TIMP1 expression in colorectal cancer: Linking prognosis, tumor immunity and molecular pathways

JIMING GU[1], DONGMING ZHU[1], FAN CHEN[2], TONGGUO SHI[3*] and SUHUA XIA[2*]

[1]Department of General Surgery, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215000, P.R. China;
[2]Department of Oncology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215000, P.R. China;
[3]Jiangsu Institute of Clinical Immunology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215000, P.R. China

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Abstract. Tissue inhibitor of matrix metalloproteinase 1 (TIMP1) is highly expressed in several cancer types, including lung, brain, prostate, breast and colon cancer, and is associated with a poor prognosis. However, further comprehensive research is required. The present study aimed to explore TIMP1 expression in colorectal cancer (CRC), its prognostic relationship and its connection to tumor immunity, using data from The Cancer Genome Atlas database and clinical samples. The present study evaluated the correlation between TIMP1 expression and CRC by integrating RNA sequencing data, and clinical information from The Cancer Genome Atlas (TCGA), UALCAN and GEPIA2 databases, as well as clinical samples. The findings demonstrated that TIMP1 is highly expressed in CRC tissues and is associated with a poor prognosis. Notably, high TIMP1 levels in CRC were positively correlated with the numbers of $CD4^+$ and $CD8^+$ T cells based on the immune scores, as well as with tumor mutation burden/microsatellite instability. In addition, TIMP1 may be involved in pathways such as those associated with epithelial-mesenchymal transition, extracellular matrix-related processes, collagen formation, angiogenesis, apoptosis and ferroptosis. TIMP1 may also be involved in pathways associated with genes upregulated by reactive oxygen species, tumor inflammation and in the TGF-β signaling pathway. Overall, the results indicate that TIMP1 is associated with prognosis, tumor immunity and several pathways in CRC, potentially offering novel theoretical insights for the treatment of CRC.

Correspondence to: Professor Suhua Xia, Department of Oncology, The First Affiliated Hospital of Soochow University, 899 Pinghai Road, Suzhou, Jiangsu 215000, P.R. China
E-mail: xiasuhua@suda.edu.cn

Professor Tongguo Shi, Jiangsu Institute of Clinical Immunology, The First Affiliated Hospital of Soochow University, 899 Pinghai Road, Suzhou, Jiangsu 215000, P.R. China
E-mail: shitg@suda.edu.cn

*Contributed equally

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Introduction

Colorectal cancer (CRC) ranks third among the most prevalent diseases and is the second deadliest malignant disease globally (1,2). In 2020, there were ~1.9 million newly reported cases of colorectal cancer (CRC) globally, with >900,000 deaths attributed to CRC (3). Key factors contributing to its high mortality include challenges in early diagnosis, tumor heterogeneity and the ineffectiveness of late-stage treatments (4-6). Tumor heterogeneity encompasses both cellular heterogeneity within the tumor and heterogeneity of the tumor microenvironment (7,8). A growing body of research indicates that the heterogeneity of CRC is among the most notable in malignant tumors and exerts a profound influence on the prognosis and treatment of the disease (5,9,10). Moreover, previous studies leveraging bioinformatics analysis for molecular classification and prognostic prediction of CRC have emerged, indicating its considerable clinical promise (11-13). However, due to its heterogeneity, CRC progression is a multifactorial, multistage and dynamic process, which imposes certain limitations on existing models. Consequently, it is imperative to explore novel molecular subtypes and models of CRC for prognostic evaluation.

Tissue inhibitor of matrix metalloproteinase 1 (TIMP1), a member of the TIMP gene family, has been reported to inhibit matrix metalloproteinase (MMP) activity (14). The TIMP1 gene is situated on human chromosome Xp11.1-p11.4 and its mRNA codes for a protein composed of 184 amino acids, exhibiting a molecular mass of 29-34 kDa (15). Accumulating evidence indicates that it interacts with several molecules to regulate biological processes such as cell proliferation, apoptosis and differentiation, and it is implicated in the pathological mechanisms of several diseases, particularly cancer (16,17). A total of >35 independent clinical studies have reported that high TIMP1 expression is positively associated with poor prognosis across several cancer types, including lung, brain, prostate, breast and colon cancer (14). Moreover, the levels of TIMP1 are elevated in advanced-stage tumors (14). Tian *et al* (18) reported that pancreatic cancer cell-derived TIMP1 activates CD63/PI3K/AKT signaling to promote perineural invasion

by stimulating Schwann cells. Moreover, TIMP1 loss has been reported to alter the senescence-associated secretory phenotype of senescent tumor cells and enhance prostate cancer metastasis via MMP activation (19). However, the role of TIMP1 in CRC remains poorly understood. Therefore, the present study aimed to explore the clinical and prognostic significance of TIMP1 in CRC.

Materials and methods

The Cancer Genome Atlas (TCGA). TCGA database (http://cancergenome.nih.gov/) was the primary resource for acquiring CRC mRNA expression data and clinical information. The present study analyzed multi-omics patient data, which were systematically organized across multiple databases, including the original TCGA datasets (http://cancergenome.nih.gov/). Subsequent databases, such as the University of Alabama at Birmingham CANcer data analysis Portal (UALCAN) (20,21) and Gene Expression Profiling Interactive Analysis (GEPIA) (22), provided comprehensive insights into several aspects. The patient records from each database were specifically utilized for diverse analyses.

Gene expression data processing. The UALCAN database (https://ualcan.path.uab.edu/) (20,21) was used to analyze TIMP1 mRNA expression levels in pan-cancer and in colon cancer samples (primary tumor, n=286; normal, n=41). GEPIA2 (http://gepia2.cancer-pku.cn/#general) (21) was used to assess TIMP1 expression in TCGA tumors by matching TCGA normal and Genotype-Tissue Expression project data for CRC (tumor, n=275; normal, n=349). The GSE24550 dataset was used to analyze TIMP1 mRNA expression levels in CRC tissues (G2, n=77) and normal tissues (G1, n=13) (23). In addition, the Clinical Proteomic Tumor Analysis Consortium (CPTAC) database within UALCAN was used to evaluate TIMP1 protein expression in CRC (normal, n=100; primary tumor, n=97).

CRC clinical pathological parameters. STAR-counts data and the corresponding clinical information for CRC tumors were downloaded from TCGA database (24). The data in transcripts per million (TPM) format were extracted and normalized using $\log_2$(TPM+1) transformation. Following filtering of the samples with both RNA sequencing (RNAseq) and clinical data, CRC samples with high TIMP1 expression (top 25%, n=156) and low TIMP1 expression (top 25%, n=157) were selected for analysis (Table I). Statistical analysis was performed using R software (version v4.0.3; The R Foundation). $P<0.05$ was considered to indicate a statistically significant difference.

