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Analysis of Expression and Prognosis Value for Matrix Metalloproteinases in Human Colorectal Carcinoma

Ping Li[1], Sijin Li[1], Xing Zhao[3], Yajie Dong[4], He Song[1], Hairu Ji[2], Peiyuan He[1,*], Zhiping Hou[2,*]

[1]Department of Gastroenterology, The Affiliated Hospital of Chengde Medical University, Chengde 067400, Hebei, China.
[2]Department of Pathology, Chengde Medical University, Chengde 067050, Hebei, China.
[3]Department of Pathology, The Affiliated Hospital of Chengde Medical University, Chengde 067400, Hebei, China.
[4]Department of Pathophysiology, Chengde Medical University, Chengde 067050, Hebei, China.

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***Corresponding author:** Peiyuan He, Department of Gastroenterology, The Affiliated Hospital of Chengde Medical University, Chengde 067400, Hebei, China; Zhiping Hou, Department of Pathology, Chengde Medical University, Chengde 067050, Hebei, China.

Abstract

Background: Matrix metalloproteinases (MMPs), a family of endogenous proteases are implicated in the progression of several cancer types. Previous studies report that MMPs participate in tumorigenesis of colorectal cancer. However, the aberrant expression of MMP members and their roles in the prognosis of colorectal cancer has not been explored. **Methods:** This study sought to explore differentially expressed genes and compared the transcriptional expression with survival data of MMPs in colorectal cancer (CRC) samples using Oncomine, cBioPortal databases, GEPIA (Gene Expression Profiling Interactive Analysis), and Kaplan-Meier Plotter online tools. **Results:** MMP1,3,7,9,11,12 and 28 showed high expression levels in CRC tissues, but only expression of MMP11 and 12 were correlated with advanced tumor stages. **Conclusion:** The findings of this study show that MMP1,3,7,9,11 and 28 are potential targets for the effective treatment of individuals with CRC, while MMP9 and 12 are promising novel prognostic signatures of CRC.

Keywords

Bioinformatics; Colon adenocarcinoma (COAD); Colorectal cancer (CRC); Matrix metalloproteinases (MMPs); Overexpression; Prognosis

1. Introduction

Matrix metalloproteinases (MMPs) are a family of endogenous proteases that require zinc and calcium for catalytic activity. These enzymes play key roles in maintaining and reconstructing extracellular matrix, such as embryonic development [1, 2], morphogenesis [3, 4], reproduction [5] and tissue remodeling [6]. It is also involved in inflammation [7, 8]. MMPs degrade most kinds of protein constituents in extracellular matrix, degrade the histological barrier of tumor cell infiltration, and play an indispensable role in tumor apoptosis, and metastasis [9]. Several studies have explored the role of MMPs in tumor infiltration along with metastasis. Notably, MMPs are considered the primary proteolytic enzyme in tumor infiltration.

Currently, 28 MMPs family members have been isolated and identified. MMPs can be divided into five categories based on the substrate and fragment homology [10]. The categories include (1) collagenase, whose main hydrolytic substrate is fibrous collagen, including type III, II and I collagen, and MMP-1, MMP-8 and MMP-13 fall in this class; (2) gelatinase, which is classified into gelatinase B (MMP-9), and gelatinase A (MMP-2). Gelatinase primarily hydrolyzes denatured collagen, and type IV collagen, which is the primary constituent of the basement membrane; (3)

stromal degradation factors including MMP-7 and MMP-26; (4) stromal dissolution factors including MMP-3, MMP-7, MMP-10 and MMP11; (5) membrane type matrix metalloproteinases (MT MMPs): including MMP-25, MMP-24, MMP-17, MMP-16, MMP-15, and MMP-14. In addition, to the five major MMPs subgroups, a few MMPs are not categorized into any of these classes, such as epilysin (MMP-28), metalloelastase (MMP-12), enamelysin (MMP-20), and RASI-1 (MMP-19) [11].

Colorectal cancer (CRC) is the most commonly diagnosed cancer worldwide [12]. In 2022, more than 1.9 million cases were diagnosed. Colorectal cancer is the second most common cause of cancer death, leading to more than 900 000 deaths per year (https://www.iarc.who.int/cancer-type/colorectal-cancer/). WHO 2010 classification of colorectal tumors of colorectal carcinoma [13, 14] reports that CRC types include adenocarcinoma (including mucinous adenocarcinoma), undifferentiated carcinoma, signet-ring cell carcinoma, small cell carcinoma, adenosquamous carcinoma, and squamous cell carcinoma. Adenocarcinoma is the most frequent CRC type; and it represents more than 95% of all CRC cases. Conventional approaches for treatment of CRC include surgery, radiotherapy, and/or chemotherapy. Notably, 10%-15% of colorectal cancer occurs in first-degree relatives (including parents, siblings, and children) who have colorectal cancer, and the genetic risk is high due to familial aggregation of colorectal cancer. Both domestic and international consensus and guidelines on colorectal cancer reported that exploring molecular markers is an important factor in making treatment decisions for metastatic CRC. In addition, these guidelines unanimously recommend that the routine detection of molecular markers should include: Ras (including KRAS and NRAS), BRAF, MMR/MSI, which effectively guide treatment and prognosis judgment [15, 16].

