Identification of the immune infiltration landscape and development of the prognostic signature on cuproptosis-related genes in colorectal cancer

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Abstract

Background: Cuproptosis is a novel mechanism of cell death induced by extra copper that was directly bound with the lipoylated proteins in the TCA cycle and could lead to the dysfunction of the mitochondrion. However, the impact of cuproptosis on TIME in colorectal cancer remains unclear. The investigation of the interplay between cuproptosis and TIME could help us decipher novel strategies for colorectal cancer diagnosis and treatment. This study aimed to construct a prognostic signature on immunotherapies and explore the novel patterns of the cuproptosis-related genes(CRGs) in colorectal cancer.

Methods: All the analyses were performed by R packages. The biological roles and functional patterns of the CRGs were performed by GO/KEGG analysis. The signature was developed with the prognostic CRGs by LASSO COX regression analysis. TIMER algorithm was selected for the immune cell infiltration analysis and the OCLR algorithm was for calculating the stem cell index. Consensus analysis was used to explore the novel landscape of the CRGs in colorectal cancer.

Results: The CRGs mainly participated in the process of the TCA cycle and energy metabolism in colorectal cancer. Five CRGs were identified with prognostic values and the correlation with immune infiltration was also demonstrated, meanwhile, two genes could be independent biomarkers for immunotherapies. By consensus analysis, the CRGs also showed a significant difference in stem cell characteristics.

Conclusion: A comprehensive analysis of functional and immune infiltration of CRGs in colorectal cancer was performed. The results also provided evidence for revealing the vulnerabilities in the correlation between cuproptosis and TIME. More data from research trials and experiments are required.

Introduction

Colorectal cancer(CRC) has become the third morbidity of malignancy with the second most common cause of cancer-induced death worldwide, seriously threatening the health of humans[1,2]. Multiple studies have shown that tumor cells' activity, proliferation, and communication are impacted not just by single tumor cells, but also by their complex surrounding environment, known as the tumor microenvironment (TME), which is made up of tumor cells and tumor-related cells[3–5]. Tumor immune microenvironment(TIME), representing the immune elements of the TME, has been demonstrated as the dynamic community and critical role in the oncogenesis and metastasis of colorectal cancer[6,7]. Immunotherapies including immune checkpoint blockades (ICBs) and others that promote the switch from a protumor to an antitumor response have given innovative treatments for colorectal cancer patients[8,9]. However, the low response rates limit the utilization of immunotherapies[10], making TIME research and precise colorectal cancer classification all the more important.

Copper-induced cell death, also termed cuproptosis, is a novel mechanism of cell death, which could lead to the proteotoxic stress response through directly binding with the lipoylated proteins that are key enzymes in the tricarboxylic acid (TCA) cycle and further induce the dysfunction of mitochondrial metabolism[11,12]. Cuproptosis could also induce the death of tumor cells by generating reactive oxygen species (ROS) to activate the apoptosis signaling pathway which makes it a novel mechanism for the antitumor studies[13]. However, to get a better understanding of how cuproptosis interact with the TIME still needs more shreds of evidence.

In this study, ten cuproptosis-related genes(CRGs) which were illustrated participating in the process of copper-induced cell death were selected to investigate the sophisticated crosstalk between cuproptosis and TIME, which could help us get a better precise classification and the application of immunotherapies for colorectal cancer.

Material And Methods

Date and sample source

The Cancer Genome Atlas(TCGA) database was selected and the TCGA-COADREAD cohort was used to access RNA-sequencing (RNA-seq) data of patients with colorectal cancer, which was released on March 15, 2022. All the obtained data were standardized to Fragments Per Kilobase per Million (FPKM) values for further analysis and performed by the packages using R software (version 4.0.3) which was shown in a simple workflow in Fig. 1.

Detecting the expression of CRGs in colorectal cancer.

Ten cuproptosis-related genes(CRGs) involved in the process of copper-induced cell death were selected. The Wilcoxon test was used to screen the expression of CRGs in colorectal cancer tissues compared with normal tissues, meanwhile, the STRING database[14] was selected to explore the correlation of these ten CRGs based on the data from TCGA.

Functional roles of the CRGs

The GO and KEGG databases were utilized to further investigate the biological roles and pathways of these CRGs in colorectal cancer and clarify the functional landscape by consensus analysis[15,16]. All the standardized data was performed by the ClusterProfiler R program[17](version 3.14.0).

Development of the signature on cuproptosis-related genes

Cox regression analysis was selected to identify the prognostic value of these CRGs. Five CRGs containing PDHB, MTF1, DLD, DLAT, and CDKN2A were finally chosen, as evidenced by a significant prognostic value by Kaplan-Meier methods. LASSO Cox regression analysis was used to construct a prognostic gene signature including these five prognostic CRGs(PDHB, MTF1, DLD, DLAT, and CDKN2A)

in colorectal cancer, using 10-fold cross-validation to determine the penalty parameter λ. Patients with colorectal cancer were subsequently divided into two categories based on the risk score: low risk and high risk, and the Kaplan-Meier method was used to assess the rate of overall survival (OS) between these two groups. To predict the diagnostic accuracy of each gene, the receiver operating characteristic (ROC) analysis was chosen. Considering the pathological characteristics, a predicted nomogram was developed to predict the 1-year, 3-year, and 5-year overall survival possibility.

Immune cells infiltration analysis of these prognostic CRGs

TIMER algorithm was utilized to investigate the correlation between prognostic CRGs and immune cell infiltration in colorectal cancer which was performed by the immuneeconv R software package[18]. Spearman correlation analysis was selected to perform the analysis of TMB(tumor mutation burden) and MSI(microsatellite-instability) of these five prognostic CRGs, as well as the correlation between the five CRGs and immune-related genes with a P-value less than 0.05 considered as statistically significant.

The consensus clusters analysis of CRGs

For a better classification of the CRGs in colorectal cancer, consensus clustering analysis was selected, the ConsensusClusterPlus R package[19](version 1.54.0) was implemented, and the pheatmap R package (version 1.0.12) was employed for clustering heatmaps[20]. The gene expression heatmap was retained for genes with SD > 0.1. If there were more than 1000 input genes, the SD was sorted, and the top 25% of the genes were retrieved. The difference in expression of mRNAs was investigated using the Limma R program (version: 3.40.2). In TCGA or GTEx, the adjusted P value was used to account for false-positive results. The findings with "Adjusted P > 0.05 and|Log (Fold Change)|>1" were chosen as the screening criterion for differential mRNA expression. Volcano graphs were created using fold-change numbers and adjusted P values. To find mRNAs that were differentially expressed between colorectal cancer and normal tissues, hierarchical clustering was performed. The immune cell infiltration between these two consensus clusters was analyzed using the TIMER algorithms. The one-class logistic regression (OCLR) machine-learning algorithm was used to calculate the mRNAsi(mRNA stemness index) of these CRGs. The mRNA stemness index of the CRGs was mapped to the range [0,1] using the Spearman method, with the minimum value subtracted and the result divided by the maximum.

Results

1 The difference in expressions of CRGs in colorectal cancer.

The expression of these ten selected CRGs in colorectal cancer was initially detected in comparison to normal colorectal tissues, and the results showed that FDX1, LIAS, LIPT1, PDHA1, GLS, DLD, DLAT, MTF1, CDKN2A was down-regulated in colorectal cancer tissues, while the expression of PDHB was up-regulated (Fig. 2A, all P < 0.05).

2 Biological functional enrichment analysis of CRGs

The protein-protein interaction (PPI) network of these ten CRGs was developed (Fig. 3A). The GO analysis which contained molecular function(MF), biological pathways(BP), and cellular components(CC) showed that these ten CRGs mainly participated in the process of cyclin-dependent protein serine/threonine kinase activity, mitochondrial matrix, oxidoreductase complex, cyclin-dependent protein kinase holoenzyme complex, tricarboxylic acid cycle, acetyl-CoA metabolic process, the acetyl-CoA biosynthetic process from pyruvate. Moreover, the KEGG analysis results indicated that the pathways in these CRGs were mainly involved were the Citrate cycle (TCA cycle), Glycolysis / Gluconeogenesis, and Pyruvate metabolism(Fig. 3B). The Kaplan–Meier survival analysis was performed for clarifying the prognostic value of these CRGs in colorectal cancer and the results illustrated CDKN2A was the protective factor, while DLD, DLAT, MTF1, PDHB were the risk factors(Fig. 3C, Table 1).