Survival prognosis analysis. The GEPIA2 database was used for the analysis of the association between TIMP1 expression, overall survival and disease-free survival in patients with CRC. In addition, the UALCAN database was used for the analysis of the association between TIMP1 expression and overall survival. CRC samples with high TIMP1 expression (median, n=309) and low TIMP1 expression (median, n=310) from TCGA database were used for further analysis. For survival analysis, the log-rank test was employed to compare survival differences between the two groups in the Kaplan-Meier analysis, obtaining the P-value, hazard ratio (HR) and 95% confidence interval (CI) using the log-rank test and univariate Cox regression. To perform Cox regression analysis, univariate and multivariate Cox proportional hazards regression analyses were performed. The 'forestplot' package was used to visually present the P-value, HR and 95% CI for each variable using a forest plot. Subsequently, based on the multivariate Cox proportional hazard model results, a Nomogram was constructed using the 'rms' package to predict the total recurrence rate over X years. All statistical analyses were performed using R software (v4.0.3).

Immune correlation analysis. To reliably evaluate immune scores in CRC, 'immunedeconv' was used, which is an R package (v4.1.3) that combines two advanced algorithms, including Tumor IMune Estimation Resource (TIMER) and Estimate the Proportion of Immune and Cancer (EPIC), applied to TCGA CRC data (n=620) (25,26). Each algorithm has been benchmarked for its unique strengths. For result analysis and visualization, the R package 'ggClusterNet' was employed. In addition, Spearman's correlation analysis was performed to assess the relationship between TIMP1 and immune cells ($CD4^+$ and $CD8^+$ T cells) in CRC using TCGA data (n=620).

Association analysis of TIMP1 expression and tumor mutation burden (TMB)/microsatellite instability (MSI). RNAseq data and clinical information of CRC (n=620) were obtained from TCGA database. Spearman's correlation analysis was used to assess the correlation between TIMP1 and TMB/MSI (25,26).

Association analysis of TIMP1 expression and pathway scores. CRC RNAseq data and the clinical information (n=620) were retrieved from TCGA database. Following compilation of the genes from the relevant pathways, the 'GSVA' package was used in R software with the parameter method 'ssgsea' used to perform single-sample gene set enrichment analysis (ssGSEA). Finally, the correlation between gene expression and pathway scores was assessed using Spearman's correlation analysis (27-29).

CRC specimens and immunohistochemical (IHC) staining. A colon cancer tissue microarray (TMA) was purchased from Hunan Aifang Biotechnology Co., Ltd., and the relevant follow-up data were included. The TMA comprised 78 pairs of colon cancer and normal tissues. The patient characteristics are summarized in Table SI. The present study was approved by the Medical Ethics Committee of Soochow University's First Affiliated Hospital (approval no. 2021-327).

IHC staining was performed as follows (30): Tissue samples were subjected to fixation utilizing a 4% paraformaldehyde solution (Beyotime Institute of Biotechnology) maintained at an ambient temperature of 25˚C for a duration of 24 h. Subsequently, the specimens were processed for paraffin embedding. Sections, each with a thickness of 5 μm, excised from the paraffin-embedded tissue blocks, underwent deparaffinization and rehydration procedures. Following antigen retrieval conducted with a 10 mM sodium citrate buffer (pH 6.0; Beyotime Institute of Biotechnology),

Table I. Tissue inhibitor of matrix metalloproteinase 1 expression and clinical features in 313 samples from patients with colorectal cancer.

Characteristic	TIMP1 expression level		P-value
	High (n=156)	Low (n=157)	
Status, n			0.0019
Alive	109 (34.8)	133 (42.5)	
Dead	47 (15.0)	24 (7.7)	
Age			
Mean ± SD	66.7±12.8	64.9±13.4	0.4630
Median (min, max)	68 (31, 90)	66 (31, 90)	0.2150
Sex, n			0.4978
Female	77 (24.6)	71 (22.7)	
Male	79 (25.2)	86 (27.5)	
Ethnicity[a]			0.0404
American Indian	1 (0.3)	0 (0.0)	
Asian	4 (1.3)	2 (0.6)	
Black	12 (3.8)	25 (8.0)	
White	89 (28.4)	69 (22.0)	
pT stage[a]			0.1457
T1	6 (1.9)	6 (1.9)	
T2	14 (4.5)	26 (8.3)	
T3	107 (34.2)	109 (34.8)	
T4	13 (4.2)	9 (2.9)	
T4a	12 (3.8)	4 (1.3)	
T4b	3 (1.0)	2 (0.6)	
Tis	0 (0.0)	1 (0.3)	
pN stage[a]			0.1345
N0	85 (27.2)	103 (32.9)	
N1	23 (7.3)	22 (7.0)	
N1a	6 (1.9)	5 (1.6)	
N1b	7 (2.2)	2 (0.6)	
N1c	1 (0.3)	2 (0.6)	
N2	18 (5.8)	19 (6.1)	
N2a	3 (1.0)	2 (0.6)	
N2b	11 (3.5)	2 (0.6)	
NX	1 (0.3)	0 (0.0)	
pM stage[a]			0.1457
M0	116 (37.1)	114 (36.4)	
M1	16 (5.1)	17 (5.4)	
M1a	6 (1.9)	1 (0.3)	
MX	14 (4.5)	22 (7.0)	
pTNM stage[a]			0.2748
I	15 (4.8)	27 (8.6)	
II	10 (3.2)	9 (2.9)	
IIA	56 (17.9)	51 (16.3)	
IIB	2 (0.6)	7 (2.2)	
III	2 (0.6)	6 (1.9)	
IIIA	5 (1.6)	2 (0.6)	
IIIB	22 (7.0)	19 (6.1)	
IIIC	15 (4.8)	12 (3.8)	
IV	15 (4.8)	14 (4.5)	
IVA	7 (2.2)	4 (1.3)	
IA	0 (0.0)	1 (0.3)	

[a]Certain cases have been excluded from the analysis for the variable due to missing values. Categorical data are presented as n (%). TIMP1, tissue inhibitor of matrix metalloproteinase 1; SD, standard deviation; p, pathological; T, tumor; N, node; M, metastasis.

the sections were treated with a 3% hydrogen peroxide solution at room temperature for 10 min to inhibit endogenous peroxidase activity and to mitigate non-specific protein interactions. The sections were then incubated with anti-TIMP1 antibodies (1:300 dilution; cat. no. 16644-1-AP; Proteintech Group, Inc.) overnight at 4˚C, succeeded by incubation with a biotinylated goat anti-rabbit secondary antibody working solution (1:500 dilution; cat. no. SA1020; Boster Biological Technology Co. Ltd.) at 37˚C for 30 min. Immunodetection was subsequently executed employing the Dako EnVision detection system (Agilent Technologies, Inc.). The prepared slides were examined and images were captured under a fluorescence microscope (Leica Microsystems). The IHC score was determined by multiplying the staining intensity (negative, 0; mild, 1; moderate, 2; and strong, 3) by the percentage of the stained area (0%, 0; ≤25%, 1; 25-50%, 2; 50-75%, 3; and >75%, 4).