In spite of the recent advancements in the management of CRC, consisting of early diagnosis and effective therapeutic approaches, 5% to 10% of individuals with CRC present with metastatic disease condition at the initial CRC diagnosis. Out of these, only a fifth survive for five years [17]. The current prognostic biomarkers are limited due to tumor diversity, therefore, there is a need to develop novel predictive biomarkers for effective prognosis and for the development of personalized treatment.

Aberrant expression of MMPs factors and correlation with clinico-pathological characteristics and the prognosis value have been reported in human colorectal cancer. However, studies have not explored the function of MMPs in CRC using bioinformatics analysis. DNA and RNA research plays a critical component in biomedical and biological fields of research and has shown tremendous advances with the incorporation of microarray technology [18]. This study sought to explore expressions and mutations of distinct MMPs factors in individuals with colorectal cancer to establish expression trends, prospective roles, and distinct prognostic significance of MMPs in colorectal cancer using gene expression and copy number data available in various databases.

2. Materials and Methods

2.1 Ethics Statement

This study was approved by the Academic Committee of Chengde Medical University. The study was conducted following the principles of the Declaration of Helsinki. All datasets were retrieved from published literature. All participants provided written informed consent.

2.2 Oncomine Data Resource Analysis

Oncomine webserver was used to explore the mRNA levels of MMPs in distinct cancers (https://www.oncomine.org/resource/login.html, an online cancer microarray database). Transcriptional expression levels of MMPs between clinical cancer and non-malignant samples were compared using the Student's t-test. The threshold P value was 0.001 and a fold change of 2.

2.3 GEPIA Analysis

GEPIA webserver was used for the analysis of RNA sequencing expression data of 9,736 cancer and 8,587 non-malignant samples retrieved from The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) and GTEx (https://commonfund.nih.gov/gtex) databases. GEPIA offers tools that can be customized. It can be used to perform survival analysis of patients, differential expression analyses between tumor and non-malignant samples, correlation analysis, profiling based on different cancer types, or pathological stages of cancer, determination of similar genes, and analyses of dimensionality reduction [19].

In this study, the expression of MMPs was profiled based on the tissue in colon cancer using a box plot, with |Log2FC| Cutoff = 1, p-value Cutoff = 0.01, and log2 (TPM + 1) was used for log-scale. Major stage (yes) or sub-stage (no) was used for plotting stage plots.

In the survival analysis of patients, genes with significant correlation with the survival of patients were determined, and MMPs expression in colon cancer were plotted.

2.4 Kaplan-Meier Plotter

The predictive value of the mRNA expression of STAT (signal transducer and activator of transcription) was explored using the Kaplan-Meier Plotter webserver (www.kmplot.com). Relapse free survival (RFS) of individuals with rectal cancer was explored. The 270 patient samples were divided into two groups based on the median expression (high versus low expression). Kaplan-Meier survival curve was used to evaluate survival of patients in the two groups, with the hazard ratio with 95% confidence intervals. JetSet best probe set of MMPs was selected to construct Kaplan-Meier curves.

2.5 Co-expression Analyses Using cBioPortal Tool

TCGA has sequencing and pathological data for 30 different cancers [20]. The colorectal adenocarcinoma (The Cancer Genome Atlas, Provisional) cohort, comprising data from 640 cases (392 colon adenocarcinoma, 169 rectal adenocarcinoma, 66 mucinous adenocarcinoma of the colon and rectum, 13 colorectal adenocarcinoma) with pathology reports, was chosen for further analyses of MMPs using cBioPortal (https://www.cbioportal.org/results). Co-expression and network analyses of the genomic profiles were performed based on the cBioPortal's online guidelines.

2.6 Immunohistochemistry

3-mm tumor slices were incubated overnight with rabbit polyclonal antibodies against MMP1, MMP3, MMP7, MMP9, MMP11, MMP12, and MMP28 (all from Santa Cruz Biotechnology; 1:100) at 4 ºC. The samples were then incubated at room temperature with HRP-labelled antibodies (Santa Cruz Biotechnology, Santa Cruz, CA; 1:500) for two hours. After incubation, samples were stained with DAB (Cat No, Vector Laboratories, Burlingame, CA), and mounted on the slides using Vectashield mounting medium (Cat No, Vector Laboratories). Samples were then observed under a light microscope (Olympus 600 auto-biochemical analyzer, Tokyo, Japan). Assays without the primary antibody served as negative controls and showed that the observed signals were specific.

2.7 Statistical Analysis

mRNAs expression levels of MMPs in the colorectal and control groups were compared using the Student's t-test; Pearson's correlation coefficient was used to determine MMPs associated with each other using their mRNA expressions. The correlation between mRNA expression levels of MMPs and the survival of colorectal cancer patients was analyzed using the Kaplan-Meier curve. The log rank method was used to compare the survival curves.