Table 1

ONTOLOGY	ID	Description	GeneRatio	BgRatio	P value	P.adjust
BP	GO:0006086	acetyl-CoA biosynthetic process from pyruvate	4/14	16/18670	3.58e-10	1.99e-07
BP	GO:0006085	acetyl-CoA biosynthetic process	4/14	22/18670	1.44e-09	3.98e-07
BP	GO:0006099	tricarboxylic acid cycle	4/14	34/18670	9.06e-09	1.42e-06
BP	GO:0006101	citrate metabolic process	4/14	35/18670	1.02e-08	1.42e-06
BP	GO:0006084	acetyl-CoA metabolic process	4/14	38/18670	1.44e-08	1.48e-06
CC	GO:0005759	mitochondrial matrix	9/14	469/19717	4.07e-12	1.02e-10
CC	GO:1990204	oxidoreductase complex	4/14	112/19717	9.45e-07	1.18e-05
CC	GO:0000307	cyclin-dependent protein kinase holoenzyme complex	2/14	42/19717	3.97e-04	0.003
CC	GO:1902554	serine/threonine protein kinase complex	2/14	88/19717	0.002	0.011
CC	GO:1902911	protein kinase complex	2/14	109/19717	0.003	0.013
MF	GO:0016620	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor	4/14	35/17697	1.27e-08	8.10e-07
MF	GO:0016903	oxidoreductase activity, acting on the aldehyde or oxo group of donors	4/14	43/17697	2.97e-08	9.51e-07
MF	GO:0004693	cyclin-dependent protein serine/threonine kinase activity	2/14	29/17697	2.33e-04	0.003
MF	GO:0030332	cyclin binding	2/14	30/17697	2.50e-04	0.003

ONTOLOGY	ID	Description	GeneRatio	BgRatio	P value	P.adjust
MF	GO:0097472	cyclin-dependent protein kinase activity	2/14	30/17697	2.50e-04	0.003
KEGG	hsa00020	Citrate cycle (TCA cycle)	4/12	30/8076	7.50e-08	4.50e-06
KEGG	hsa00620	Pyruvate metabolism	4/12	39/8076	2.24e-07	6.71e-06
KEGG	hsa00010	Glycolysis / Gluconeogenesis	4/12	67/8076	2.04e-06	2.90e-05
KEGG	hsa05218	Melanoma	4/12	72/8076	2.72e-06	2.90e-05
KEGG	hsa04115	p53 signaling pathway	4/12	73/8076	2.88e-06	2.90e-05

3. The nomogram constructed by prognostic CRGs.

To clarify the correlation between these five prognostic CRGs(PDHB, MTF1, DLD, DLAT, and CDKN2A) and pathological characteristics in colorectal cancer, a prognostic nomogram was created. The results showed that CDKN2A and DLAT were identified as the biomarkers that could alter the prognosis of the patients with colorectal cancer by the univariate analysis, meanwhile, the pT, pN, pM were the factors associated with these five prognostic by univariate and multivariate analyses, as shown in Fig. 4A-B. Moreover, the 3-year and 5-year overall survival (OS) rates could be predicted better in the entire cohort, as shown in Fig. 4C-D.

4 Developing the signature of the CRGs in colorectal cancer.

With the prognostic value and correlation with the pathological characteristics in colorectal cancer, a prognostic gene signature was created containing these five prognostic CRGs(PDHB, MTF1, DLD, DLAT, and CDKN2A) using the LASSO Cox regression analysis (Fig. 5A-B). Two groups including high-risk and low-risk were subsequently separated calculating with the formula of the risk score=(-0.2954)*DLAT+(0.1097)*CDKN2A. The expression, risk score distribution, and survival status of the final prognostic signature in colorectal cancer were all present and the survival curves showed that the death risks increased with their risk score raised, as shown in Fig. 5C. The Kaplan-Meier survival curves for the signature demonstrated that the overall survival (OS) rate was higher in colorectal cancer patients with low-risk scores (median time = 4.2 and 8.2 years) (P = 0.000264), with AUCs of 0.645, 0.671, and 0.696 in the 1-year, 3-year, and 5-year ROC curves, respectively (Fig. 5D, 5E)

5 CRGs were associated with immune cell infiltration in colorectal cancer.

The TIMER algorithm which contained six immune infiltration cells: CD8 + T cell, CD4 + T cell, B cell, myeloid dendritic, macrophage, and neutrophils was used for analyzing the correlation between the five prognostic CRGs(PDHB, MTF1, DLD, DLAT, and CDKN2A) and the infiltration of the immune cells. The results demonstrated the correlation between the five CRGs and the number of immune infiltration cells, especially the B cell, macrophage, as well as CD8 + T cell showed a significant correlation with four CRGs, more interestingly, the MTF1 showed the correlation with all these six immune infiltration cells(Fig. 6A). To further screen the immune roles of these five CRGs in colorectal cancer, the correlation between the genes of the immune suppressor, immune checkpoints(Fig. 6B), MHC(major histocompatibility complex) (Fig. 6C), Chemokines(Fig. 6D), as well as Chemokines receptor(Fig. 6E) and these five CRGs were also performed. The analysis results illustrated a significant relation between these five CRGs and most immune-related genes in colorectal cancer, especially the MTF1.

7. The analysis results of application for immunotherapies in CRGs.

To identify whether these five prognostic CRGs could serve as the independent biomarkers and predict the potential response of the immunotherapies in colorectal cancer, the analysis of tumor mutation burden (TMB), as well as microsatellite instability (MSI) analyses were further performed, and the final results revealed a significant positive correlation between TMB and MTF1(Fig. 7B, P = 0.042), as well as a positive correlation between MSI and MTF1(Fig. 7G, P = 1.12e-5), DLAT(Fig. 7I, P = 0.0039). The above results illustrated that the MTF1 and DLAT could be the biomarkers for immunotherapies in colorectal cancer.

8. The consensus clusters of CRGs in colorectal cancer

Consensus cluster analysis was performed to explore the novel patterns of these CRGs in colorectal cancer based on the characteristics such as biological function, metabolism, and immune infiltration by the data from TCGA. The delta area curve of the consensus clusters represents the relative change in area under the cumulative distribution function (CDF) curve for each category number k when compared to k-1 consistency analysis, and the abscissa of the CDF represents category number k, as well as the ordinate, represents the relative change in the area(Fig. 8A-B). When the k number was two, all the selected data were best suited for further analysis, and two clusters were finally divided(Fig. 8C-D).

9 Functional landscape of the CRGs in colorectal cancer.

The difference in mRNA expression between these two clusters in colorectal cancer was first analyzed, and the results showed that 4135 mRNAs were up-regulated which was shown in red compared with 331

mRNAs that were down-regulated in blue(Fig. 9A-B). The GO analysis results of these two clusters in colorectal cancer suggested that the up clusters were mainly related to the process of cell cycles such as the regulation of cell cycle, proteasomal protein, catabolic process, cell cycle checkpoint, chromosome segregation, DNA replication, RNA splicing and the metabolism process like phase transition regulation of DNA metabolic process, proteasomal protein catabolic process, and ncRNA metabolic process, while the down was associated with the protein targeting to membrane, ATP synthesis coupled electron transport, oxidative phosphorylation, protein targeting, and ATP metabolic process by the GO analysis(Fig. 9C). From the results of the KEGG analysis, the up mRNAs were associated with the p53 signaling pathway, colorectal cancer, spliceosome, and cell cycle signaling pathways, while the down clusters were mainly related to glutathione metabolism, oxidative phosphorylation, and 2 − oxocarboxylic acid metabolism signaling pathways(Fig. 9D).