Statistical analysis. R software (version 4.0.3) and GraphPad Prism 8.0 (Dotmatics) were used for both data analysis and visualization. The data are presented as the mean ± SD from a minimum of three replicates. Differences between two groups were assessed using an unpaired t-test, Wilcoxon rank-sum test or paired t-test, as appropriate. Fisher's exact test or χ^2 test were used to analyze the associations between the expression of TIMP1 and clinicopathological features. Spearman's correlation analysis was performed to assess the correlation between TIMP1 and immune cells ($CD4^+$ and $CD8^+$ T cells) in CRC using TCGA data (n=620). $P<0.05$ was considered to indicate a statistically significant difference.

Results

TIMP1 is highly expressed in CRC tissues. Using the UALCAN database, TIMP1 mRNA expression was assessed across TCGA cancer tissues. The results indicated that, compared with that in normal tissues, its expression was markedly upregulated in several tumor types, including CRC. In 15/24 tumors, TIMP1 was overexpressed (Fig. 1A). Based on a database visualization website of UALCAN with TCGA, the mRNA expression levels of TIMP1 were also significantly higher in colon cancer samples compared with those in normal samples (Fig. 1B). The GSE24550 dataset was also evaluated, where the specimens of patients with colon cancer exhibited high TIMP1 expression compared

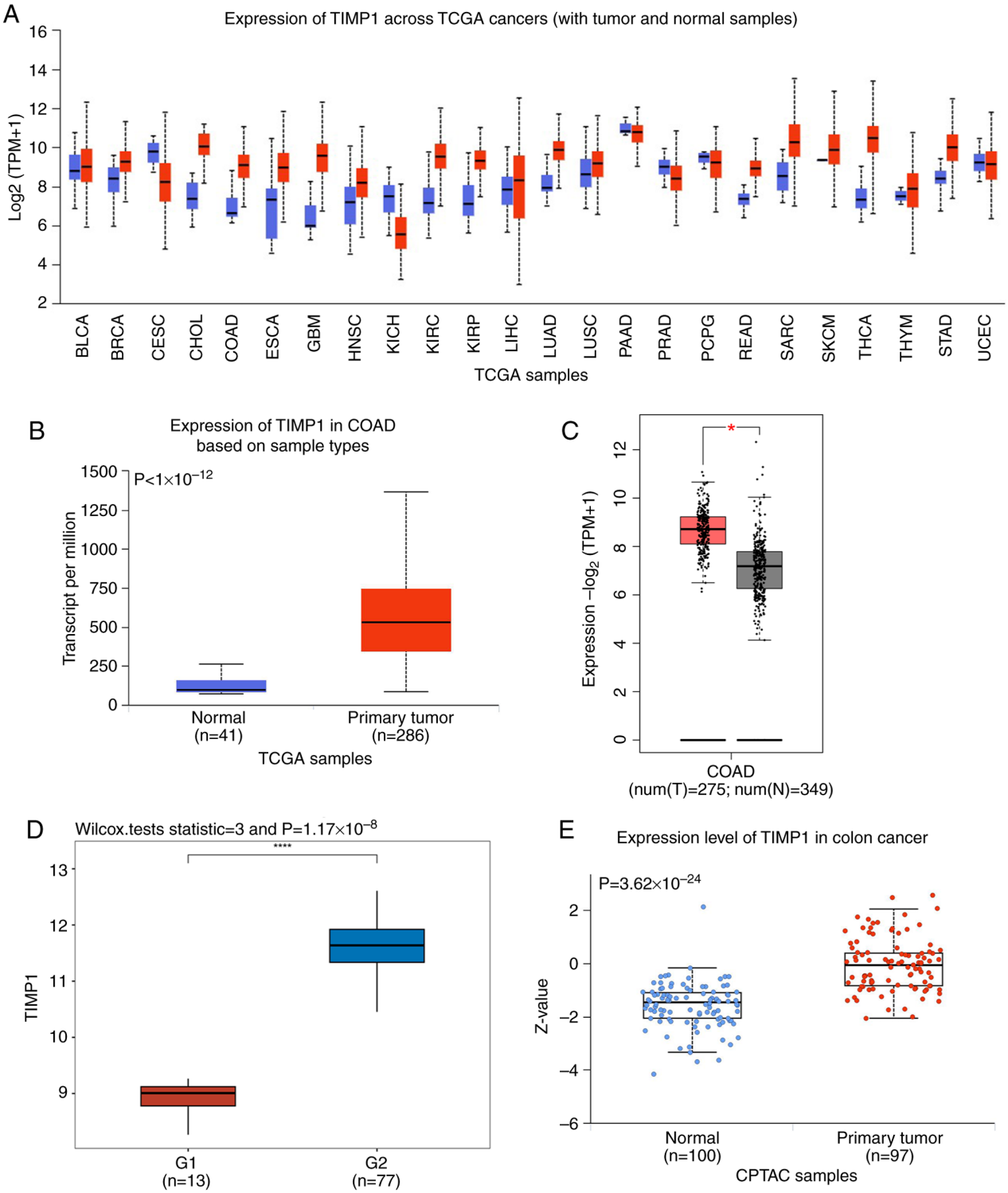

Figure 1. TIMP1 expression in several types of cancer. (A) UALCAN database was used to analyze the expression levels of TIMP1 in several types of pan-cancer tissues (indicated by the red box) and their corresponding normal tissues (indicated by the blue box). (B) Expression of TIMP1 was significantly upregulated in colon cancer tissues compared with that in normal tissues. (C) Expression level of TIMP1 was significantly upregulated in colon tissues compared with that in normal tissues, based on TCGA data from the Gene Expression Profiling Interactive Analysis 2.0 database. (D) Expression level of TIMP1 was significantly upregulated in colon tissues (G2) compared with in normal tissues (G1) in the GSE24550 dataset. (E) Protein expression of TIMP1 in colon tissues from CPTAC data based on the UALCAN database. *P<0.05; ****P<0.0001. TIMP1, tissue inhibitor of matrix metalloproteinase 1; UALCAN, University of Alabama at Birmingham CANcer data analysis Portal; TCGA, The Cancer Genome Atlas; CPTAC, Clinical Proteomic Tumor Analysis Consortium; TPM, transcripts per million; G, grade; COAD, colon adenocarcinoma.

with those of normal controls (Fig. 1C). Furthermore, TCGA data analysis of GEPIA confirmed this result (Fig. 1D). Moreover, CPTAC data revealed that the protein expression of TIMP1 exhibited significant upregulation specifically in the tumor tissue, in comparison with that in normal tissues (Fig. 1E).