3. Results

3.1 Transcriptional Levels of MMPs in Patients with Colon Adenocarcinoma (COAD)

mRNA levels of MMPs in cancers were compared with those in non-malignant samples using the Oncomine data resource (Figure 1). The transcriptional expression level of MMP1 was significantly upregulated in patients with COAD in seven out of ten datasets, and 14 out of 25 analyses met the fold change 2. MMP3 mRNA expression level was significantly higher in 7 out of 11 datasets compared with controls, and 13 out of 33 analyses met the fold change 2. MMP7 was significantly upregulated in patients with COAD in 9 out of 13 datasets, and 20 out of 35 analyses met the fold change 2. Notably, MMP9 expression in all the datasets showed no significance difference compared with the control. MMP11 was significantly upregulated in patients with COAD in 9 out of 11 datasets, and 15 out of 24 analyses met the fold change 2. MMP12 was significantly upregulated in patients with COAD in 7 out of 8 datasets, and 13 out of 23 analyses met the fold change 2. Further, MMP28 was significantly upregulated in normal tissue in 7 out of 8 datasets, and 20 out of 23 analyses met the fold change 2.

Overexpressed MMPs in each dataset are presented in appendix Table 1 with the fold change, p-value, and source.

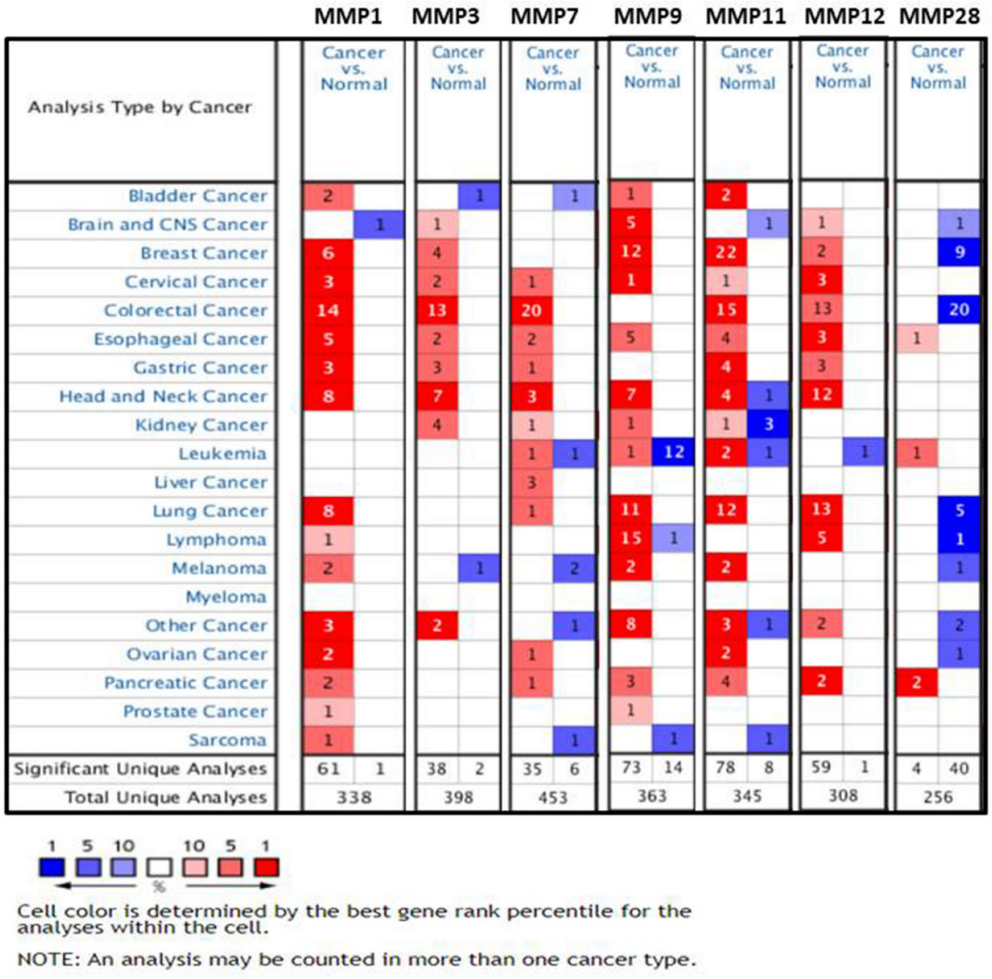

Figure 1. Transcription Levels of MMPs in Different Types of Cancers (using Oncomine database).

