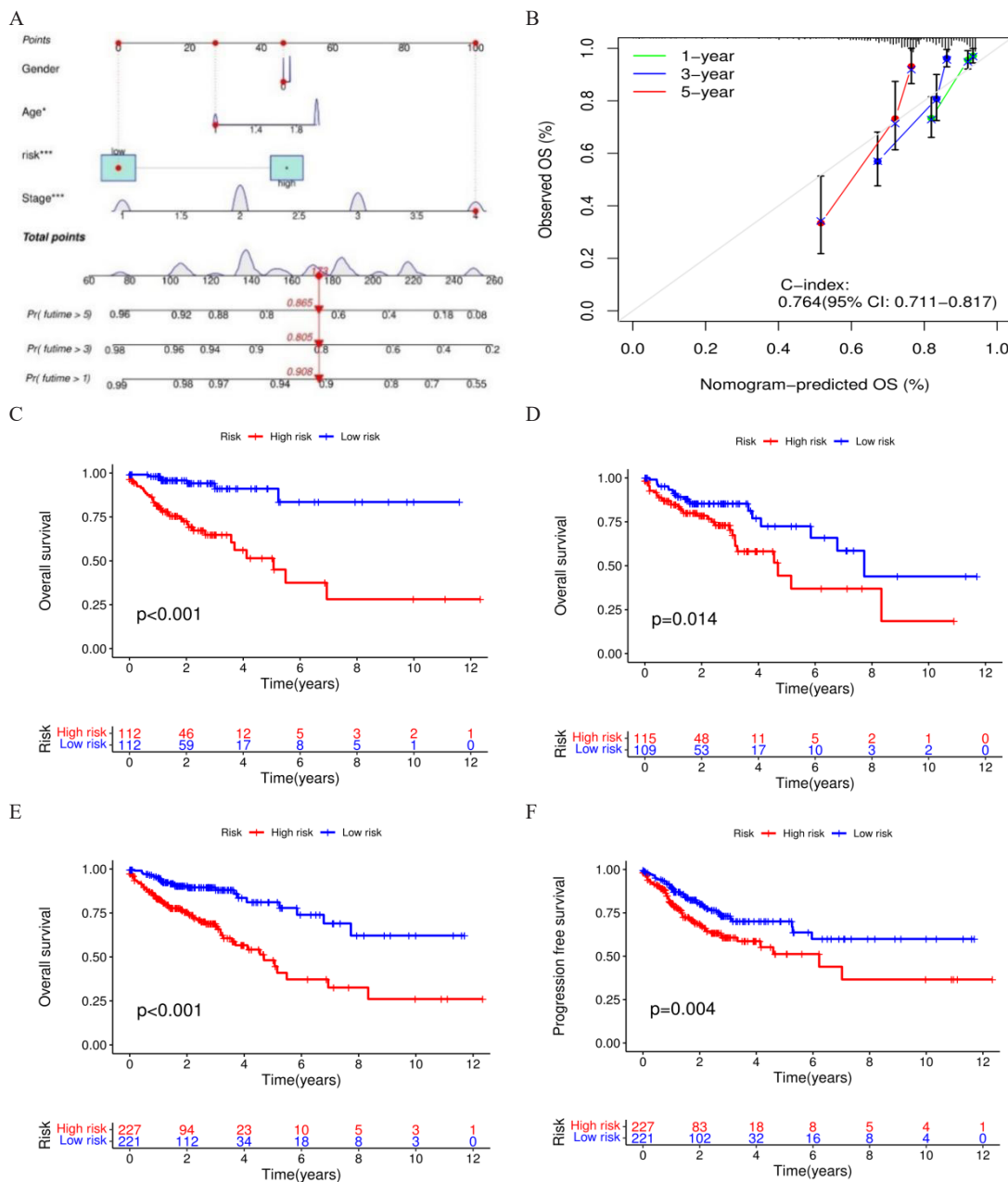
Figure 5. Predicting the performance of characteristics. (A, D, E) The risk score, survival status distribution, and expression level of patients in the training set. (B, E, H) The risk score, expression level, and survival status distribution of patients in the test set. (C, F, I) The risk score, expression level, and survival status distribution of patients in a complete set.

3.4. Subgroup and clinical feature prediction plots of Kaplan Meier survival curves for COAD

Given the strong predictive ability of the risk score, we decided to combine it with clinical information to construct the survival curve. We validated the survival curves in a randomly selected cohort of patients, which showed 1-year, 3-year, and 5-year survival rates of 0.865, 0.805, and 0.908, respectively (Fig.6A). The model demonstrated good predictive performance, as indicated by the C-index of 0.764 in the B curve (Fig.6B).

After constructing a multivariable Cox regression model to identify prognostic features, we further investigated the prognostic potential of six DEGs in colorectal cancer. In addition, according to the results of the survival analysis, the

OS and PFS of patients in the low-risk group were significantly better than those in the high-risk group (**Fig.6C-F**). Additionally, according to the OS results of the clinically staged patient model, the model is applicable to early and mid-to-late-stage COAD patients (**Fig. 6G, H**). The results showed significant statistical differences ($p < 0.05$).