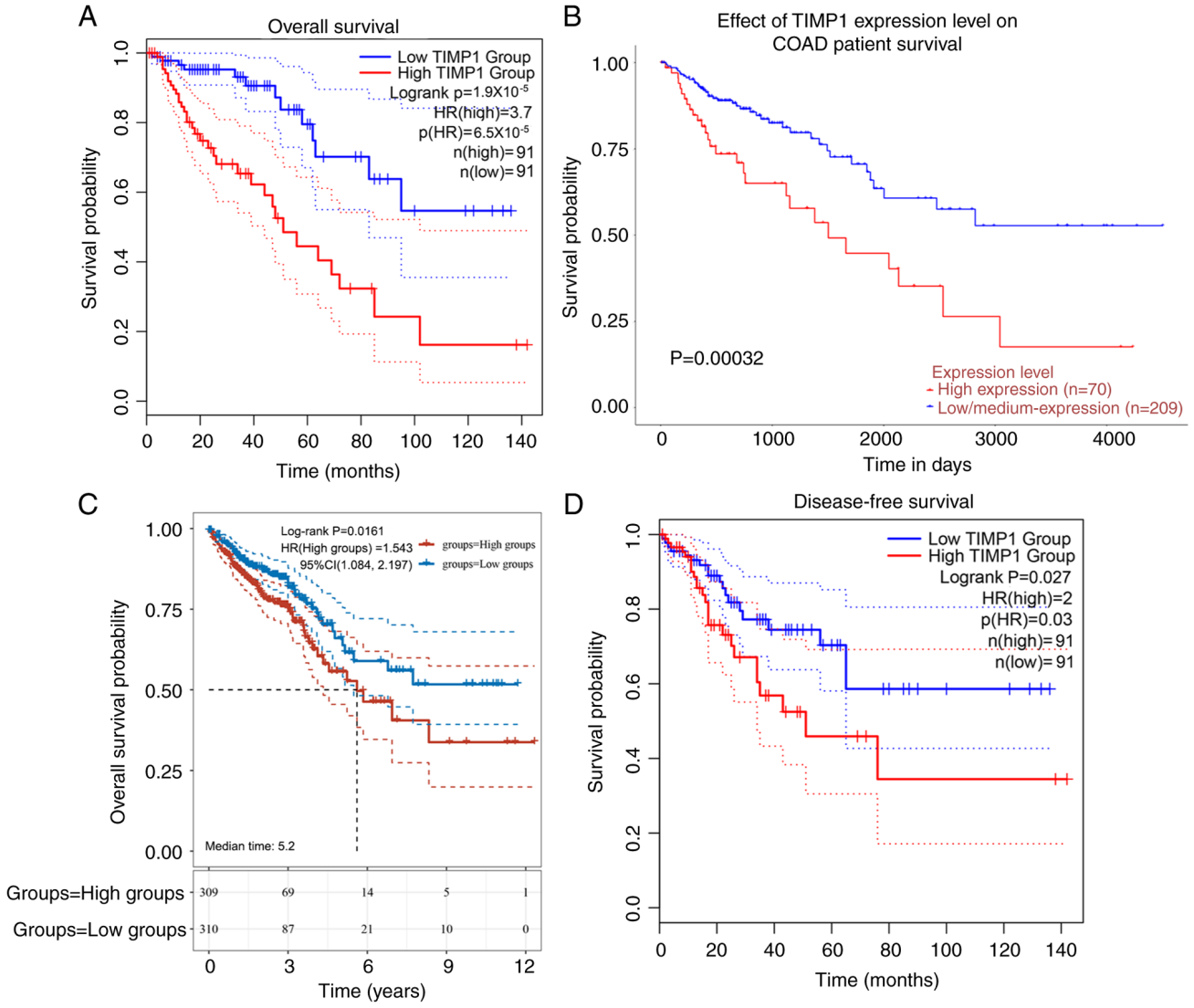

Figure 2. Association between TIMP1 expression and survival prognosis of patients with CRC. (A) Association between TIMP1 expression and the overall survival of patients with CRC was analyzed using the GEPIA 2.0 database. (B) Association between TIMP1 expression and the overall survival of patients with colon cancer was analyzed using the University of Alabama at Birmingham CANcer data analysis Portal database. (C) Kaplan-Meier survival curve for TIMP1 in CRC-The Cancer Genome Atlas data, with different groups compared using the log-rank test. (D) Association between TIMP1 expression and the disease-free survival of patients with CRC was analyzed using the GEPIA 2.0 database. TIMP1, tissue inhibitor of matrix metalloproteinase 1; CRC, colorectal cancer; GEPIA, Gene Expression Profiling Interactive Analysis; HR, hazard ratio; CI, confidence interval; COAD, colon adenocarcinoma.

Associations between TIMP1 and clinicopathological features. To assess the association between TIMP1 expression levels in CRC tissues and the clinicopathological characteristics of patients with CRC, STAR-counts data and relevant clinical information were obtained from TCGA database. The results revealed that TIMP1 expression was significantly associated with survival (P=0019) and ethnicity (P=0.0404); however, no significant associations were noted between TIMP1 levels and age, sex, pathological (p) tumor (T)-node (N)-metastasis (M) stage, pT stage, pN stage or pM stage (Table I).

Association between TIMP1 expression and prognosis. Patients with CRC and high TIMP1 expression exhibited shorter overall survival compared with those with low expression (Fig. 2A-C). Notably, TIMP1 expression was significantly associated with disease-free survival (Fig. 2D). Univariate Cox regression analysis identified TIMP1 expression, age, pT stage (T4 vs. T1), pN stage (N1/2 vs. N0), pM stage (M1/X vs. M0) and pTNM stage (III vs. I and IV vs. I) as significant predictors of overall survival in patients with CRC (Fig. 3A). The results of multivariate Cox regression analysis further confirmed that TIMP1 expression, age, pN stage (N1/2 vs. N0) and pTNM stage (III vs. I, IV vs. I) were independent prognostic factors for overall survival (Fig. 3B). Based on these factors, nomograms were constructed using TCGA datasets to calculate prognostic scores for patients with CRC, yielding a survival probability of 0.715 (Fig. 3C). The calibration curves for TCGA cohorts were closely aligned with the 45-degree diagonal line (Fig. 3D), indicating optimal concordance between predicted and actual survival probabilities.