Table 1. The Significant Changes of MMPs Expression in Transcription Level Between Different Types of COAD and Normal Tissues (Oncomine Database)

	Type of colorectal cancer versus normal tissue	Fold change	p value	source and /or reference
MMP1	Colon Adenocarcinoma vs. Normal	7.778	3.33E-06	**Notterman Colon Statistics**
	Colorectal Carcinoma vs. Normal	6.222	5.60E-12	**Skrzypczak Colorectal Statistics**
	Colorectal Adenocarcinoma vs. Normal	4.763	2.97E-10	
	Rectal Adenoma vs. Normal	3.91	1.71E-07	**Sabates-Bellver Colon Statistics**
	Colon Adenoma vs. Normal	3.982	1.30E-10	
	Rectal Adenocarcinoma vs. Normal	4.274	2.44E-26	**Gaedcke Colonectal Statistics**
	Rectal Mucinous Adenocarcinoma vs. Normal	3.745	5.50E-06	**TCGA Colorectal Statistics**
	Colon Mucinous Adenocarcinoma vs. Normal	4.911	3.93E-07	
	Rectal Mucinous Adenocarcinoma vs. Normal	4.510	1.18E-04	
	Rectal Adenocarcinoma vs. Normal	3.937	1.78E-04	**Kaiser Colon Statistics**
	Colon Mucinous Adenocarcinoma vs. Normal	4.786	1.80E-05	
	Colon Carcinoma Epithelia vs. Normal	15.694	8.88E-08	**Skrzypczak Colorectal 2 Statistics**

Table 1 Continued

	Colon Adenoma Epithelia vs. Normal	6.491	8.62E-06	
	Colorectal Carcinoma vs. Normal	27.017	4.85E-20	**Skrzypczak Colorectal Statistics**
	Colorectal Adenocarcinoma vs. Normal	13.364	5.83E-17	
	Rectal Adenocarcinoma vs. Normal	41.182	1.73E-38	**Gaedcke Colorectal Statistics**
	Colon Mucinous Adenocarcinoma vs. Normal	58.870	4.55E-15	
	Rectal Adenocarcinoma vs. Normal	21.790	2.57E-23	
	Colon Adenocarcinoma vs. Normal	25.277	3.05E-24	**TCGA Colorectal Statistics**
MMP3	Rectosigmoid Adenocarcinoma vs. Normal	68.696	2.60E-05	
	Cecum Adenocarcinoma vs. Normal	16.008	2.64E-08	
	Colon Adenoma vs. Normal	25.992	4.57E-08	**Skrzypczak Colorectal 2 Statistics**
	Colon Carcinoma Epithelia vs. Normal	44.776	3.64E-06	
	Colorectal Carcinoma vs. Normal	2.152	2.85E-05	**Graudens Colon Statistics**
	Colon Adenoma vs. Normal	24.729	1.80E-12	**Sabates-Bellver Colon Statistics**
	Colon Adenocarcinoma vs. Normal	4.456	2.02E-05	**Kaiser Colon Statistics**
	Colorectal Adenocarcinoma vs. Normal	18.579	6.03E-27	**Skrzypczak Colorectal Statistics**
	Colorectal Carcinoma vs. Normal	19.121	2.70E-18	
	Colon Adenoma vs. Normal	58.095	5.66E-25	**Sabates-Bellver Colon Statistics**
	Rectal Adenoma vs. Normal	63.567	1.32E-05	
	Colon Adenocarcinoma vs. Normal	14.234	4.89E-08	**Notterman Colon Statistics**
	Colon Adenocarcinoma vs. Normal	10.031	1.67E-19	**Ki Colon Statistics**
	Rectal Adenocarcinoma vs. Normal	67.268	4.28E-31	
	Colon Mucinous Adenocarcinoma vs. Normal	62.702	8.32E-16	
	Colon Adenocarcinoma vs. Normal	75.587	1.87E-30	**TCGA Colorectal Statistics**
	Cecum Adenocarcinoma vs. Normal	44.624	1.93E-15	
MMP7	Rectal Mucinous Adenocarcinoma vs. Normal	92.438	8.74E-07	
	Colon Adenocarcinoma vs. Normal	8.969	1.73E-16	
	Cecum Adenocarcinoma vs. Normal	7.246	1.42E-08	**Kaiser Colon Statistics**
	Colon Mucinous Adenocarcinoma vs. Normal	9.613	2.11E-06	
	Rectal Adenocarcinoma vs. Normal	8.445	7.74E-05	
	Colon Carcinoma Epithelia vs. Normal	13.656	4.77E-12	
	Colon Carcinoma vs. Normal	16.825	2.03E-09	**Skrzypczak Colorectal 2 Statistics**
	Colon Adenoma vs. Normal	46.498	2.15E-06	
	Rectal Adenocarcinoma vs. Normal	53.898	4.09E-40	**Gaedcke Colonectal Statistics**
	Colorectal Carcinoma vs. Normal	21.244	9.49E-16	**Hong Colorectal Statistics**
MMP9		NA		
MMP11	Colorectal Carcinoma vs. Normal	6.664	1.59E-11	**Graudens Colon Statistics**
	Colon Adenocarcinoma vs. Normal	3.868	7.42E-19	**Kaiser Colon Statistics**