10 The comparison of differences in immune infiltration between the consensus clusters.

The TIMER algorithm was selected to compare the infiltration of the immune cells between these two clusters in colorectal cancer, and the results illustrated that there was a significant difference in the B cells, CD8 + T cells, Neutrophil, Macrophage, Myeloid dendritic cells between these two clusters(Fig. 10A). For the comparison of the immune checkpoints between these two clusters, eight genes were selected as the immune checkpoint-relevant transcripts, and the analysis results illustrated that between these two clusters, five genes including CD274, CTLA4, HAVCR2, PDCD1LG2, and TIGIT showed the significant difference(Fig. 10B). The OCLR algorithm[21] was used for comparing the stemness which reflects the index stem cell-like characteristics of tumor cells between these two clusters in colorectal cancer. The final OCLR scores in these two groups were shown in Fig. 10C and the results showed a significant difference between these two clusters of the stemness in colorectal cancer(P = 0.0012).

Discussion

With the huge advancement in the area of bioinformatics algorithms and multi-omics research methodologies[22,23], the researchers could get a better understanding of colorectal cancer. The studies of the characteristics and heterogeneity of the tumor immune microenvironment(TIME) which was illustrated as a complex and dynamic community, could help get a more precise classification and novel clinical strategies for patients with colorectal cancer[24–26]. Copper is a critical factor for the metabolism of all cells, however, when the organism has more copper than its homeostasis, the excess copper would directly bind to the lipoylated proteins of the tricarboxylic acid (TCA) cycle and further induce the dysfunction of the mitochondria which could finally lead to the death of cells[11]. In tumor cells, three pathways containing reactive oxygen species(ROS) accumulation, proteasome inhibition, and antiangiogenesis were illustrated as the mechanisms correlated with the process of copper-induced cell death, also termed cuproptosis[13]. The exploration of the correlation between copper-induced cell death

and TIME could help find the vulnerabilities and novel biomarkers for the immunotherapies of colorectal cancer.

In this study, all these ten cuproptosis-related genes(CRGs) significantly showed different expressions in the colorectal cancer tissues, and the functional enrichment analysis suggested that these CRGs were mainly involved in the function and signaling pathways of tricarboxylic acid(TCA) cycle, mitochondrial metabolism, and the metabolism-related protein complex activities which were related with the disorders of energy metabolism and dysfunction of mitochondria of colorectal cancer[27,28]. Five CRGs containing PDHB, MTF1, DLD, DLAT, and CDKN2A were demonstrated with prognostic value in colorectal cancer, meanwhile, the CDKN2A and DLD were demonstrated with a significant correlation with the pathological characteristics in patients with colorectal cancer by the univariate analysis. Moreover, based on the prognostic value of these five CRGs, a gene signature containing these genes was developed by the LASSO Cox regression analysis, and according to the calculated results of the signature, the high-risk score group was associated with shorter overall survival(OS) rate. The above results revealed that these CRGs not only have a significant expression in colorectal cancer tissues but also could have a correlation with the prognosis and pathological characteristics of the patients with colorectal cancer.

To clarify whether these five prognostic CRGs(PDHB, MTF1, DLD, DLAT, and CDKN2A) were associated with the TIME which was the immune parts of TME in colorectal cancer, the TIMER algorithm was first selected for estimating the immune cell infiltration of these five CRGs, meanwhile, the genes of immune suppressor, MHC, Chemokines, and Chemokines receptor were selected to detect the immune roles of CRGs in colorectal cancer. The results of the TIMER analysis revealed the correlation of these CRGs with the infiltration of the immune cells and four CRGs were illustrated the significant correlation with the B cell, CD8 + T cell, as well as the macrophage which was illustrated as the critical roles in the oncogenesis, metastasis and cell death such as apoptosis and pyroptosis of the tumor cells[29,30]. The immune suppressor and immune checkpoints have been demonstrated with a significant impact on the escape from the immune surveillance of the tumor cells and immunotherapies in colorectal cancer[31,32], MHC genes have shown the correlation with the cell death and function of the immune cells such as CD4 + T cells and CD8 + T cells[33,34], chemokines and chemokines receptor genes were associated with the energy metabolism and the macrophage polarization in tumor cells[35,36]. The above immune-related estimation illustrated the significant correlation between CRGs and TIME in colorectal cancer, especially the MTF1. Moreover, the TMB and MSI analysis results of these five CRGs in colorectal cancer demonstrated that MTF1 and DMAT could serve as the independent biomarkers for the immunotherapies of colorectal cancer[37,38]. All the above analysis results suggested that these five prognostic CRGs have a significant correlation between the TIME and immune infiltration cells in colorectal cancer, more interestingly, the correlation between the CRGs and the immune genes suggested the CRGs could have an impact on the polarization of macrophages, function of B cell and CD8 + T cell in colorectal cancer and the metastasis of the tumor cells.

For further screening of the immune and functional landscape of these CRGs in colorectal cancer, consensus clustering analysis was selected to explore the novel patterns of these CRGs in TCGA and two clusters were finally identified. The different expression of mRNAs between these two clusters was first detected, and the results revealed the significant characteristics for the classification of CRGs in colorectal cancer. The comparison of the function between these two clusters was subsequently performed by KEGG and GO databases, and the results were summarized with up-regulated and down-regulated. The up-regulated mRNAs were mainly associated with the process of the cell cycle and the metabolism by GO analysis, meanwhile, the pathway patterns by the KEGG database revealed the up clusters mainly participate in the pathways of colorectal cancer, p53 signaling pathway, and the cell cycle pathway which were related with the oncogenesis and metabolism of tumor cells[39–41]. While the down-regulated mRNAs were mainly related to protein targeting like targeting to membrane, the process of TCA cycle and mitochondrion metabolism such as oxidative phosphorylation and process of ATP metabolism by GO analysis, and the pathways analysis revealed the down-regulated mRNAs mainly participated in the glutathione metabolism and oxidative phosphorylation signaling pathways which were associated with the cell death as well as the dysfunction of the mitochondrion in tumor cells[42–44]. For screening the difference in immune infiltration landscape between these two clusters, the TIMER algorithm was selected and the results showed a significant difference in the immune infiltration cells, moreover, five immune-checkpoints genes expression between these two clusters also showed a statistically significant difference. The difference of mRNAsi calculated by the OCLR algorithm illustrated the significant difference in stem cell-like characteristics between these two clusters which were associated with the behavior, growth, communication, and immune response of tumor cells in colorectal cancer[45–47].

The above results revealed that the CRGs were mainly characterized by the process of the TCA cycle and energy metabolism of tumor cells in colorectal cancer. The results of immune infiltration patterns and mRNA stemness index could provide the clues that the immune checkpoints and stem cell-like characteristics might play key roles in the precise classification of the copper-induced cell death in colorectal cancer, respectively. Meanwhile, the relationship between CRGs and immune cell infiltration also suggested the directions for anti-cancer research such as immunotherapies in colorectal cancer.

There are also some limitations to this research. All studies were carried out with the TCGA-COADREAD cohort, and more data from in vivo, in vitro research, and clinical studies could be used to corroborate the results.

Conclusion

A comprehensive analysis of the functional and immune landscape of the cuproptosis-related genes(CRGs) in colorectal cancer(colorectal cancer) was performed, and a gene signature was developed based on the prognostic value of the CRGs. TCA cycle and energy metabolism were the pathways these CRGs characterized, meanwhile, the significant correlation between CRGs and immune cells infiltration was illustrated. Moreover, the stemness index could also serve for the classification of the CRGs in colorectal cancer. This research provided new perspectives on cuproptosis with the tumor immune

microenvironment(TIME) and novel biomarkers for the immunotherapies as well as the precise classification for colorectal cancer. More data and investigations are required to corroborate these findings.

Abbreviations

CRC: Colorectal cancer

CRGs: Cuproptosis-related genes

TME: Tumor microenvironment

TIME: Tumor immune microenvironment

ICBs: immune checkpoint blockades

TCA: tricarboxylic acid

ROS: Reactive oxygen species

OS: Overall survival

ROC: Receiver operating characteristic

TMB: Tumor mutation burden

MSI: Microsatellite-instability

mRNAsi: mRNA stemness index

OCLR: One-class logistic regression

MF: Molecular function

BP: Biological pathways

CC: Cellular components

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed in this study are available from the corresponding author upon reasonable request.

Competing interests

The authors report no conflicts of interest in this work.

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Authors' contributions

All authors participated in the analysis of the data, drafted or revised the article, gave final approval to the version to be published, and agreed to take responsibility for all aspects of the work.