Association between TIMP1 and immunity. Subsequently, the role of TIMP1 in immunity was used to evaluate its potential

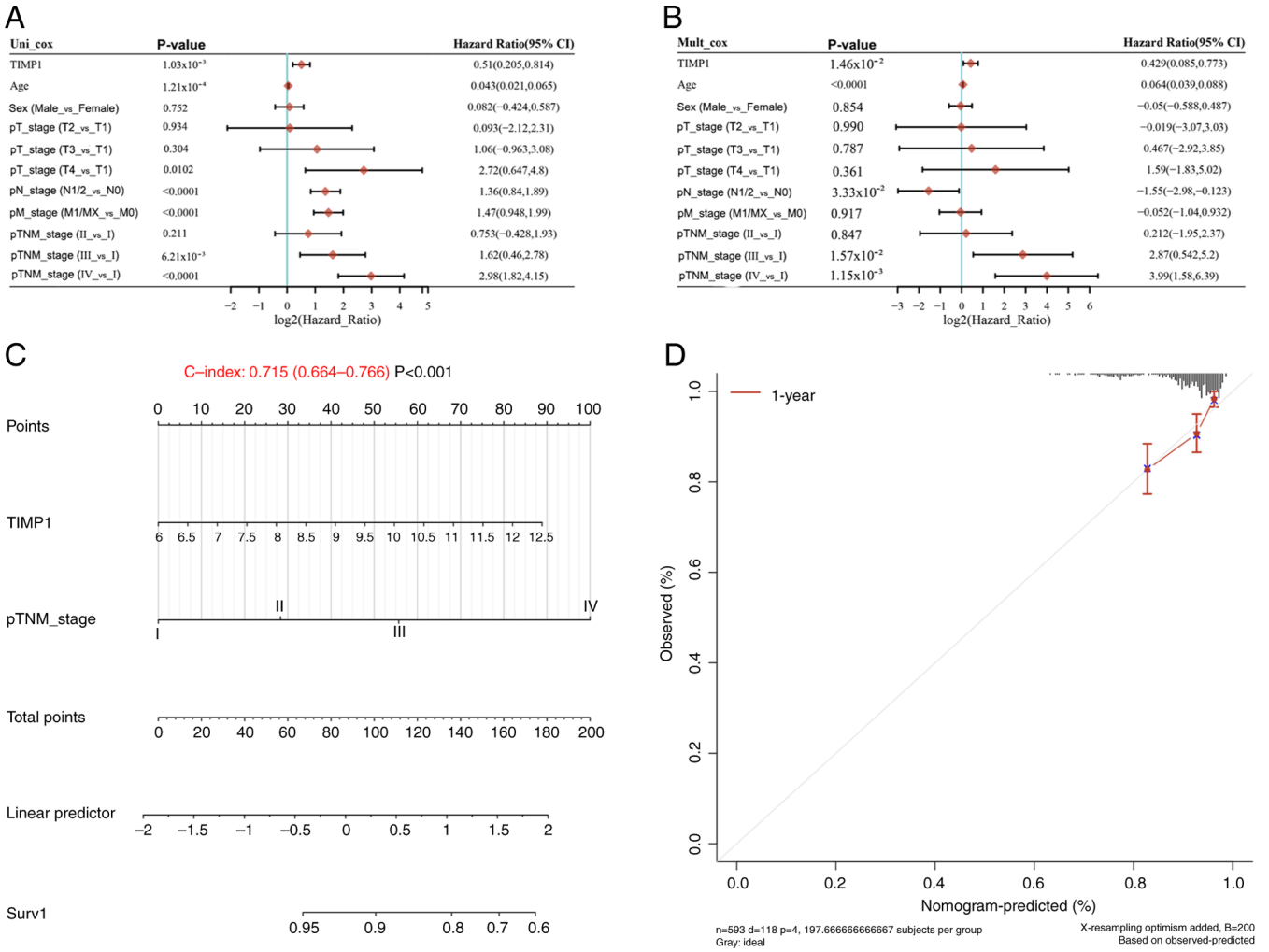

Figure 3. TIMP1 serves as a prognostic factor for overall survival in CRC. (A) Univariate and (B) multivariate Cox analyses of TCGA data of patients with CRC for TIMP1 expression and clinical characteristics. (C) Nomogram formulated to predict 1-year overall survival for patients with CRC from TCGA data. (D) Calibration curve for the overall survival nomogram model in the discovery cohort, where the diagonal dashed line denotes the ideal nomogram, and the red line represent the observed 1-year nomograms. TIMP1, tissue inhibitor of matrix metalloproteinase 1; CRC, colorectal cancer; TCGA, The Cancer Genome Atlas; HR, hazard ratio; CI, confidence interval; p, pathological; T, tumor; N, node; M, metastasis.

in CRC immunotherapy. Network connection diagrams and heatmaps illustrated the link between TIMP1 expression and immune scores, where red/blue intensity and ring size reflected correlation strength (Fig. 4A and B). Red lines indicate the positive correlations and green lines the negative. Both TIMER and EPIC scores revealed the positive correlation of TIMP1 with $CD4^+$ and $CD8^+$ T cells. Spearman's correlation analysis using TCGA CRC samples further confirmed this significant association (Fig. 4C).

Previous studies have reported that TMB and MSI are valuable biomarkers that provide insights into cancer prognosis and treatment decisions (31-33). Both TMB and MSI serve roles in cancer prognosis, treatment selection and immunotherapy development (34). The findings of the present study demonstrate a significant but weak positive correlation between TIMP1 expression and TMB and MSI in CRC (Fig. 4D). This suggests that TIMP1 can potentially serve as a biomarker to identify patients who may benefit from immune checkpoint inhibitors, particularly in tumors where high TMB and MSI predict a favorable response to immunotherapy.

TIMP1 and pathways. The ssGSEA algorithm was applied to compute the enrichment fraction of each sample for specific pathways, thereby exploring the sample-pathway relationship (27). The calculated fractions intuitively reflected the interaction and association between samples and pathways. The results indicated that in CRC, TIMP1 may be positively correlated with epithelial-mesenchymal transition (EMT) markers, extracellular matrix (ECM)-related genes, collagen formation, angiogenesis, apoptosis, ferroptosis, the upregulation of the expression levels of genes caused by reactive oxygen species (ROS), tumor inflammation and the TGF-β signaling pathway (Fig. 5). Overall, these findings indicate that TIMP1 may be associated with multiple pathways and may regulate the progression of CRC.