Table 1 Continued

	Colon Mucinous Adenocarcinoma vs. Normal	4.464	6.48E-09	
	Cecum Adenocarcinoma vs. Normal	2.934	2.48E-07	
	Rectosigmoid Adenocarcinoma vs. Normal	3.497	6.11E-05	
	Colon Adenocarcinoma vs. Normal	8.496	6.42E-36	
	Rectal Adenocarcinoma vs. Normal	8.190	4.90E-30	**TCGA Colorectal Statistics**
	Cecum Adenocarcinoma vs. Normal	5.187	1.97E-13	
	Colon Mucinous Adenocarcinoma vs. Normal	5.845	1.67E-11	
	Colorectal Carcinoma vs. Normal	3.903	5.43E-16	**Skrzypczak Colorectal Statistics**
	Colon Adenocarcinoma vs. Normal	2.455	3.47E-06	**Notterman Colon Statistics**
	Rectal Adenocarcinoma vs. Normal	10.925	1.10E-31	**Gaedcke Colonectal Statistics**
	Colon Adenocarcinoma vs. Normal	2.149	3.97E-14	**Ki Colon Statistics**
	Colon Carcinoma vs. Normal	8.530	1.58E-08	**Skrzypczak Colorectal 2 Statistics**
	Colorectal Carcinoma vs. Normal	4.543	1.40E-07	**Hong Colorectal Statistics**
	Colon Adenocarcinoma vs. Normal	7.778	3.33E-06	**Notterman Colon Statistics**
	Colorectal Carcinoma vs. Normal	6.222	5.60E-12	**Skrzypczak Colorectal Statistics**
	Colorectal Adenocarcinoma vs. Normal	4.763	2.97E-10	
	Rectal Adenoma vs. Normal	3.91	1.71E-07	**Sabates-Bellver Colon Statistics**
	Colon Adenoma vs. Normal	3.982	1.30E-10	
	Rectal Adenocarcinoma vs. Normal	4.274	2.44E-26	**Gaedcke Colonectal Statistics**
MMP12	Rectal Mucinous Adenocarcinoma vs. Normal	3.745	5.50E-06	**TCGA Colorectal Statistics**
	Colon Mucinous Adenocarcinoma vs. Normal	4.911	3.93E-07	
	Rectal Mucinous Adenocarcinoma vs. Normal	4.510	1.18E-04	
	Rectal Adenocarcinoma vs. Normal	3.937	1.78E-04	**Kaiser Colon Statistics**
	Colon Mucinous Adenocarcinoma vs. Normal	4.786	1.80E-05	
	Colon Carcinoma Epithelia vs. Normal	15.694	8.88E-08	**Skrzypczak Colorectal 2 Statistics**
	Colon Adenoma Epithelia vs. Normal	6.491	8.62E-06	
MMP28		NA		

3.2 Association Between MMPs mRNA Levels and Clinico-pathological Features of Individuals with COAD

mRNA expression levels of MMPs were compared between colon cancer and matched colon tissues or non-malignant tissue using GEPIA tool. Analysis showed that MMP1, MMP3, MMP7, MMP9, MMP11, and MMP12 expression levels were significantly higher in colon cancer tissues compared with the matched colon tissues and non-malignant tissue. MMP28 expression was down-regulated in the colon cancer tissues compared with the matched non-malignant tissues (Figure 2A, 2B). In addition, association between MMPs levels and colon cancer tumor stage was explored. MMP11 and MMP12 levels significantly varied with tumor stage. On the other hand, MMP1, MMP3, MMP7, MMP9, and MMP28 groups showed no significantly variation across different tumor stages (Figure 3).

Immunohistochemistry was used to explore protein expression level of MMPs in colon cancer tissues and adjacent non-malignant tissues. MMP1, MMP3, MMP7, MMP9, MMP11, and MMP12 proteins showed significantly higher protein expression levels in colon cancer tissues compared with the levels in the normal tissues (Figure 4).