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Figures

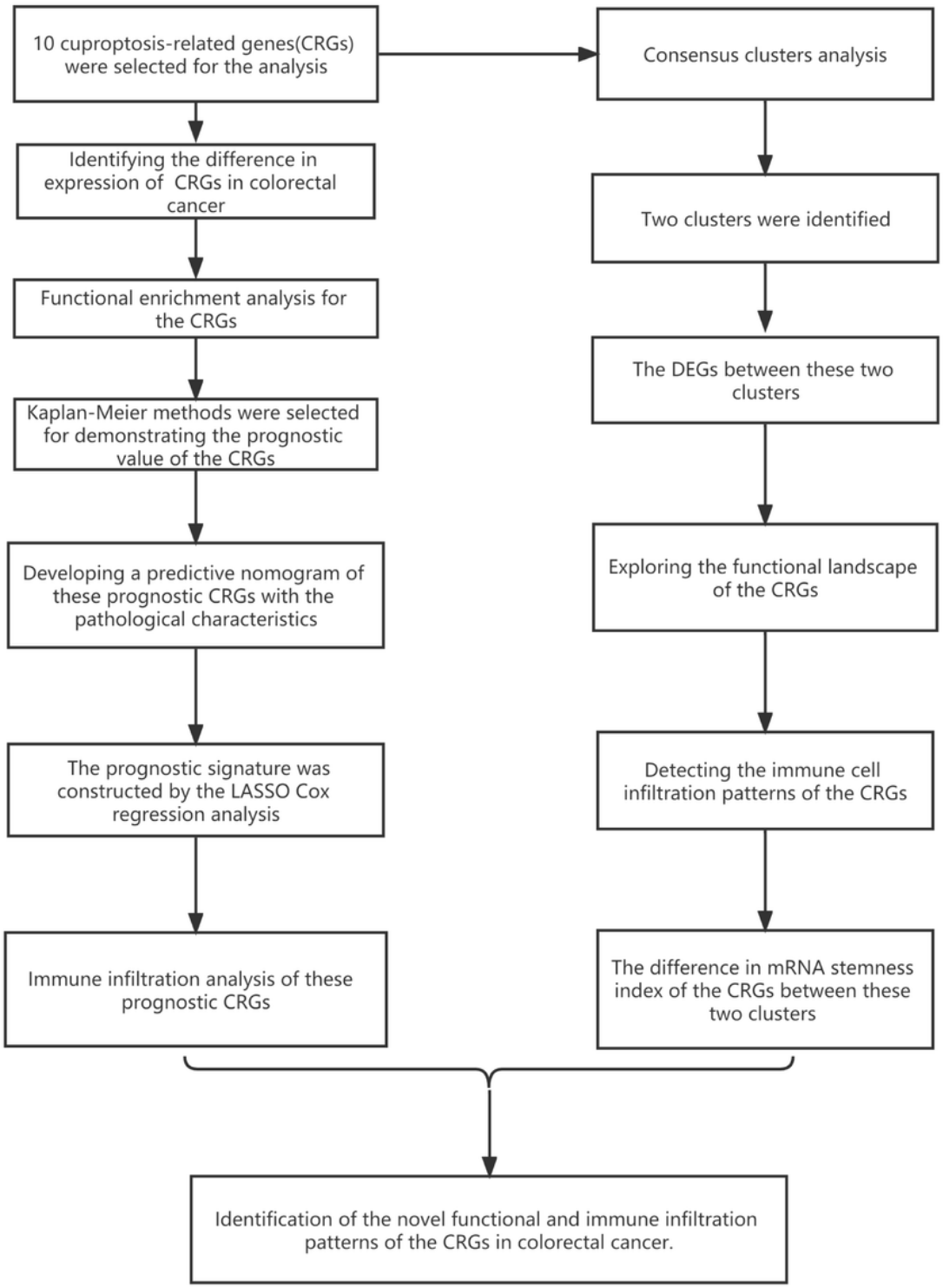

Figure 1

The workflow of this study.

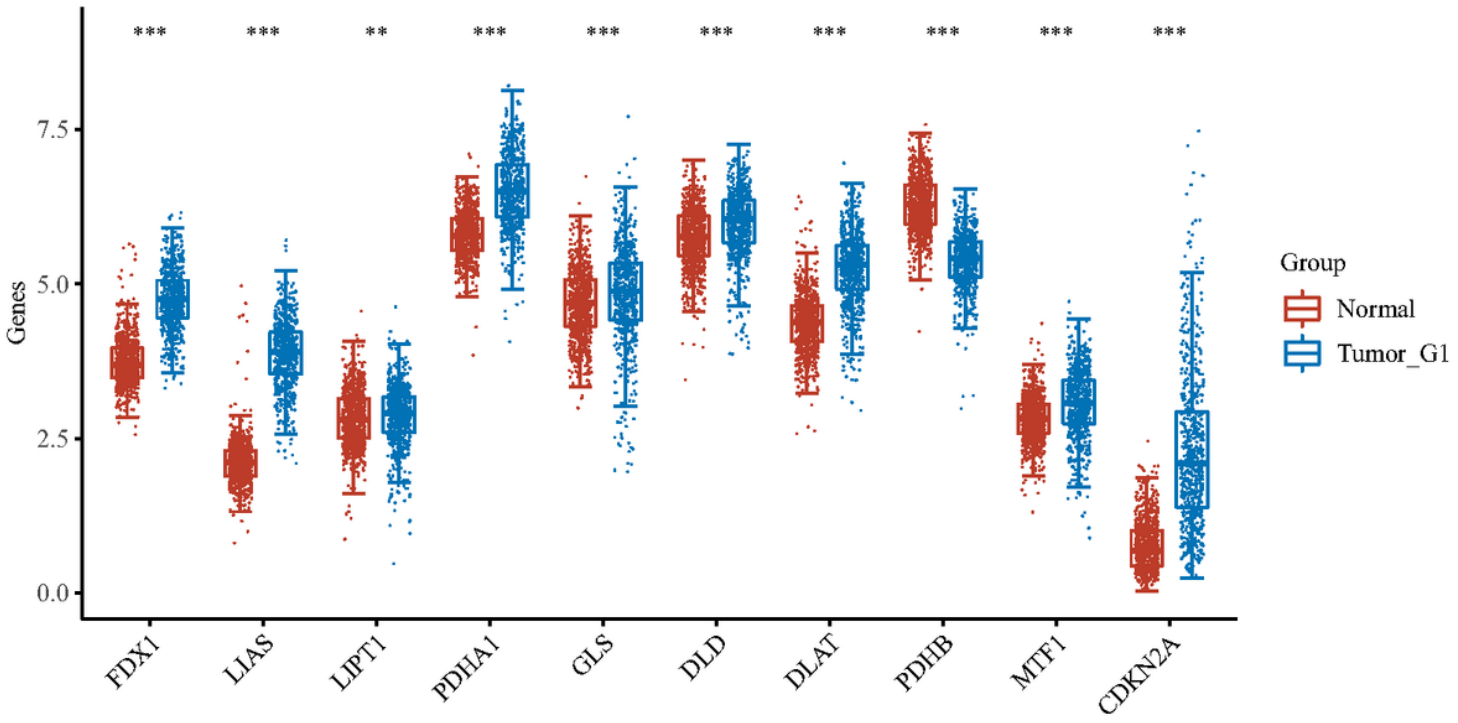

Figure 2

The expression of these 10 CRGs in colorectal cancer compared with normal tissues. Tumor, red; Normal, blue (*p < 0.05, **p < 0.01, ***p < 0.001).