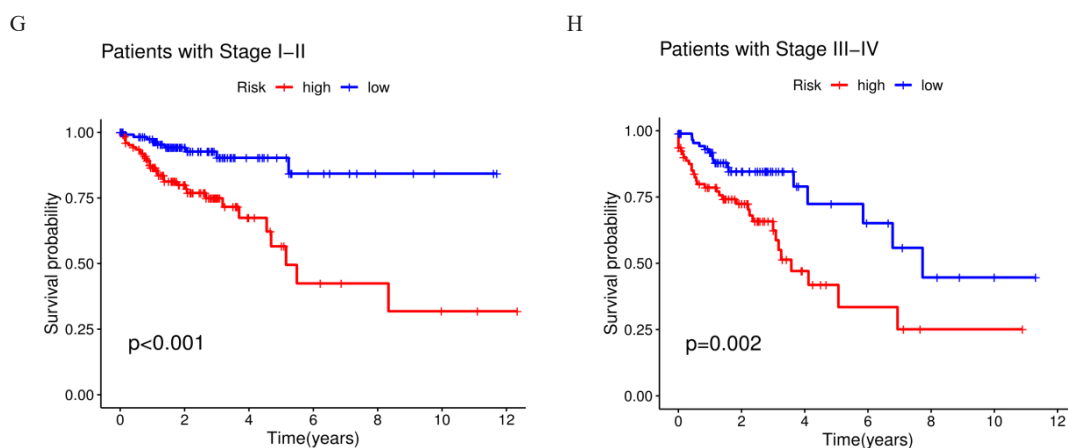
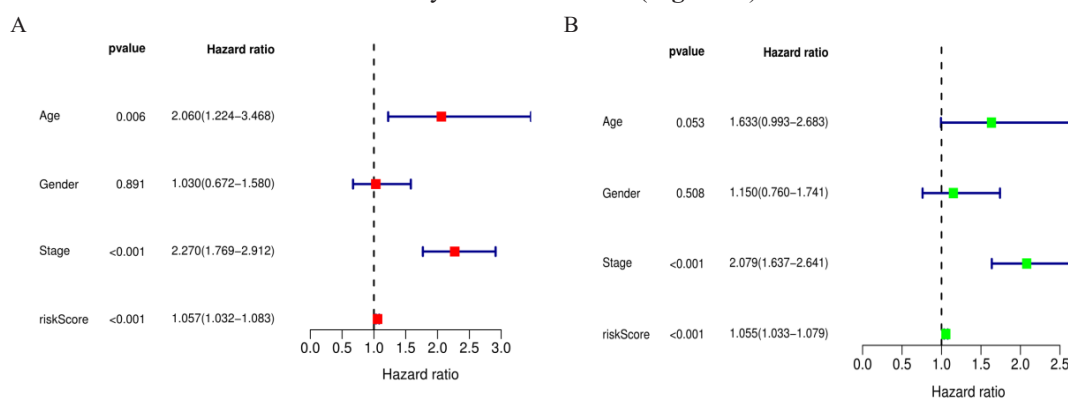


Figure 6. Kaplan-Meier survival analyses of COAD patients. (A) Column line graphs of OS. (B) Calibration curve at different times. (C) Testing set in OS. (D) The training is set in OS. (E) All set in OS. (F) All set in PFS. (G, H) Survival plots depicting the outcomes of COAD patients in both the initial and advanced disease stages.

3.5. Independent analysis of prognostic factors in COAD

Multivariate Cox regression analysis can be used to identify the risk markers as independent prognostic factors. To assess the predictive capacity of the COAD model, we performed the Cox regression analysis. The Cox regression result presented in **Fig.7A** and **B** shows that the risk score ($p < 0.01$), and the HRs were 1.057 (1.032-1.083) and 1.055 (1.033-1.079), respectively. This result proved that the risk markers were independent prognostic factors in COAD patients regardless of age and gender. The ROC curve showed that the risk score is the optimal indicator for predicting prognosis (**Fig.7C**), indicating that risk prediction has reliable diagnostic significance. The results of time-dependent ROC analysis showed that this feature had good predictive ability for survival in different years in all cohorts (**Fig.7D-F**).



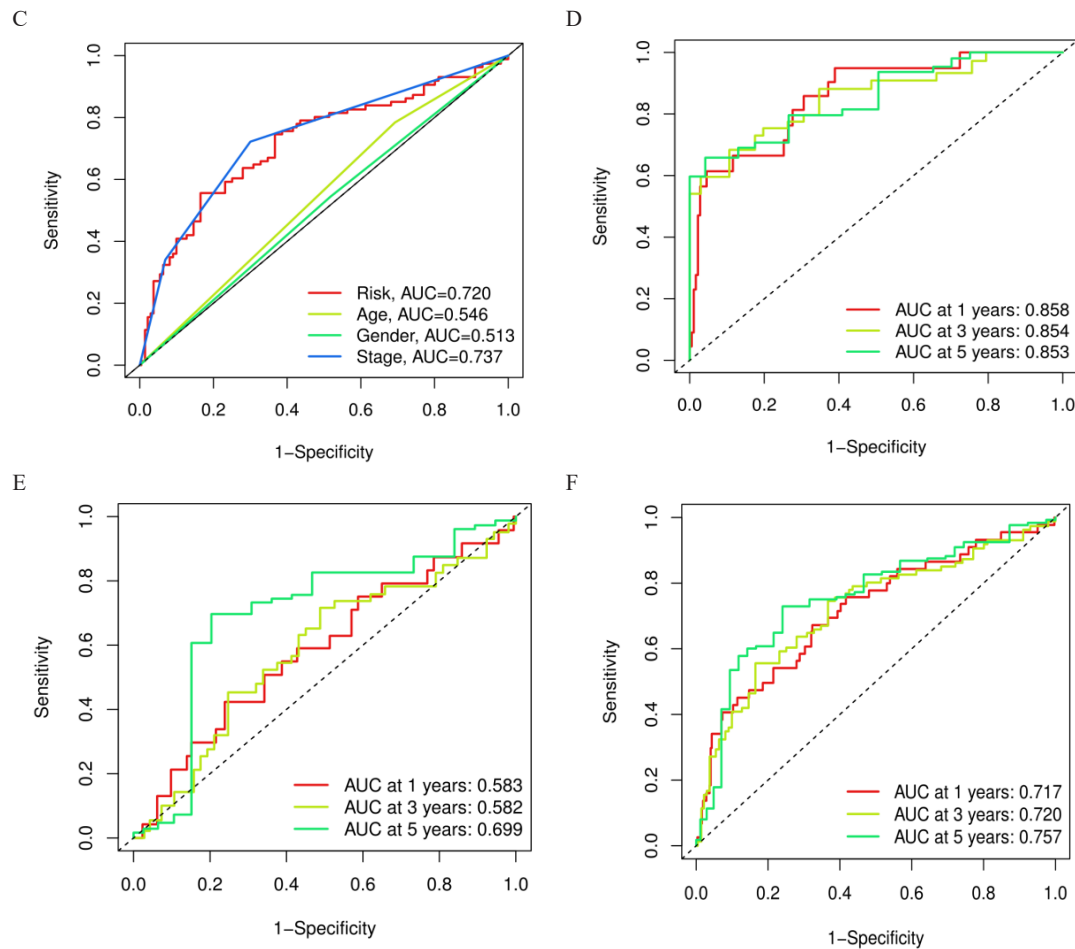


Figure 7. Independent analysis of prognostic factors in patients with COAD. (A-B) Univariate independent and Multi-variate independent prognostic analysis. (C) The complete set of AUC signs the area under the ROC curve. (D-F) The ROC curve of survival rates in different years.

3.6. Tumor mutational burden analysis based on DRGs

According to the research findings, TMB can be considered as a viable option for assessing prognosis and serving as a biomarker for immunotherapy in COAD patients(21). **Fig.8A** and **B** illustrate the top 15 frequently mutated genes, namely APC, TP53, KRAS, TTN, PIK3CA, FAT4, SYNE1, MUC16, ZFHX4, RYR2, OBSCN, CSMD3, DNAH5, LRP1B, and PCLO. Based on the research findings depicted in Figure 8C, we investigated the differences in TMB between different risk groups and identified significant disparities between the groups.