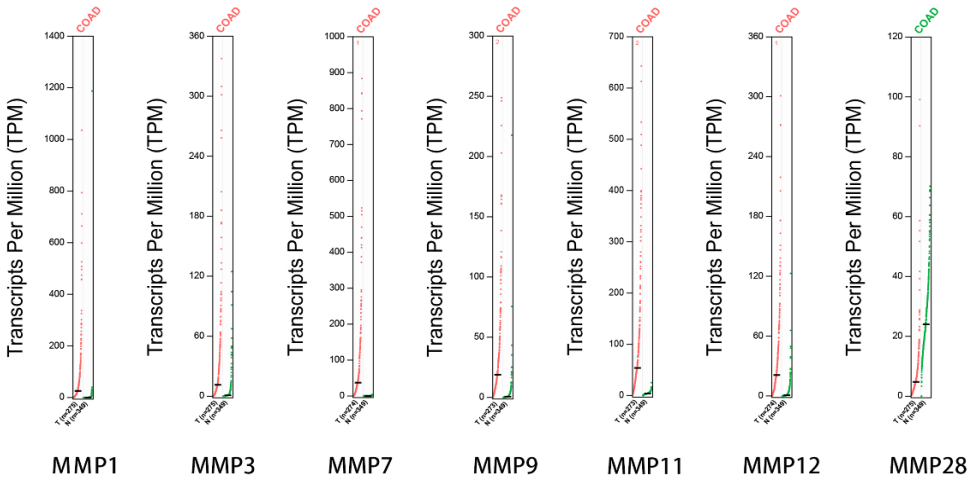

Figure 2A

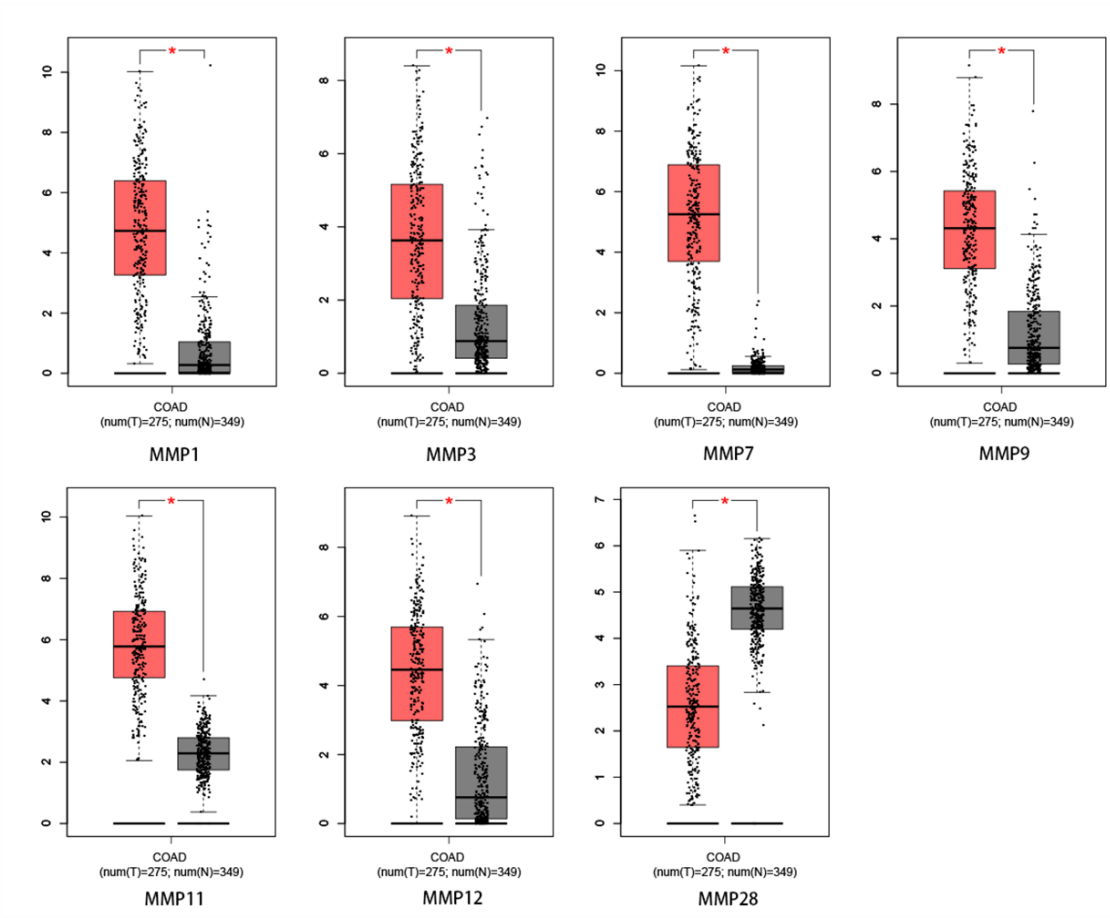

Figure 2B

Figure 2. Expression levels of MMPs in COAD (GEPIA). A: scatter diagram; B: boxplot.