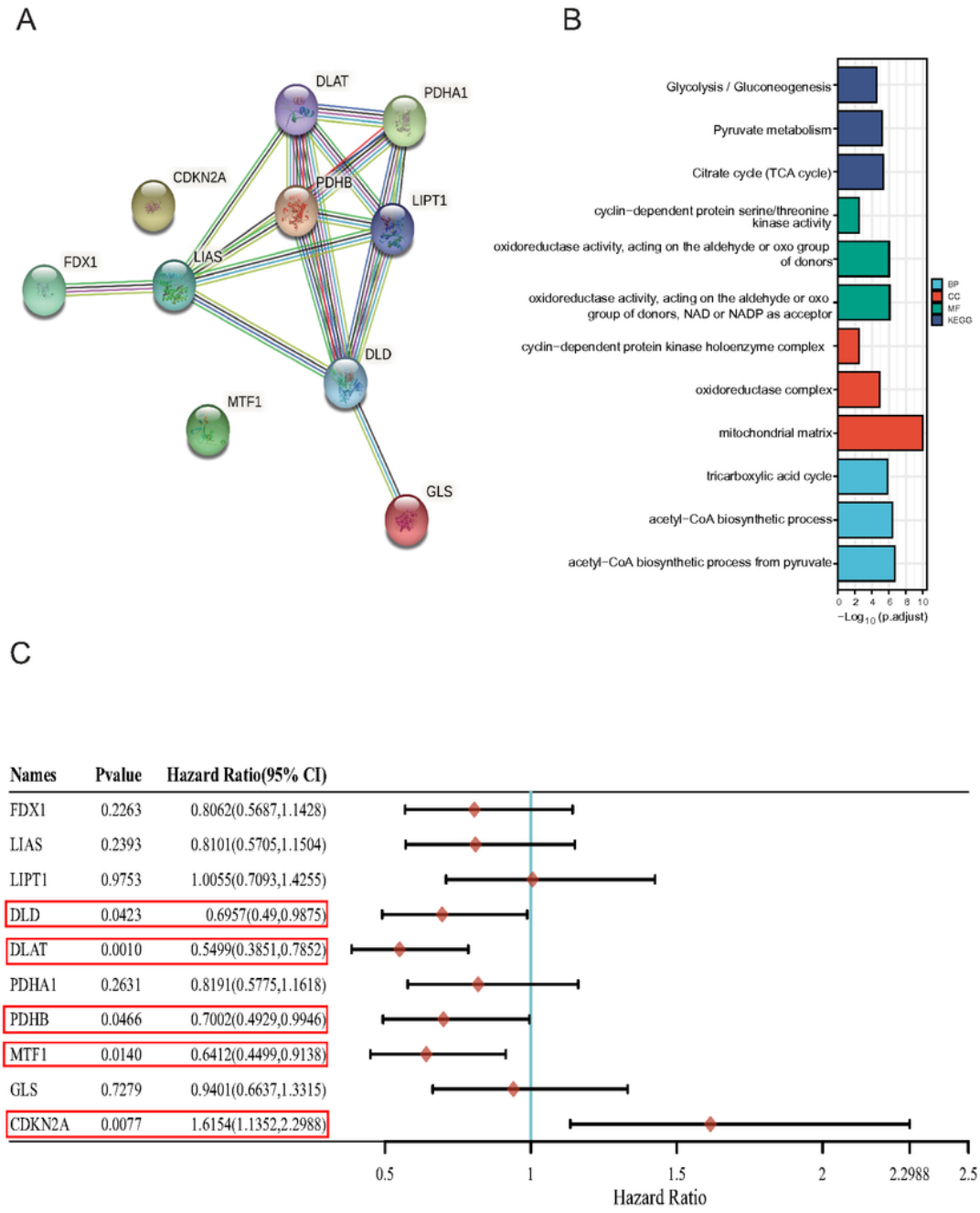

Figure 3

A The protein-protein interaction network of CRGs using the STRING database. **B** The enriched item in gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The size of columns represented the number of genes enriched. BP, biological process; MF, molecular function. **C** The prognostic value of CRGs in colorectal cancer.

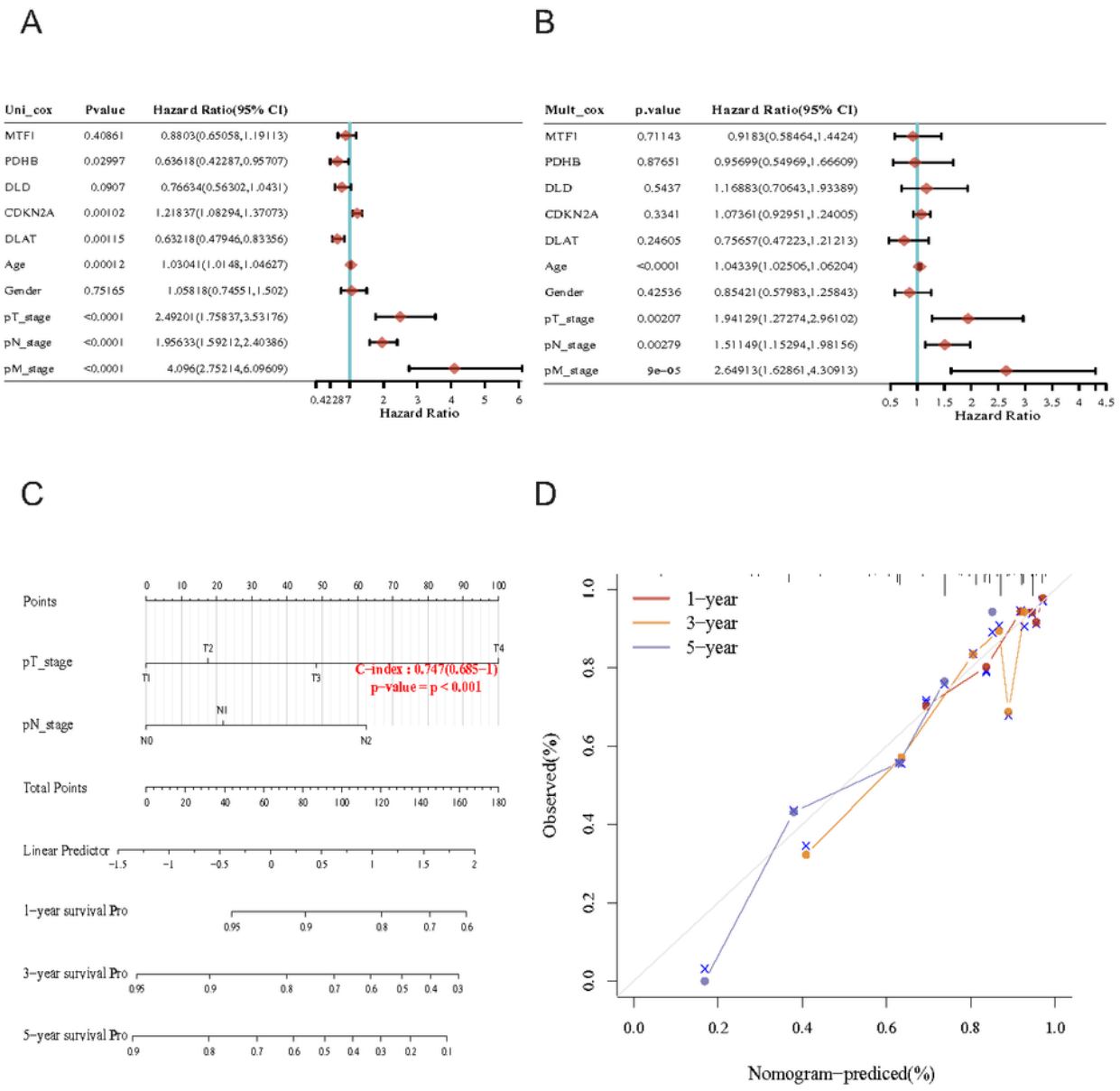

Figure 4

The correlation between the clinical characteristics and PDHB, MTF1, DLD, DLAT, and CDKN2A in colorectal cancer