TIMP1 protein expression in CRC tissues and prognosis. The IHC assay indicated that the expression levels of TIMP1 were significantly higher in colon cancer tissues than those in adjacent normal tissues (Fig. 6A and B). Notably, individuals with high TIMP1 expression (IHC score >4) exhibited a significantly reduced survival rate compared with those

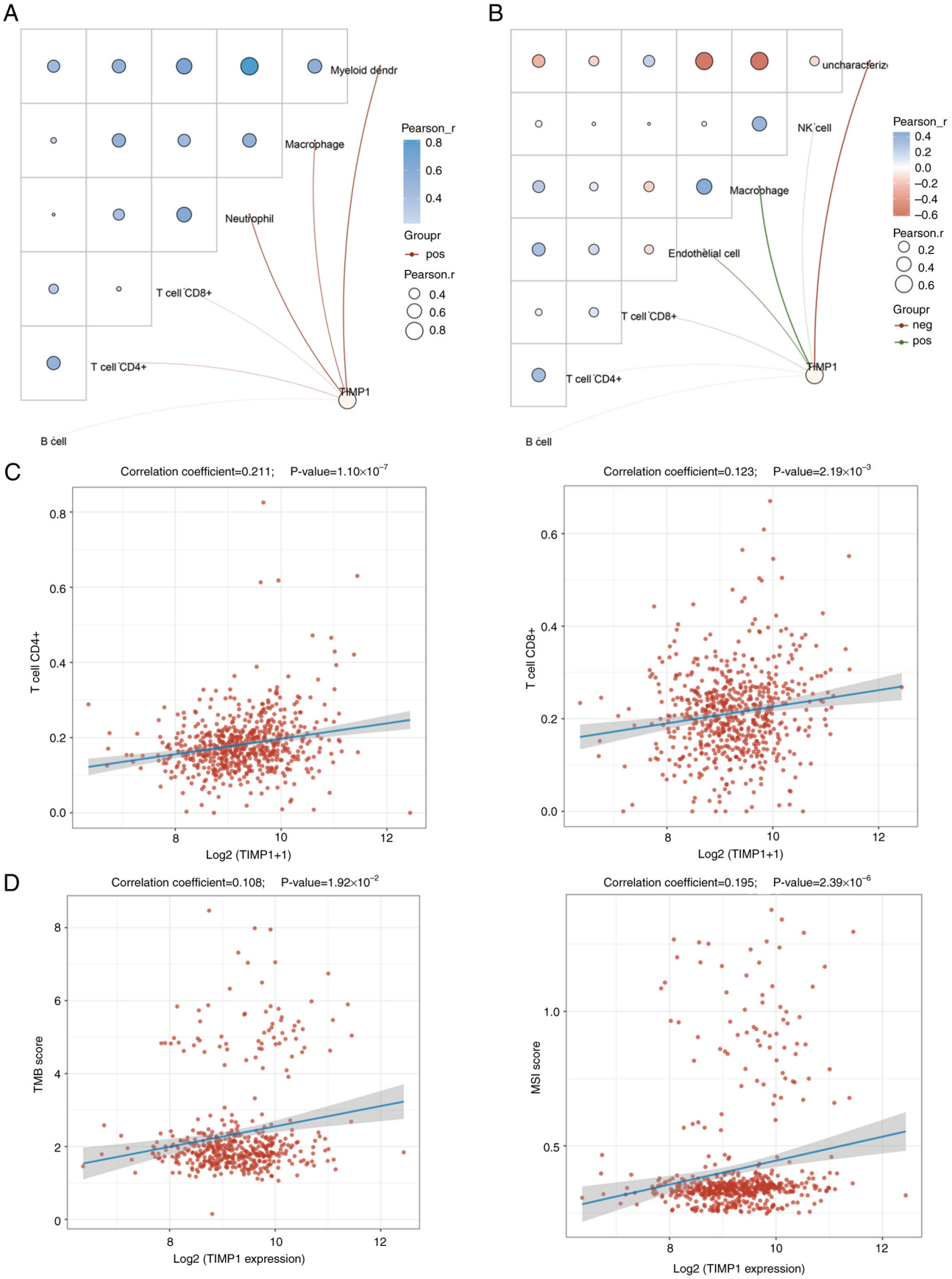

Figure 4. Association between TIMP1 expression and immunity in CRC. Heatmap illustrates the correlation analysis of TIMP1 expression with (A) TIMER and (B) EPIC scores. Red signifies a positive correlation, whilst blue indicates a negative correlation. The intensity of the red or blue color reflects the strength of the correlation, and the size of the circle indicates the magnitude of the correlation. In the schematic, a red line denotes a negative correlation between model scores/gene expression and immune scores, whereas a green line denotes a positive correlation. (C) Spearman's correlation analysis of TIMP1 expression with $CD4^+$ and $CD8^+$ T cells in CRC from The Cancer Genome Atlas database. (D) Spearman's correlation analysis of TIMP1 expression with TMB/MSI score distribution is shown. TIMP1, tissue inhibitor of matrix metalloproteinase 1; CRC, colorectal cancer; TMB, tumor mutation burden; MSI,/microsatellite instability; NK, natural killer.