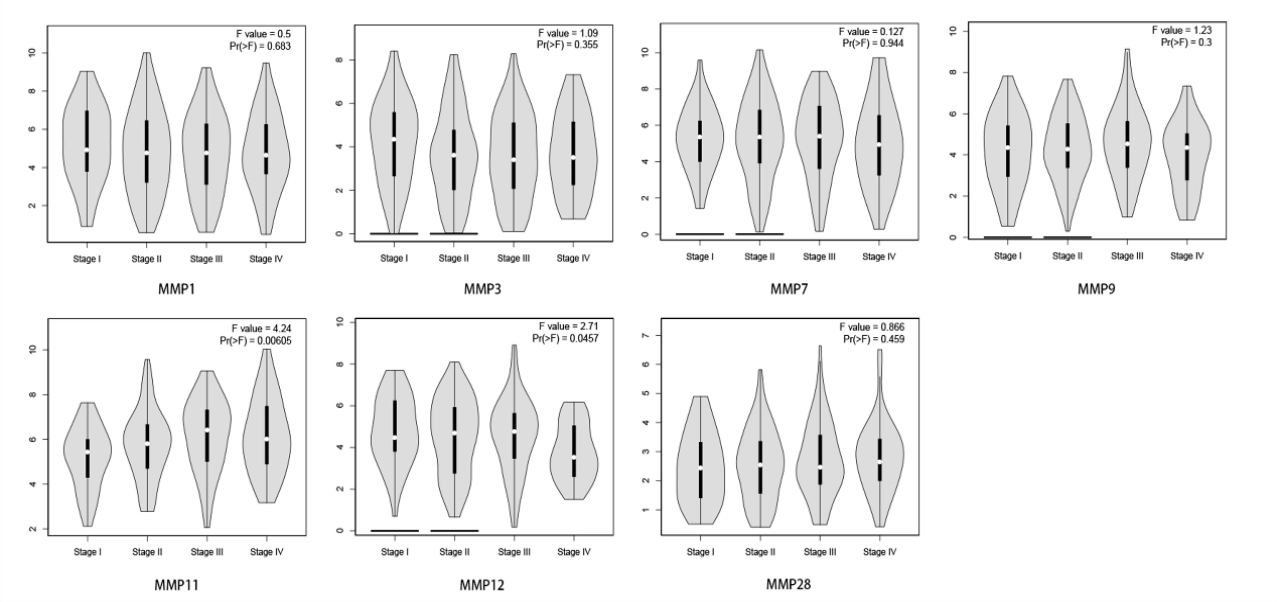

Figure 3. Correlations between MMPs expression and Tumor Stage in Colorectal Cancer Patients (analyzed using GEPIA).

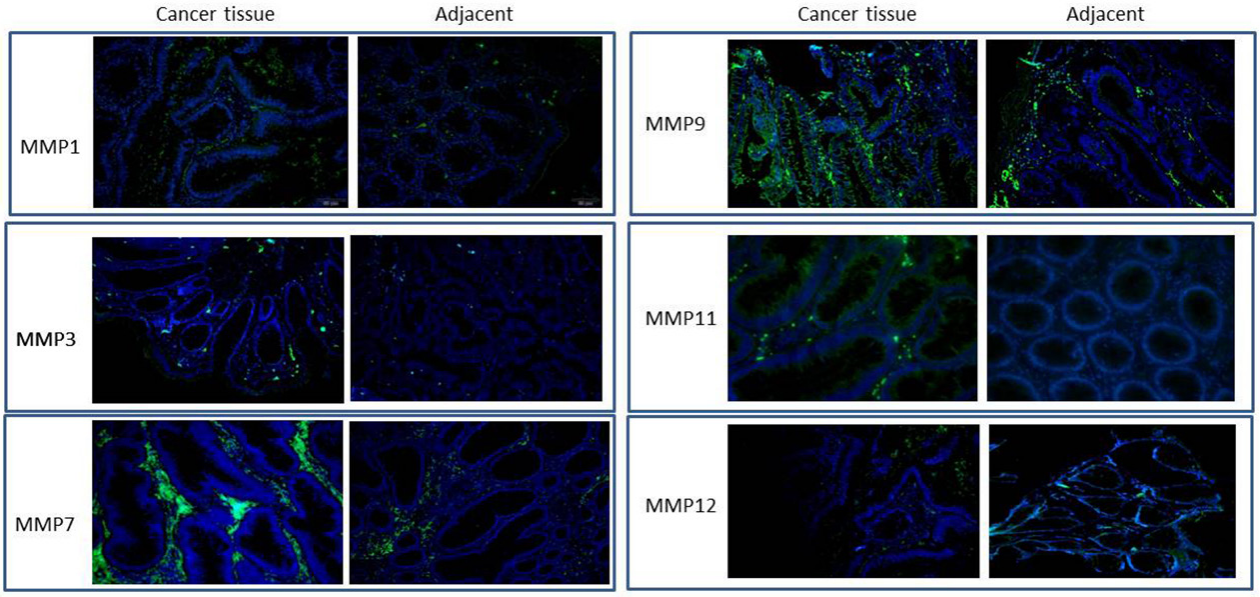

Figure 4. Expression of MMPs using fluorescence immunohistochemistry in colorectal carcinoma patients.

3.3 Relationship Between Elevated mRNA Expression of MMP1, 3, 7, 9, 11, 12 and Low mRNA Expression of MMP28 with Improved Prognosis of Individuals with CRC

The critical value of MMPs in the survival of individuals with COAD was explored using the GEPIA dataset. Association between mRNA expression levels of MMPs and the overall survival (OS), and RFS were determined using log rank test analyses. Analysis showed that expression of MMP was not significantly correlated with OS and RFS ($P > 0.05$) (Figure 5).