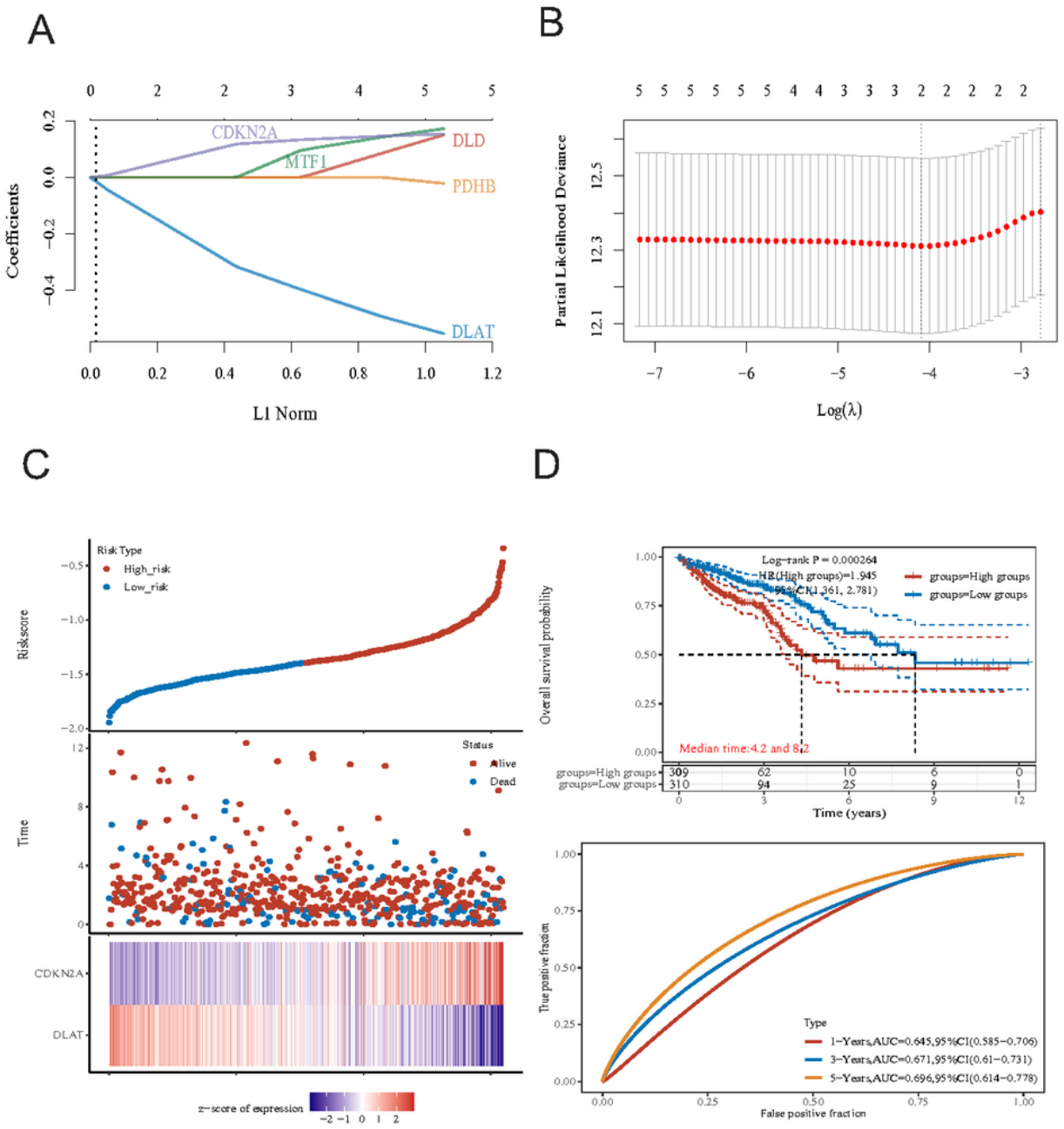

Figure 5

The construction of a signature with prognostic CRGs. **A** LASSO coefficient profile of the five CRGs. **B** Plots of the ten-fold cross-validation error rates. **C** Distribution of risk score, survival status, and the expression of five prognostic CRGs in colorectal cancer. **D** Overall survival curves for colorectal cancer patients in the high- or low-risk group and the ROC curve of measuring the predictive value.

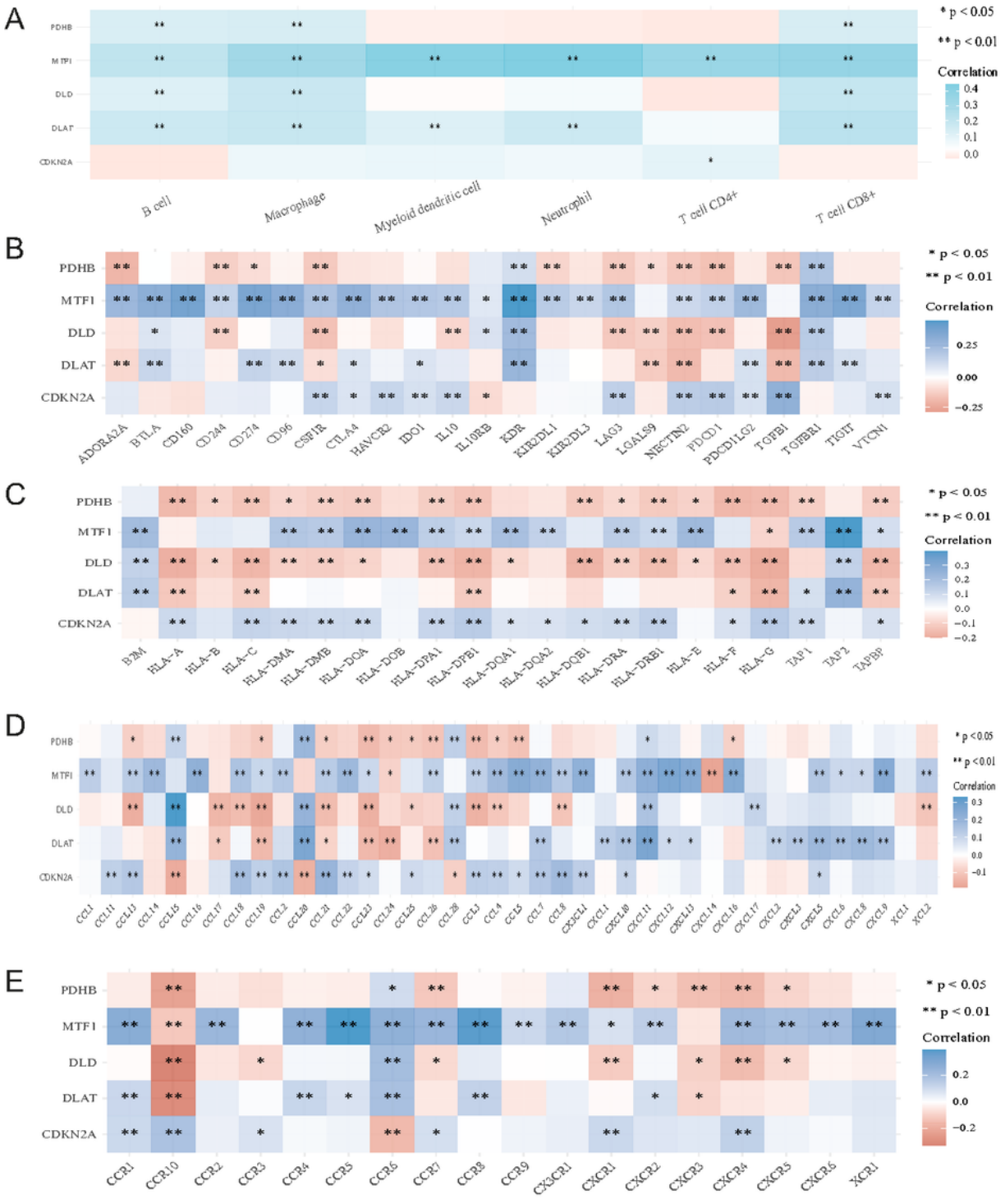

Figure 6

A Immune cell infiltration analysis for the CRGs by the TIMER algorithm. The correlation between the prognostic CRGs and **B** the immune suppressor and immune checkpoints, **C** MHC genes **D** Chemokines genes **E** Chemokine receptor genes. Asterisks represent levels of significance (blank for no significance, *p < 0.05, **p < 0.01, ***p < 0.001).