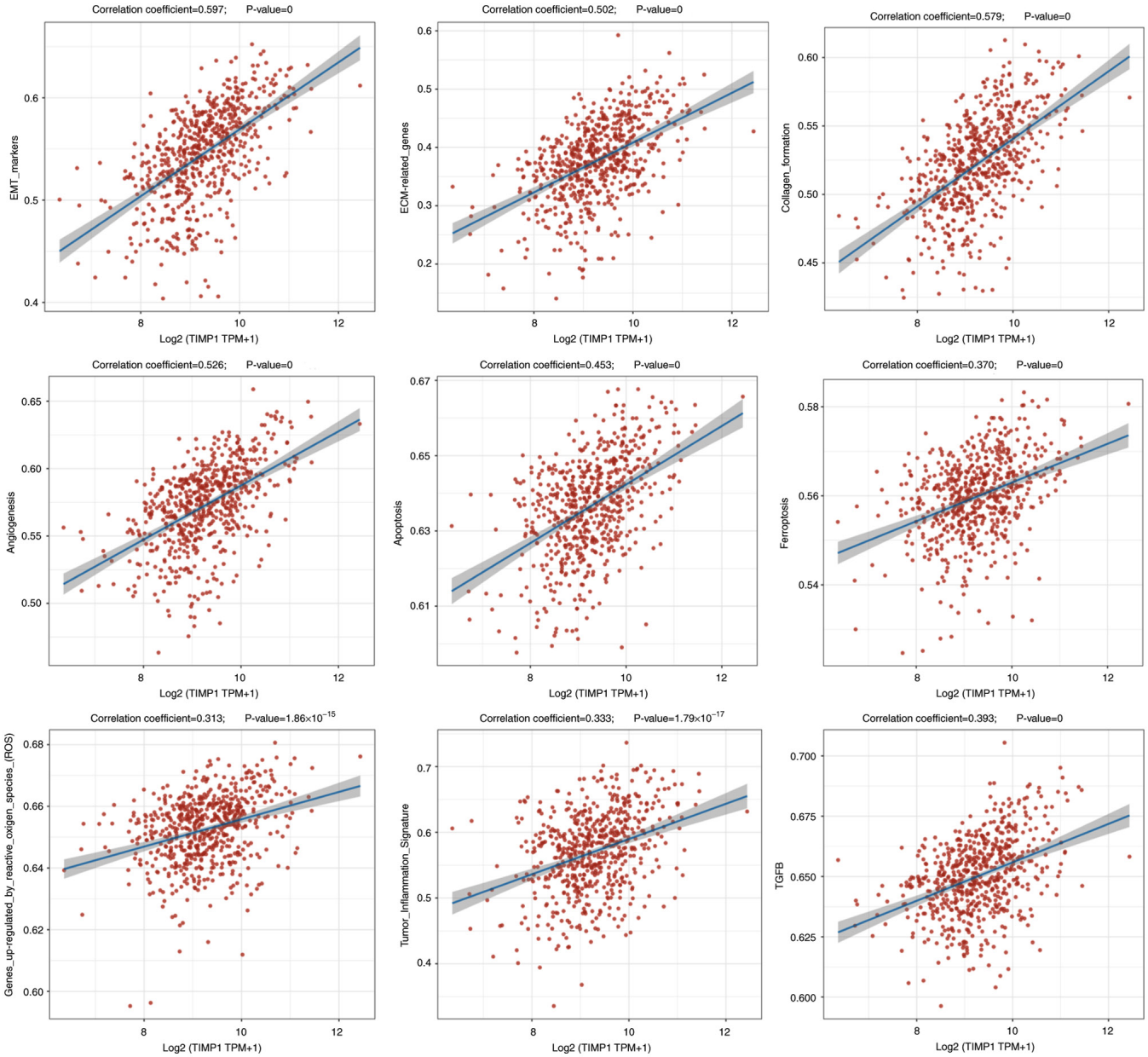

Figure 5. Correlation of TIMP1 and signaling pathways in CRC. Spearman's correlation analysis plots reveal the correlation between pathway scores and TIMP1 expression in CRC samples from The Cancer Genome Atlas database. TIMP1, tissue inhibitor of matrix metalloproteinase 1; CRC, colorectal cancer; TPM, transcripts per million; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix.

with low TIMP1 expression (IHC score ≤4) in colon cancer (Fig. 6C).

Discussion

Most patients with CRC are diagnosed at an advanced stage, which contributes to the poor prognosis commonly noted in this patient population (35,36). However, previous research indicates that an early diagnosis of CRC can markedly improve patient outcomes (37). As such, the identification of effective biomarkers for early CRC detection is crucial, as they can serve a pivotal role in enhancing the prognosis of patients with CRC.

TIMP1 belongs to the TIMP family, which includes other members such as TIMP2, TIMP3 and TIMP4 (14). Research has indicated that TIMP1 expression is upregulated across multiple tumor types and this upregulation is associated with poor prognosis and reduced survival in patients with cancer (16). For example, TIMP1 expression has been associated with gastric cancer differentiation and poor prognosis in patients with gastric cancer (38). Similarly, high TIMP1 expression in lung cancer tumors is associated with an unfavorable prognosis (39). Macedo *et al* (40) also reported that elevated TIMP1 levels in patients with colon and gastric cancer are associated with poor prognosis. These reports are consistent with the findings of the present study, which revealed that both TIMP1 mRNA and protein levels were markedly elevated in CRC tissues, and positively associated with worse overall and disease-free survival in patients with CRC. Univariate and multivariate Cox regression analyses further established high