Additionally, we investigated the association between TMB and risk scores. We confirmed their positive correlation as concluded from the results depicted in Fig.8D. In addition, we performed survival analysis on TMB and observed a significantly improved overall survival rate in the high TMB group as compared to the low TMB group, as depicted in Fig.8E. Fig.8F illustrates the risk analysis of TMB, dividing the entire patient cohort into “L-TMB + High-risk,” “H-TMB + High-risk,” “H-TMB + Low-risk,” and “L-TMB + Low-risk” groups, and com-

paring their survival rates. The results indicate significant prognostic differences among the four groups.

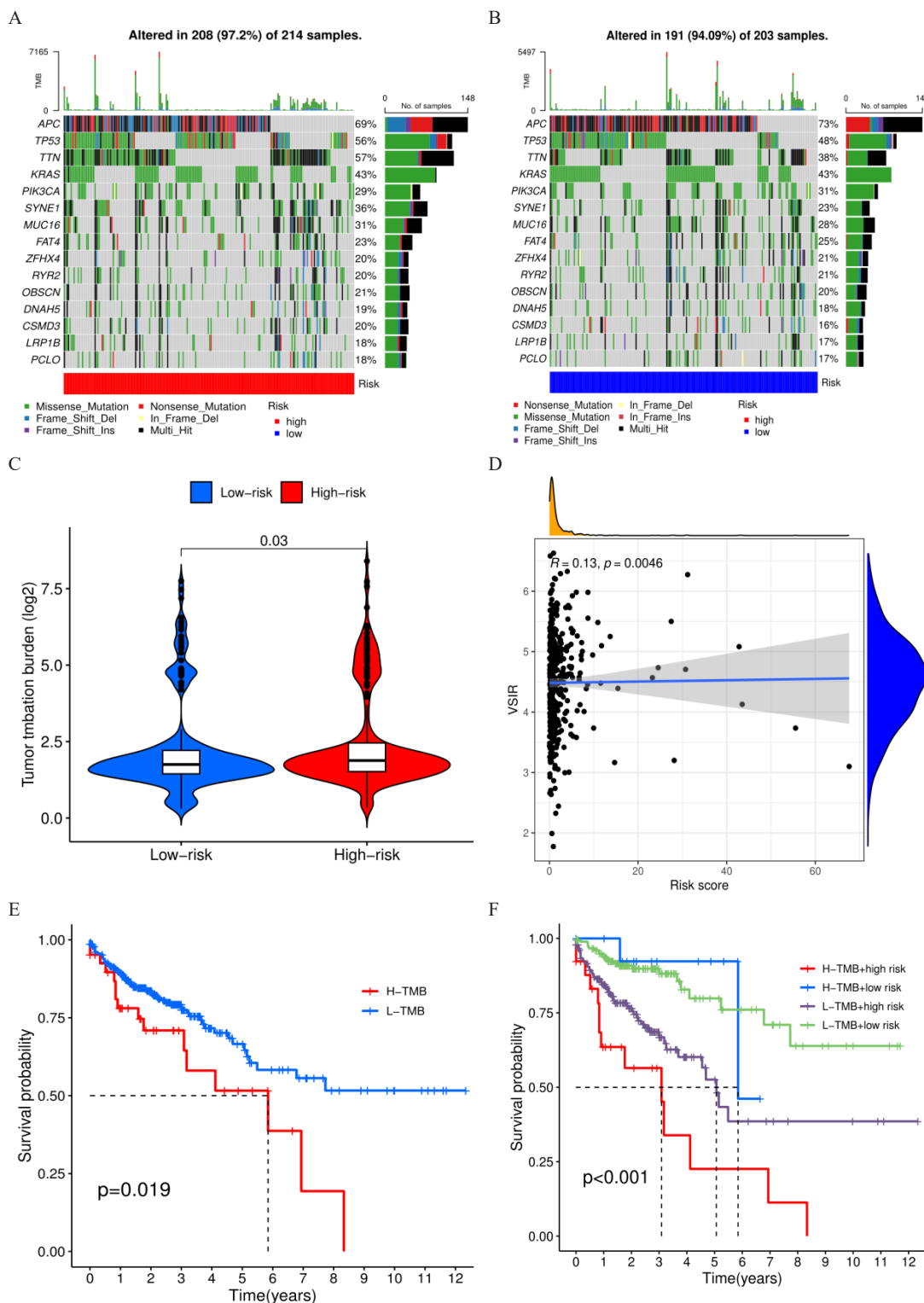


Figure 8. TMB analysis in COAD. (A, B) waterfall plot was made of the TMB levels of 15 genes in high and low-risk groups. (C) Differences in TMB between low- and high-risk groups. (D) Correlation between risk score and TMB. (E)

Survival curves for the low- and high-TMB groups. (F) Combined TMB-risk survival curve. COAD, TMB tumor mutation burden ($p < 0.01$).

3.7. Principal Component Analysis

The PCA method was utilized to investigate the differences in the expression profile of DRGs related to disulfide bonds among different risk groups. **Fig. 9A-D** demonstrate the results of samples being divided into two risk groups based on all genes, disulfide bond-related DRGs, DRGs, and the model we established. The study results indicate that the model we constructed exhibited the best discriminative ability, distinctly categorizing patients into two risk sets.