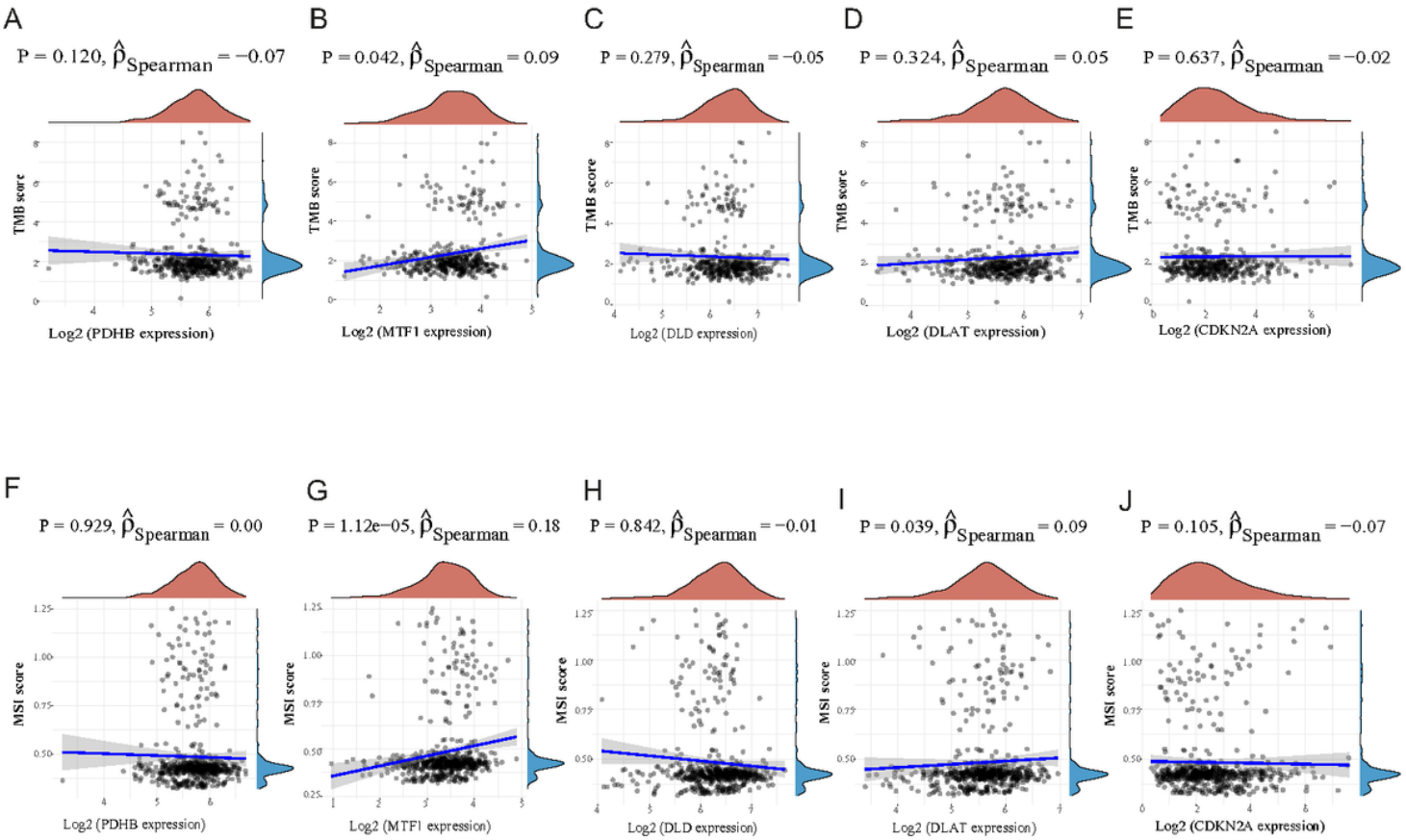

Figure 7

A-E TMB analysis of the prognostic CRGs(PDHB, MTF1, DLD, DLAT, and CDKN2A) in colorectal cancer. **F-J** MSI analysis of the prognostic CRGs(PDHB, MTF1, DLD, DLAT, and CDKN2A) in colorectal cancer. TMB, tumor mutation burden; MSI, microsatellite instability.

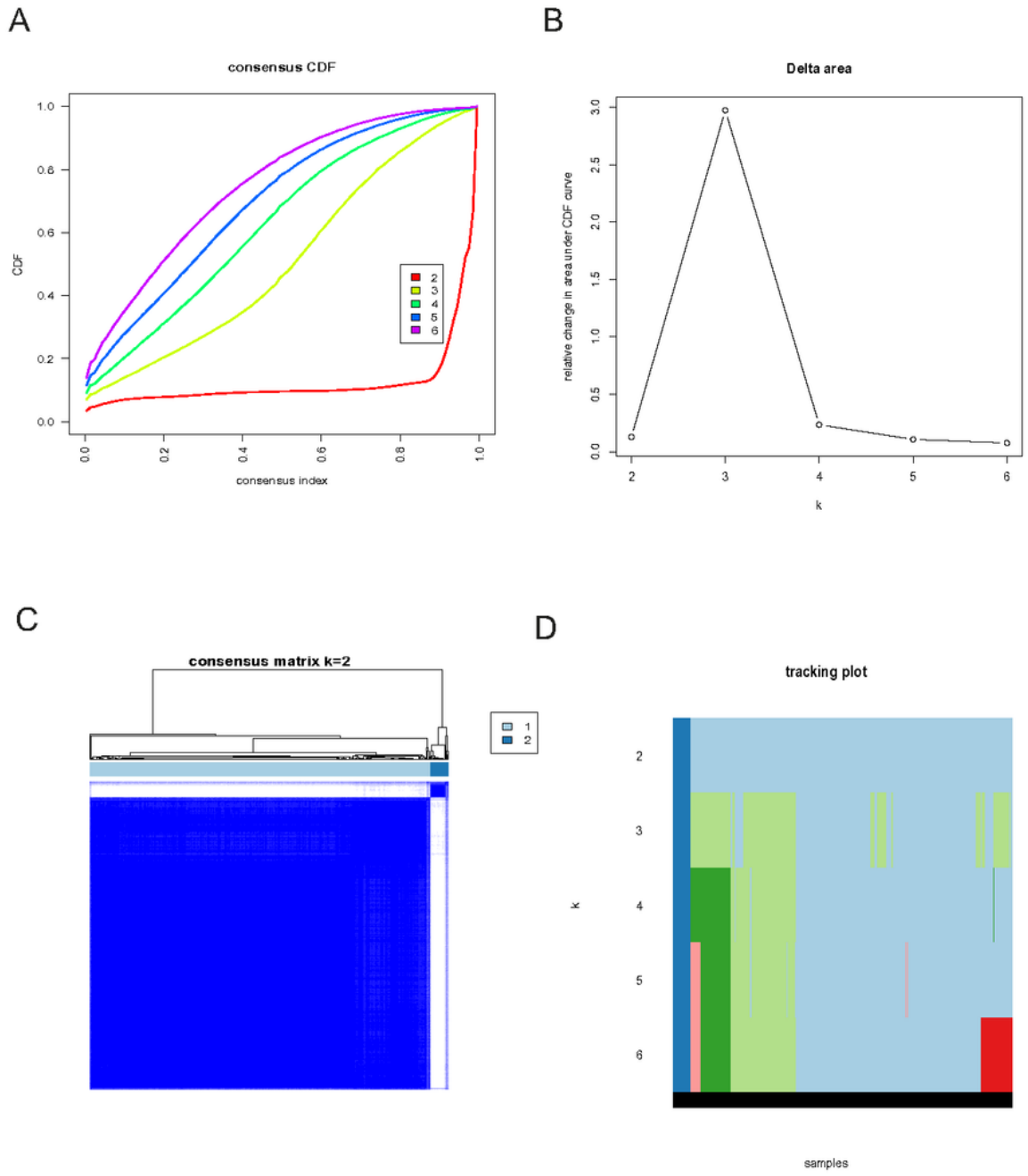

Figure 8

Consensus clusters analysis of cuproptosis-related genes in colorectal cancer **A** Cumulative distribution function (CDF) of consensus clustering by consistency analysis; **B-D** Consensus matrices of the sarcoma patients for k = 2.