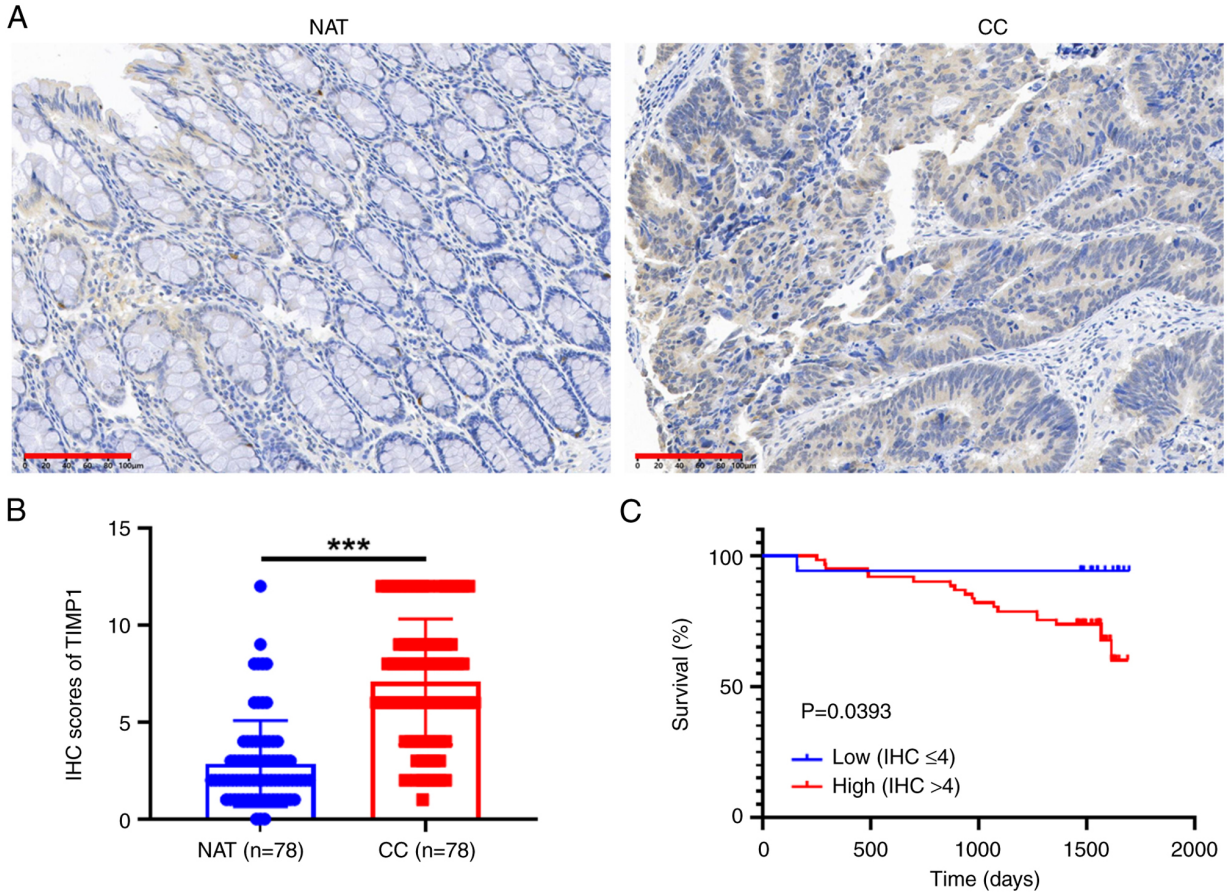

Figure 6. TIMP1 protein expression in the colorectal cancer cohort in the present study. (A) Representative images of IHC analysis of TIMP1 protein expression of NAT (n=78) and CC (n=78) tissue sections. Scale bar, 100 μm. (B) TIMP1 protein expression based on their staining index in NAT and CC specimens. (C) Patients with CC with high TIMP1 expression (IHC score ≥4) had a significantly worse prognosis than patients with low TIMP1 expression (IHC score <4). ***P<0.001. TIMP1, tissue inhibitor of matrix metalloproteinase 1; IHC, immunohistochemistry; NAT, nonmalignant adjacent tissues; CC, colon cancer.

TIMP1 expression as an independent prognostic indicator for overall survival in CRC. These results indicate that TIMP1 expression is likely a predictor of CRC prognosis.

Previous studies have established immunity as a prognostic indicator for cancer progression (41-43). Emerging mechanistic studies identify TIMP1 as a critical tumor immune modulator (44,45). In thyroid cancer, TIMP1 promotes cancer cell progression by inducing macrophage phenotypic polarization via the PI3K/AKT signaling pathway (44). In glioblastoma, the expression of TIMP1, induced by Sp1, is markedly elevated in tumor-infiltrating lymphocytes and is associated with cancer progression (45). Moreover, TIMP1 expression in gliomas is associated with tumor immune infiltration and immune checkpoint-related gene expression (46). Wang *et al* (47) reported that intracellular TIMP-1-CD63 signaling directs immune escape and metastasis evolution in KRAS-mutated pancreatic cancer cells. In the present study, network connection diagrams and heatmaps visualized the association between TIMP1 expression and immune scores. Notably, TIMP1 indicated a significant but weak correlation with $CD4^+$ and $CD8^+$ T cells in CRC. Overall, these findings suggest that TIMP1 may modulate the CRC tumor immune environment by influencing $CD4^+$ and $CD8^+$ T cell infiltration and function.

Immunotherapy, particularly the use of immune checkpoint inhibitors (ICIs), has transformed cancer treatment by leveraging the capacity of the immune system to combat tumors (48). However, in contrast to cancer types, such as non-small-cell lung cancer and melanoma, which are known to be sensitive to immunotherapy, there is a lack of reliable predictive biomarkers in multiple cancer types (49). TMB and MSI serve as genomic biomarkers to identify patients likely to benefit from ICIs, and ICIs have shown promise in colon cancer treatment, notably in patients with high TMB and MSI (34). The present study indicated a significant association between TIMP1 expression and TMB and MSI in CRC. This suggests that TIMP1 may be a potential predictive biomarker for colon cancer immunotherapy.

Furthermore, previous studies have reported that TIMP-1 can regulate tumor cell behavior by inducing signaling pathways associated with cell growth, proliferation and survival (14,16). For example, Song *et al* (50) reported that inhibiting TIMP1 expression reduced proliferation and metastasis, whilst promoting apoptosis by targeting the FAK-PI3K/AKT and MAPK pathways. The use of short hairpin RNA to knockdown TIMP1 expression was associated with a notable reduction in cell proliferation and invasion in right-sided patient-derived organoids from both left- and right-sided CRCs. This effect was achieved by modulating the FAK/AKT signaling pathway (51). In the present study, the ssGSEA algorithm revealed a positive correlation between TIMP1 expression in CRC and multiple biological processes and pathways, including EMT markers, ECM-related genes, collagen formation, angiogenesis,

apoptosis, ferroptosis, ROS-upregulated genes, tumor inflammation signatures and the TGF-β pathway. Whilst further comprehensive experimental validation is required to evaluate the pathways associated with TIMP1, the existing data strongly suggest that TIMP1 serves pivotal roles in driving CRC progression and metastasis.

Moreover, whilst the present study highlights the clinical significance of TIMP1, certain limitations are present. Firstly, specific analyses rely on retrospective public-database data; therefore, prospective studies are required to verify the clinical relevance of the findings. Given the complexity of CRC and the varied histological phenotypes, more detailed mechanistic and clinical research is essential to explore the roles of TIMP1 across CRC subtypes. In addition, the sample size of the present CRC cohort is limited. In future work, the validation cohort will be expanded by constructing a larger, multi-center TMA with balanced representation of major CRC subtypes (such as CMS, MSI-H and BRAF-mutant), on which subtype-stratified analyses will be performed to determine if the role of TIMP1 is universal or subtype-specific. Furthermore, comprehensive *in vitro* and *in vivo* experiments are required to clarify the mechanistic role of TIMP1 in tumor progression and its interaction with the TME. Despite these limitations, the present study guides future research, including clinical work and basic experiments, which will be part of the subsequent research focus.

In conclusion, high TIMP1 expression in CRC is associated with a poor prognosis for patients with CRC. TIMP1 may also modulate the CRC immune microenvironment and facilitate CRC progression and metastasis. The data suggest that TIMP1 represents a promising diagnostic biomarker and therapeutic target for CRC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SX, DZ and TS contributed to the study conception and design. JG contributed to performing experiments, acquisition of data and interpretation of data. FC performed experiments. JG and TS wrote the original manuscript. DZ and SX revised the manuscript. JG and TS confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was granted approval by the Institutional Review Board of the First Affiliated Hospital of Soochow University (Suzhou, China; approval no. 2021-327). Written informed consent was obtained from all participating patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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