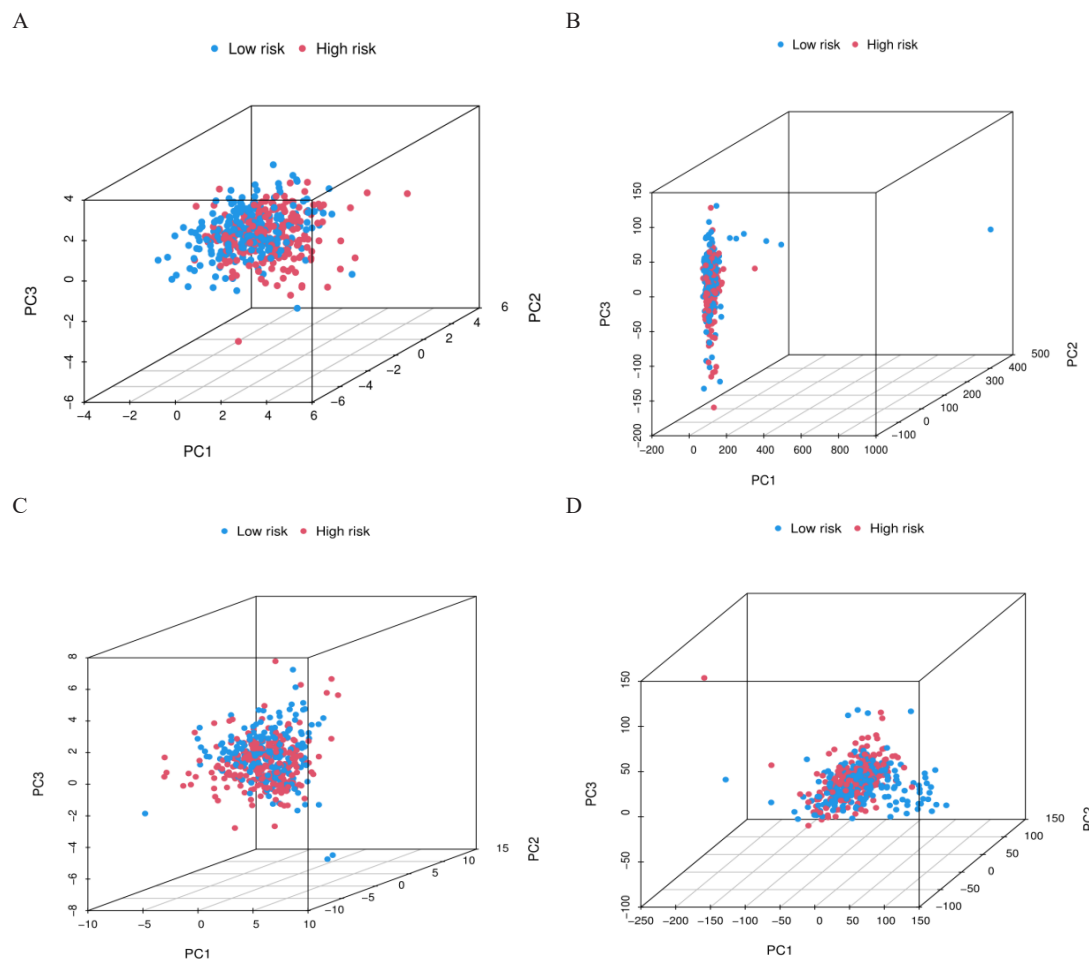


Figure 9. Principal Component Analysis. A. PCA of risk lncRNAs. B. PCA of all genes. C. Disulfidptosis-related genes. D. Disulfidptosis-associated lncRNAs.

3.8. Therapeutic Drug Sensitivity

Drug intervention plays a crucial role in the treatment of COAD. Drug sensitivity analysis provides a method for identifying effective personalized therapies. We studied six potential drugs, revealing different therapeutic effects of these drugs on high-risk and low-risk patients (**Fig.10A-F**). The results indicate a certain sensitivity of the drug Doramapimod in low-risk patients with colorectal

cancer, as shown in **Figure 10A**. Conversely, in high-risk patients, we observed sensitivity to five drugs (including Talazoparib, VX-11e, Ulixertinib, PD0325901, and MK-1775), as shown in **Figure 10B-F**.