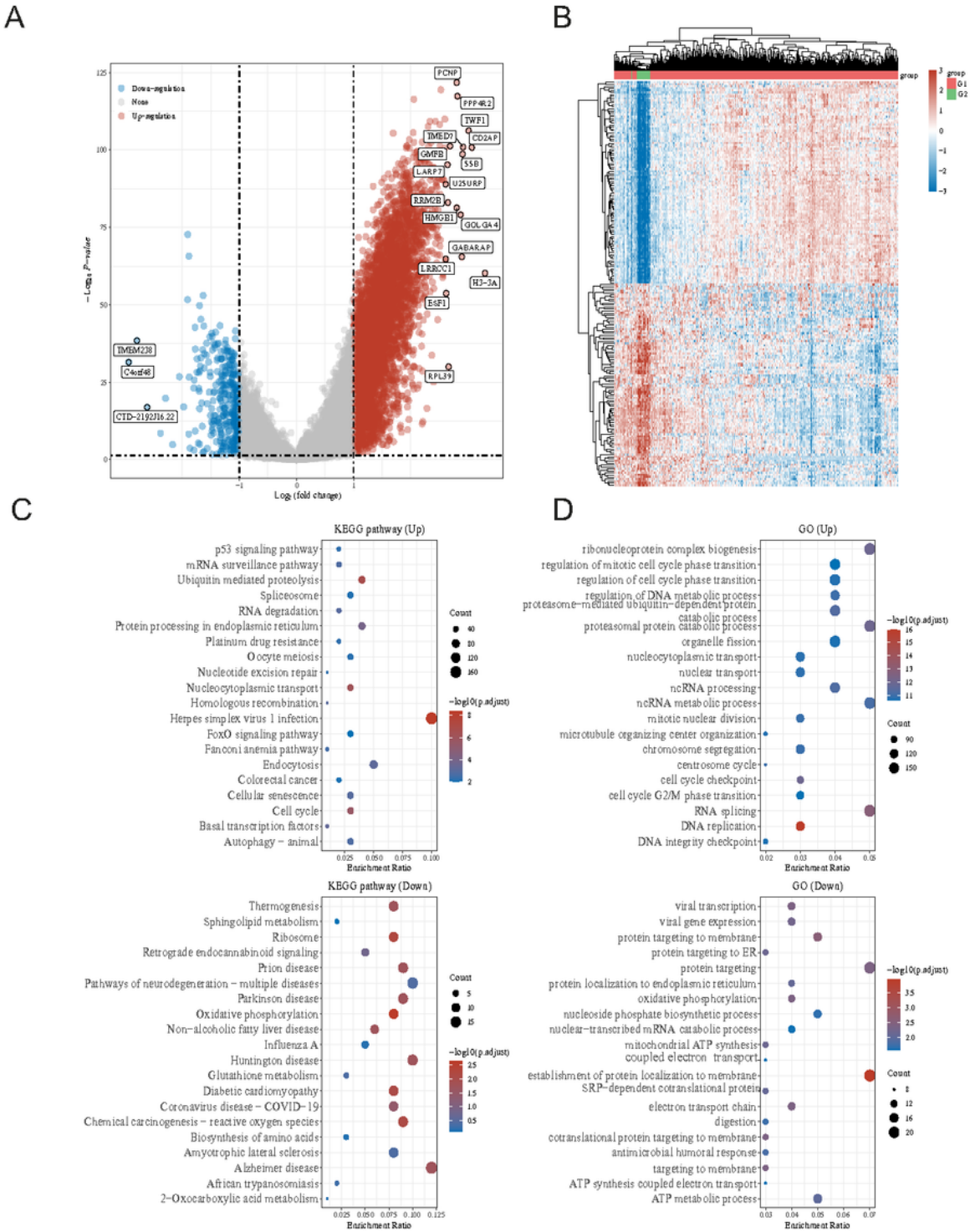

Figure 9

A Volcano plots of clustering analysis of mRNAs, **B** Hierarchical clustering analysis of mRNAs, **C-D** The enriched item in gene ontology (GO) analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of consensus clusters. The size of circles represented the number of genes enriched.

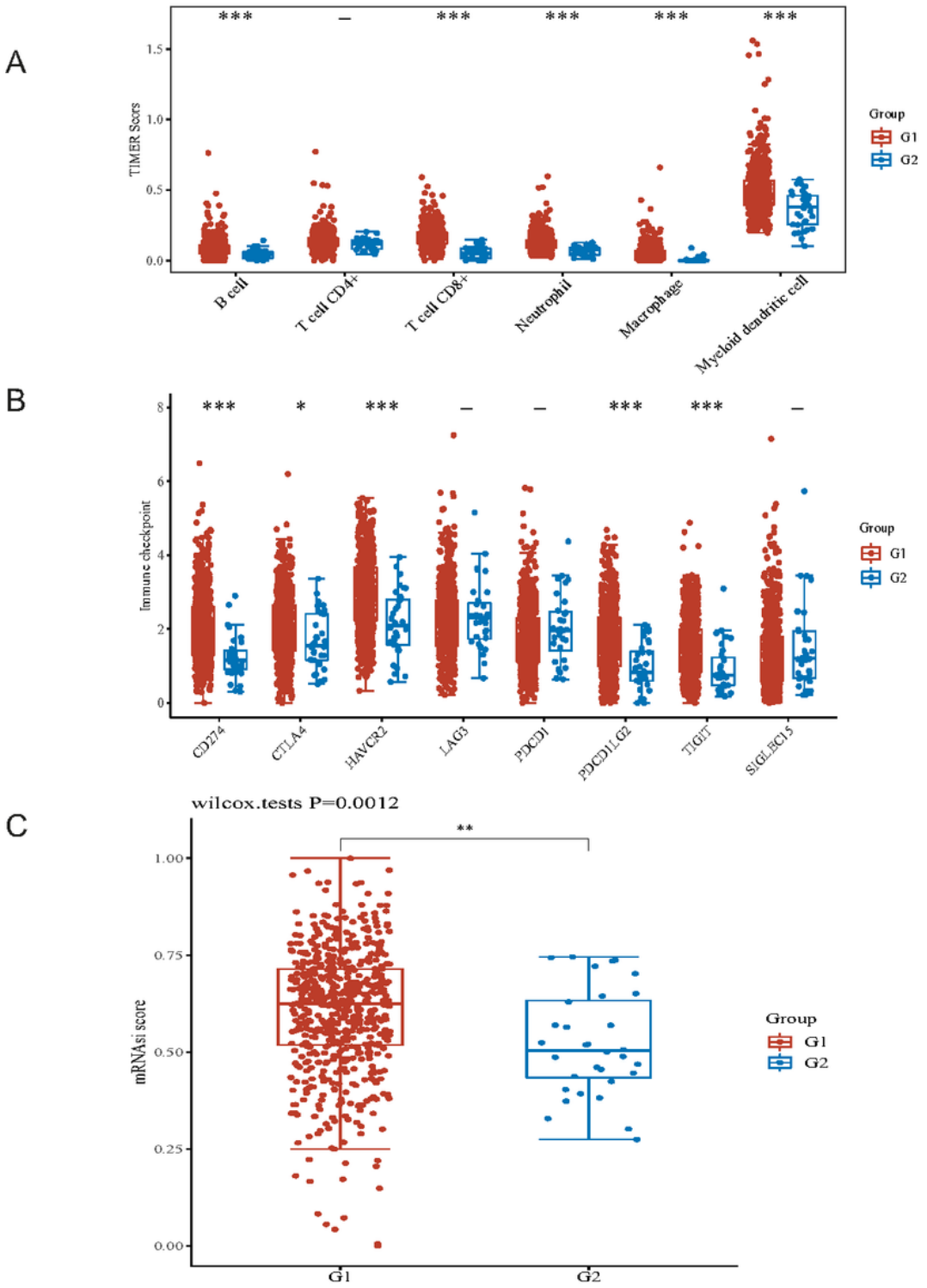

Figure 10

The immune infiltration difference between these two consensus clusters by the TIMER algorithm. **B** The estimate between the two CRGs consensus clusters and the immune checkpoints(SIGLEC15, TIGIT, CD274, HAVCR2, PDCD1, CTLA4, LAG3, and PDCD1LG2). **C** The comparison in mRNA stem index between these two clusters. Asterisks represent levels of significance (*p < 0.05, **p < 0.01, ***p < 0.001,-p, no significance).

Supplementary Files

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- CrosscheckreportbyiThenticate.pdf
- Therawdataforthisstudy.xlsx