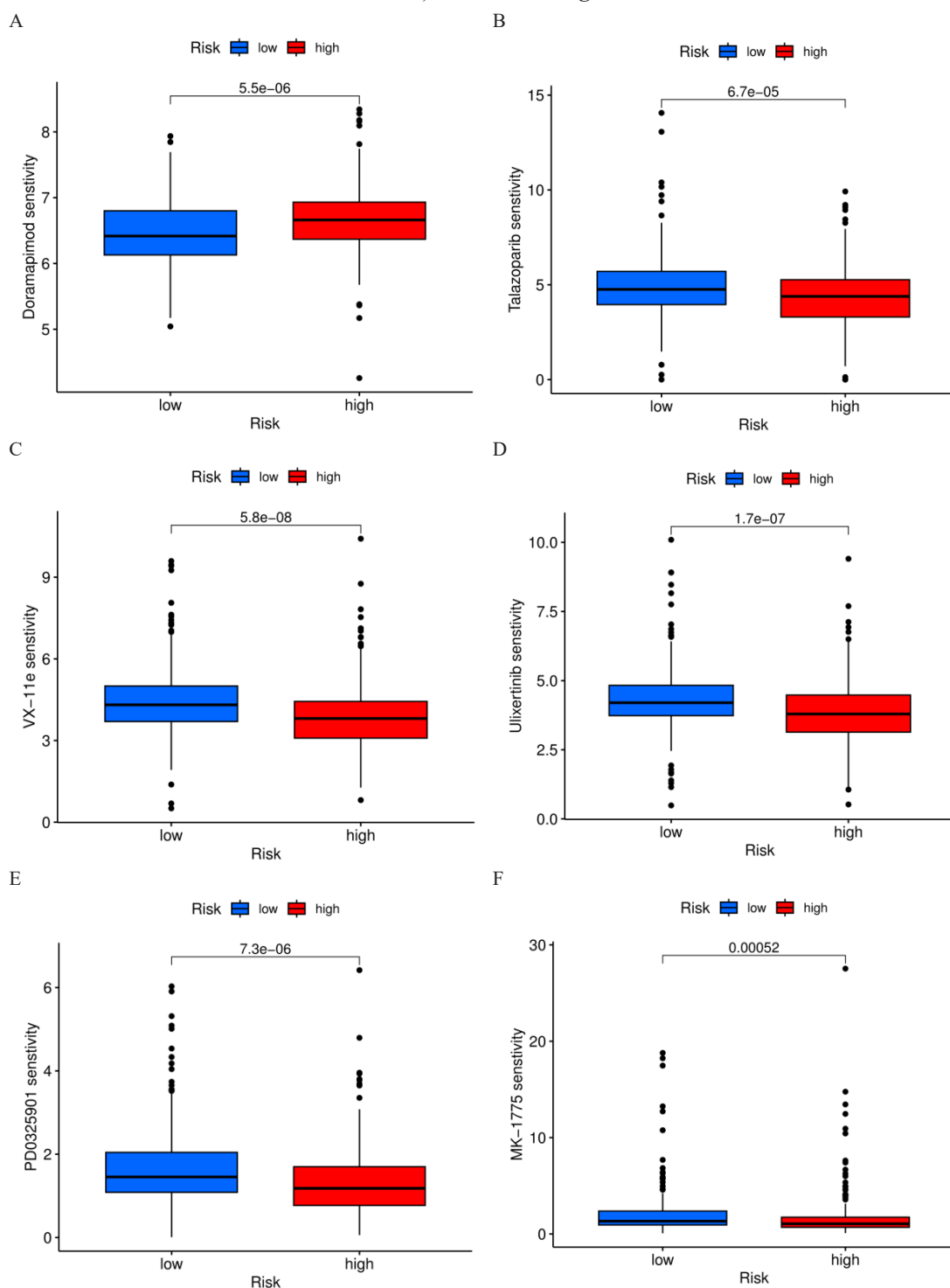
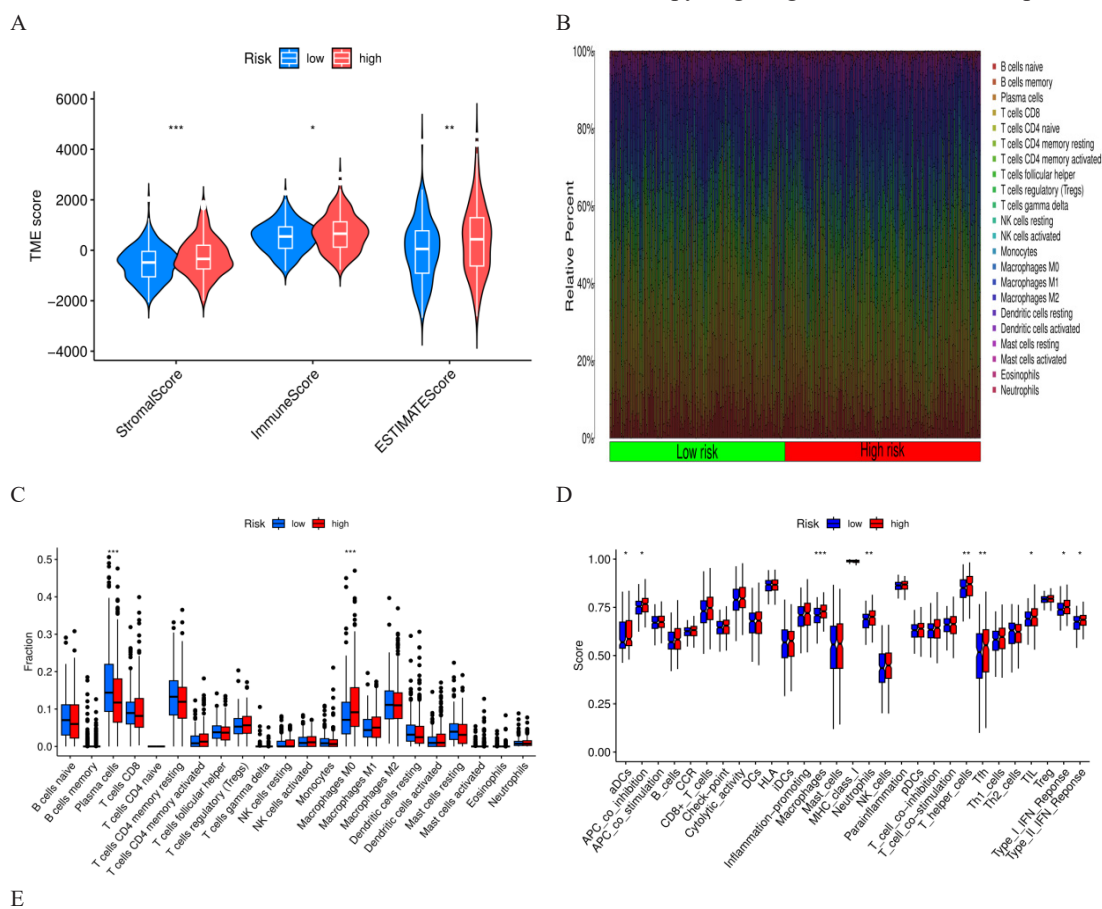


Figure 10. Six significantly different drugs were analyzed for drug sensitivity with different risk score groups. (A) Doramapimod (B) Talazoparib (C) VX-11e (D) Ulixertinib (E) PD0325901 (F) MK-1775.

3.9. Risk prognostic model and immune microenvironment analysis in COAD with OS

According to the TME analysis results, high-risk colorectal adenocarcinoma patients have lower immune scores and total scores compared to low-risk patients (**Fig.11A**). The proportion of immune cells in the low-risk group and the high-risk group is shown in **Fig.11B**. The box plots further demonstrated that, compared to low-risk patients, the expression of T cells CD4 and Plasma cells memory resting in the high-risk group was significantly downregulated ($p < 0.01$) (**Fig. 11C**). Furthermore, single-sample genome enrichment analysis revealed that in the high-risk group, 9 immune-related functions, including iDCs and Th2 cells, were significantly downregulated (**Fig.11D**). In addition, we assessed the expression of 49 immune checkpoints, comparing two groups of patients with different levels of risk scores (**Fig.11E**). Differential expression was observed in 29 immune checkpoints between these two groups ($p < 0.01$). Of particular note, HHLA2 and LGALS9, two immune checkpoints, were significantly higher expressed in patients in the low-risk group. This suggests that classifying patients based on low DRGS scores may have potential benefits and warrants consideration for the use of immunotherapy targeting these immune checkpoints.



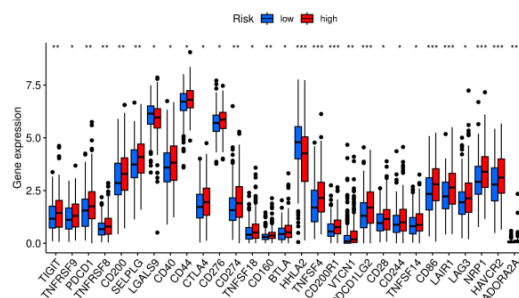


Figure 11. Correlation between prognosis model of DRGs and tumor microenvironment, immune cell infiltration, and immune function. (A) Differences in TME between high-risk and low-risk groups. (B) Proportion of immune cells in low-risk and high-risk individuals. (C) Results of immune cell infiltration analysis. (D) Results of differential analysis of immune function. (E) Differential expression of immune checkpoint markers between different risk groups. (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$)

3.10. Validation of the expression and changes of six DRGs types in COAD tissues

The results of the study conducted on six specific DRGs in cancerous and non-cancerous tissues derived from COAD patients in the TCGA database are presented in **Fig. 12**. The findings indicate significantly higher expression levels of RPA2, TIMP1, POLR3K, KTI12, and RTKN in tumor samples compared to normal samples ($p < 0.001$). At the same time, WDR1 exhibited lower expression in tumor samples. Moreover, the HPA analysis revealed distinct chromosomal section images for RPA2, TIMP1, WDR1, POLR3K, KTI12, and RTKN, specifically in colon cancer tissues as opposed to normal colon tissues (**Fig. 13** and **14**). It is worth noting that WDR1 is lowly expressed in tumor patients and highly expressed in normal patients, indicating that changes in the immune microenvironment are related to WDR1 expression.

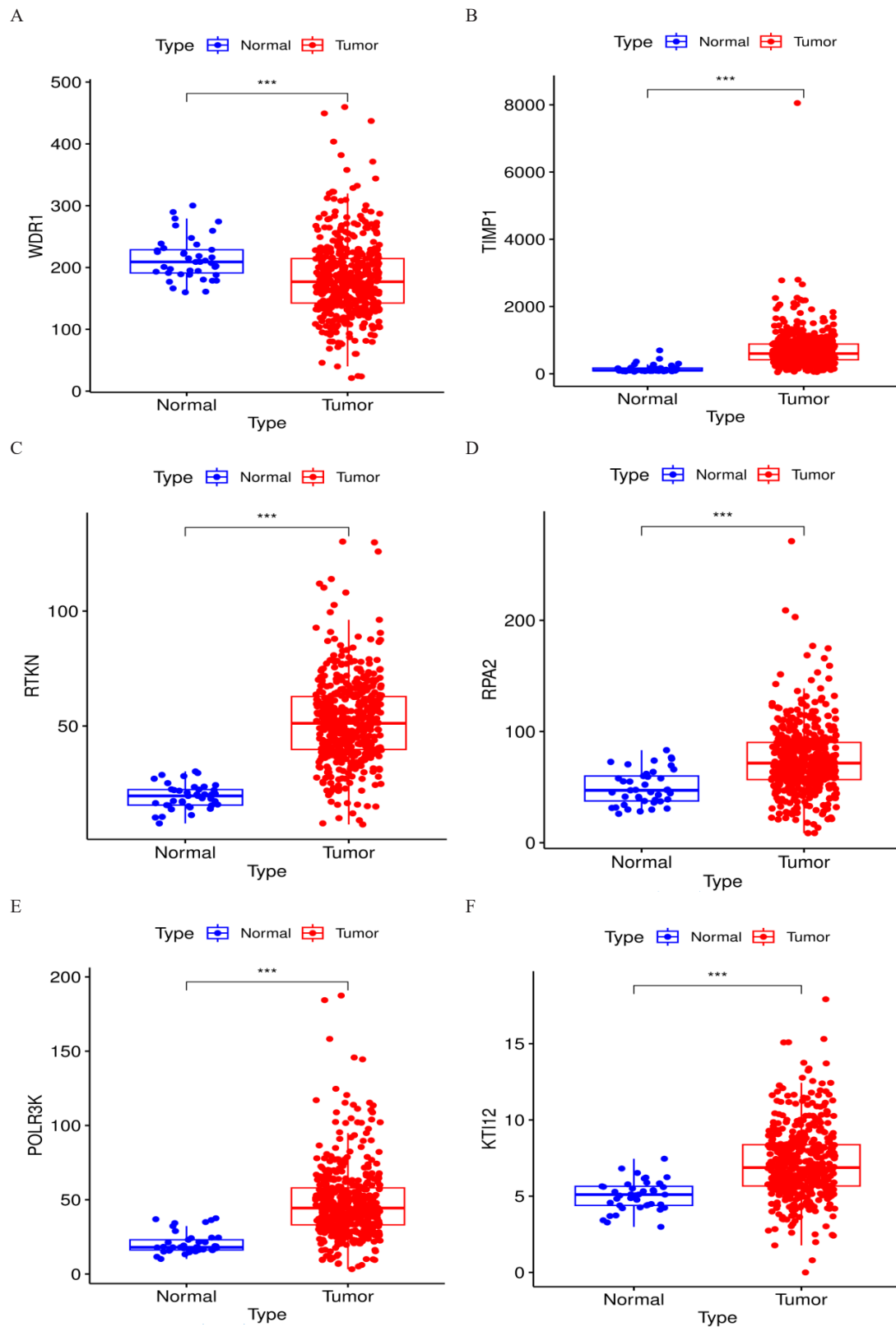


Figure 12. The expression levels of RPA2, TIMP1, WDR1, POLR3K, KTI12, and RTKN in cancer and normal samples of COAD patients in TCGA. (***:p <0.001)

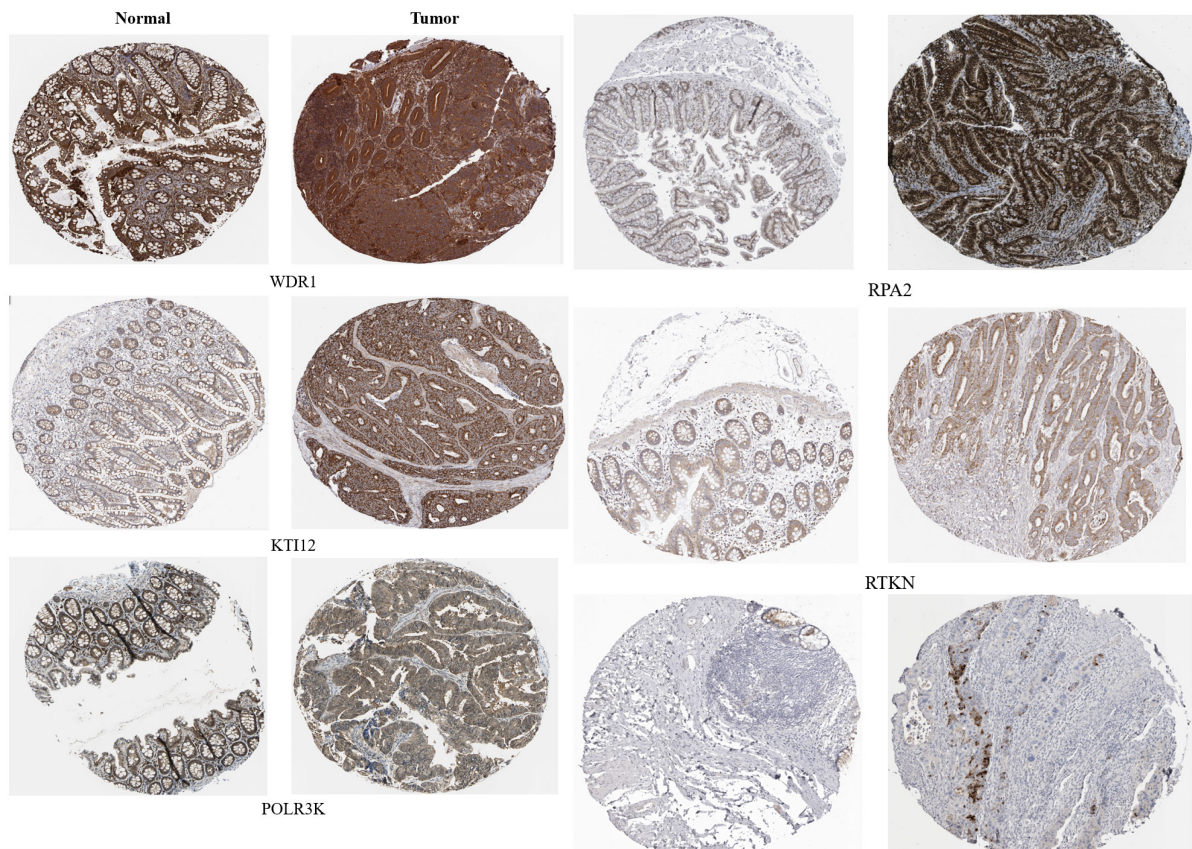


Figure 13. Expression of six DRGs in COAD tumor tissue and normal tissue. Data is sourced from the HPA database.

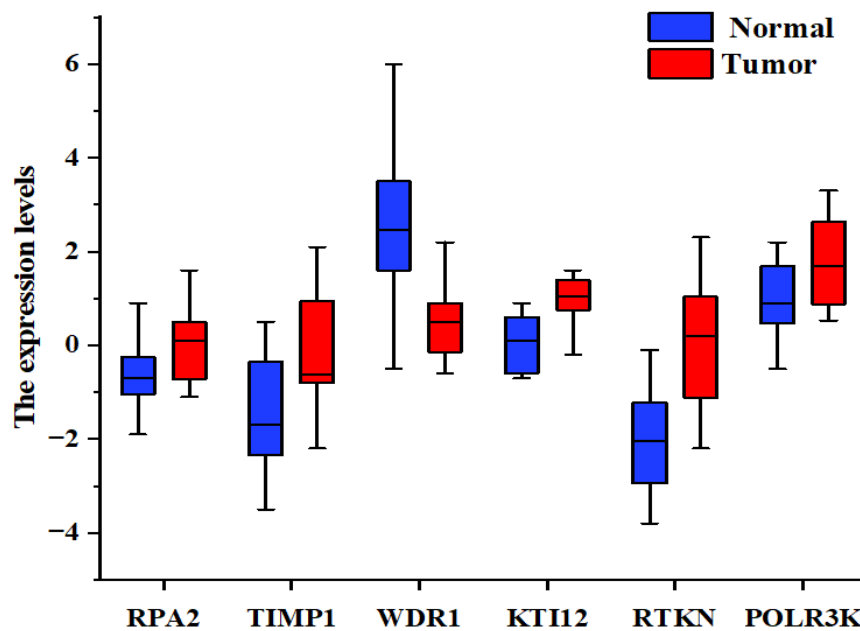


Figure 14. Relative expression levels in cancer and adjacent samples of COAD patients in HPA.

4. Discussion

COAD is a common and lethal malignant tumor caused by colon endothelial cells, which is a significant global health issue(22)the third most common cancer and the second most deadly cancer in the world. About 9.4% of cancer-related deaths in 2020 were due to COAD. Anoikis is a specialized form of programmed cell death that plays an important role in tumor invasion and metastasis. The presence of anti-anoikis factors is associated with tumor aggressiveness and drug resistance. Various bioinformatic methods, such as differential expression analysis, and functional annotation analysis, machine learning, were used in this study. RNA-sequencing and clinical data from COAD patients were obtained from the Gene expression omnibus (GEO). In recent years, the global incidence and mortality rates of COAD have been steadily rising, particularly in Asia. According to estimated data, approximately 1.4 million new cases of COAD occur each year, resulting in nearly 700,000 deaths, which is classified as the third most common cancer type in the world(23). The World Health Organization predicts that by 2030, the global number of COAD cases will increase to 1.5 million. Due to a lack of early symptoms, most COAD patients are diagnosed at middle or advanced stages, resulting in few opportunities for curative surgery. Before 2008, approximately 108,070 new COAD cases and 49,960 deaths occurred annually. However, with the development of technology and effective prevention measures, the mortality rate of COAD has significantly decreased. Investigations into the prognosis of COAD patients show that the 5-year survival rate for stage I COAD is 91%; survival rates gradually decrease with disease severity, reaching a low of 14% for stage IV COAD. Currently, the main treatments for COAD include surgical resection, immunotherapy, chemotherapy, and radiotherapy. For early-stage patients, surgical resection is the preferred treatment option(24).

However, the efficacy of other therapeutic approaches is less significant compared to current chemotherapy drugs(25)epidermal growth factor receptor inhibitor (EGFRi). Once patients develop resistance to chemotherapy regimens, the treatment process encounters severe challenges. Research reveals that colorectal cancer biomarkers assist physicians in formulating personalized drug treatment plans, effectively reducing patient mortality and morbidity. Exploring new colorectal cancer biomarkers is crucial for early detection and more efficient diagnosis and treatment, highlighting the urgent need for novel therapeutic strategies and predictive tools in colorectal cancer treatment. Consequently, this study employs newly discovered biomarkers to investigate the treatment and prognostic status of colorectal cancer.

Aberrant expression of DRGs is associated with various diseases and plays a critical role in tumorigenesis and tumor progression(26)their association in colon adenocarcinoma (COAD). Notably, a growing body of research has unveiled the significant role of DRGs in the pathogenesis of COAD. DRGs participate in processes such as drug release systems, apoptosis, and cellular autophagy, serv-

ing as potential biomarkers for disease and targets for cancer therapy. Bisulfide death can induce various redox defects and cell death. Immune cell-mediated immune responses are predominantly dependent on redox reactions, which play a pivotal role in tumorigenesis and tumor progression.

Additionally, several studies (27,28) have shown a close correlation between the treatment response and prognosis of COAD and the infiltration status of immune cells. However, research on the relationship between bisulfide death and pancreatic cancer immunity remains relatively limited. Therefore, this study conducted an exploratory analysis of COAD using TCGA data.

Disulfide death triggers a series of redox defects and induces cell death, whereas the related immune response of immune cells largely depends on the occurrence of redox reactions (29). Furthermore, the redox state serves as a crucial mechanism underlying tumorigenesis and development(30). Liu et al. (13) reported that disulfide death is associated with tumors and identified DRGs, proposing that induction of disulfide death may represent an effective therapeutic strategy for tumors. Concurrently, studies (31,32) a recently discovered form of cell death, is caused by copper levels exceeding homeostasis thresholds. Although Cu has a potential role in colon adenocarcinoma (COAD have shown a close relationship between the therapeutic response and prognosis of COAD and the infiltration status of immune cells. However, current research on the immunological correlation between disulfide death and COAD is relatively limited. This study, therefore, explored and analyzed relevant data using the TCGA database. It found that 6 genes among the 24 DRGs were associated with prognosis. This may be attributed to the activation of the tumor cell's redox system when disulfide bonds accumulate in tissues and cells, thereby protecting tumor cells from degradation and death. This revelation highlights disulfide death as a key antitumor mechanism. Furthermore, GO and KEGG analyses indicated that disulfide death-related differentially expressed genes are enriched in various tumor-related pathways, consistent with research findings in thyroid cancer (33) mainly represented by the anaplastic thyroid carcinoma (ATC, breast cancer (34), and hepatocellular carcinoma (35).

With the continuous advancement of data collection and storage technologies, an increasing number of cancer cases can be utilized for in-depth analysis and exploration, thereby revealing the patterns of disease progression and evaluating disease risks. Artificial intelligence is increasingly important in the fields of cancer diagnosis, treatment, and prognosis, particularly in the areas of cancer prediction, genetic sequencing data analysis, and personalized treatment(36). Given the advantages of large-scale data processing, potential pattern mining, accuracy enhancement, and personalized treatment plan formulation, big data, and artificial intelligence have brought about superior medical care and treatment options for cancer patients(37). In this study, we employed artificial intelligence and big data technologies to construct a model for predicting DRGs in COAD. With the aid of the TCGA-COAD cohort, we conducted in-depth investigations and predictions on various aspects of COAD, including mutations, immune sta-

tus, chemotherapy drug sensitivity, and prognosis. This comprehensive approach provides a new perspective for achieving precise and personalized treatment strategies in COAD therapy. With continuous technological breakthroughs and improvements in research methodologies, the contributions of bioinformatics and machine learning to the study of cell death will be strengthened, thereby driving greater advancements in the field of disease treatment.

In our study, we conducted a preliminary analysis of the gene levels of DRG in COAD and normal tissues, identifying 18 differentially expressed DRGs. Among these, SLC7A11, RPN1, SLC3A2, NCKAP1, NDUFA11, LRPPRC, MYH9, NUBPL, OXSM, MYH10, and QGAP1 showed upregulated expression in COAD, while NDUFS1, FLNA, LAMC1, MYL6, CAPZB, and PDLIM1 exhibited downregulated expression. Co-expression analysis revealed that in patients with COAD, numerous gene regulatory factors were associated with prognostic prediction models. Additionally, univariate Cox regression and LASSO regression analyses identified a genomic signature composed of 6 DRGs (RPA2, TIMP1, WDR1, POLR3K, KTI12, and RTKN), from which a prognostically valuable risk score model was established. Subsequent validation indicated the high efficiency of these epigenetic features in predicting survival rates of COAD patients. The model exhibited enhanced prognostic value in patients aged 65 and older, possibly due to these genes primarily functioning in the metastatic stage of cancer rather than the primary stage.

We investigated in depth the molecular mechanism of DRGs associated with disulfide bonds, exploring its influence on the occurrence and development of COAD. According to the results of the GSEA analysis, the pathways related to phagocytosis recognition and the B cell receptor signaling pathway showed an obvious enrichment trend in high-risk groups. Specifically, the high-risk group displayed significant enrichment for the ECM receptor interaction pathway. In contrast, in the low-risk group, functional pathways related to Spliceosomal TRL snRNP complex assembly Y exhibited significant enhancement. Additionally, pathways such as the citrate cycle, TCA cycle, and oxidative phosphorylation also showed significant enrichment in the low-risk group.

Malignant tumors are cellular proliferative diseases with autonomous invasion and metastatic capabilities, which are fundamentally driven by genetic alterations at the organism level, leading to aberrations in key cellular biological processes such as cell cycle regulation and apoptosis. When these mutations in genes disrupt the homeostasis of key cellular functions, they promote tumorigenesis (38)predominantly affecting males of older age. The overall (5 years. Recent research indicates that TME and TMB serve as prognostic and immunotherapeutic biomarkers, playing a crucial role in evaluating the prognosis of patients (39). This study aimed to analyze the differences in TMB scores between high- and low-risk groups and to explore the predictive value of risk factors for the efficacy of immunotherapy. In order to analyze the mutation spectrum of high-risk and low-risk populations, we downloaded mutation data for COAD from the TCGA

database(40,41). The top 15 frequently mutated genes were identified, namely APC, KRAS, TTN, TP53, SYNE1, MUC16, FAT4, CSMD3, ZFHX4, PIK3CA, RYR2, OBSCN, DNAH5, LRP1B, and PCLO. The results showed that there was a significant difference in TMB between the two groups, with TMB being positively correlated with risk scores. Subsequent survival analysis of TMB revealed significantly lower overall survival rates in the high TMB group compared to the low TMB group. TME analysis demonstrated lower immune scores and total scores in high-risk COAD compared to low-risk patients. The box plot further demonstrates that the expression levels of high-risk groups T cells CD4 and Plasma cells are significantly decreased compared to those of low-risk patients ($p < 0.05$).

Furthermore, the HPA and qRT-PCR experimental results clearly indicate significant differential expression of RPA2, TIMP1, POLR3K, KTI12, and RTKN in COAD tumors were higher than those in normal tissues. In contrast, WDR1 showed the opposite situation ($p < 0.01$). In summary, these results demonstrate the reliability and validity of the identified risk characteristics.

However, this study also has some limitations. First, all data for this study are from public databases, which lack independent experimental validation. Secondly, more independent cohorts and larger sample sizes are needed to validate the accuracy of the model. Thirdly, this study is a retrospective study on COAD and does not involve functional and mechanistic validation of genes, which will be the direction of our future efforts. In conclusion, disulfide death, as a novel form of cell demise, exhibits a close correlation between its gene expression and the prognosis as well as immune therapeutic response in COAD, thereby indicating a potential avenue for targeted DRG therapy, offering a new perspective for COAD diagnosis and treatment.

5. Conclusion

This study successfully identified six DRGs closely associated with disulfide-induced cell death, revealing novel prognostic biomarkers and potential therapeutic targets for patients with COAD. Additionally, we have developed a predictive model of DRGs based on deep learning models, providing a new direction for clinical treatment and prognosis mechanism research in COAD.

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Ethics approval and consent to participate: This manuscript uses public data, so there is no need for ethics.

Data Availability Statement

The original contributions of the study are included in the paper/appendix; for further inquiries, please contact the corresponding authors directly.

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Conflicts of Interest

The authors declare no conflict of interest.

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