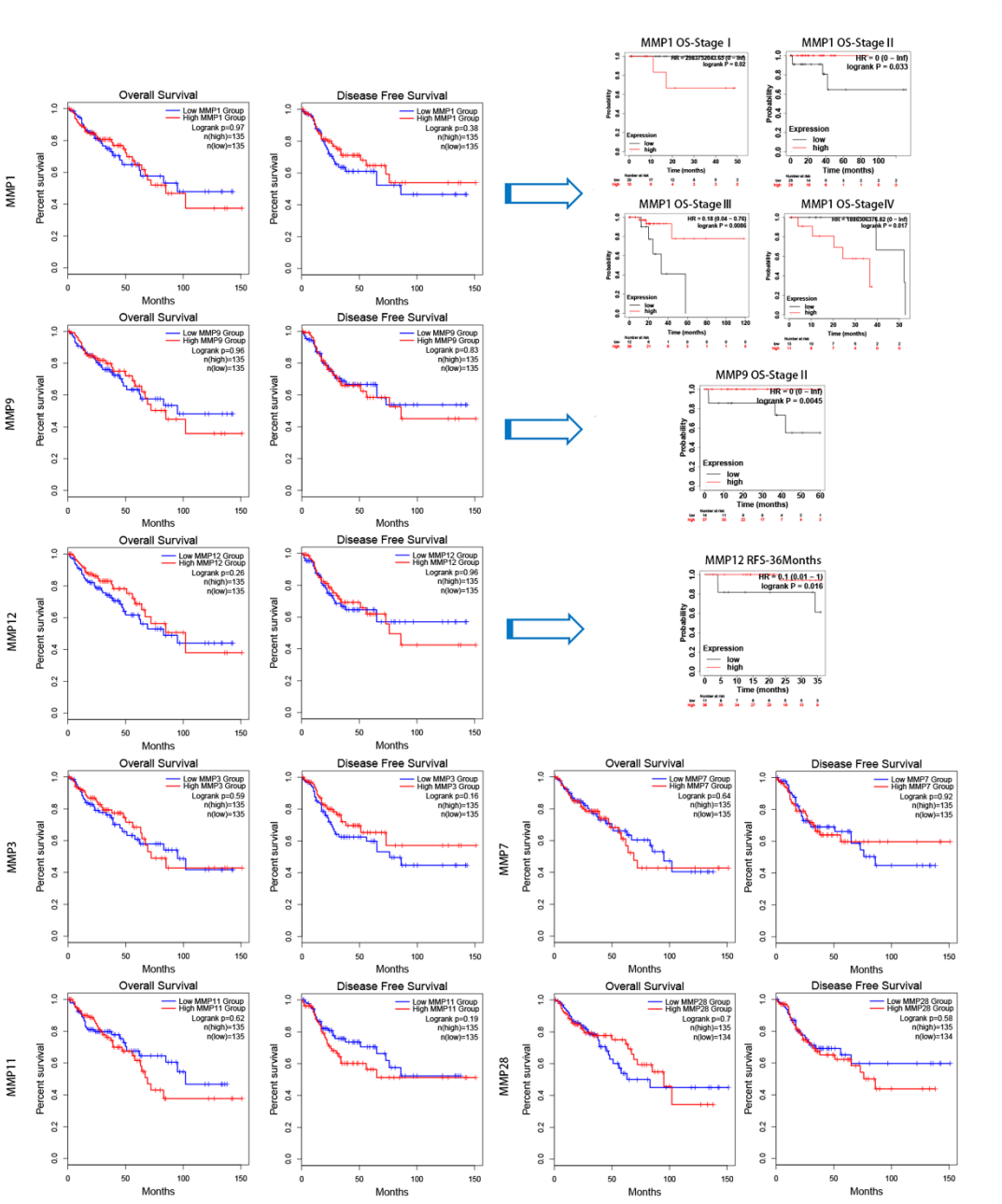

Figure 5. Prognostic value of mRNA Level of MMPs in colorectal cancer patients (Kaplan-Meier Plotter, and GEPIA analysis).

3.4 Predicted Roles and Cascades Associated with the Changes in MMP Factors and Their Commonly Altered Neighboring Genes in COAD

MMPs alterations, networks, and correlations were explored using the cBioPortal web tool for the COAD dataset (The Cancer Genome Atlas, Firehose Legacy (The Cancer Genome Atlas, Firehose Legacy); https://www.cbioportal.org/study/summary?id=coadread_tcga). MMPs levels were determined in 392 colon adenocarcinoma, 169 rectal adenocarcinoma, 66 mucinous adenocarcinoma of the colon and rectum, and 13 colorectal adenocarcinoma. Three out of four categories were explored based on genomic alterations, including mutation, amplification, and deep deletion. Analysis showed a total of 69 genomic alteration in 163 rectal adenocarcinoma samples, 23 genomic alterations in 66 mucinous adenocarcinoma of the colon and rectum, and 131 genomic alterations in 382 colon adenocarcinoma samples (Figure 6A). MMPs expressions were altered in 616 samples from 640 COAD patients (96.25%). MMP9

showed the highest mutation rate (10%) in all selected molecular profiles including missense mutation, amplification and deep deletion, followed by MMP7 (1.5%), MMP11 (1%), MMP1 and MMP3 (0.8%), and MMP12 and MMP28 (0.5%) (Figure 6A).

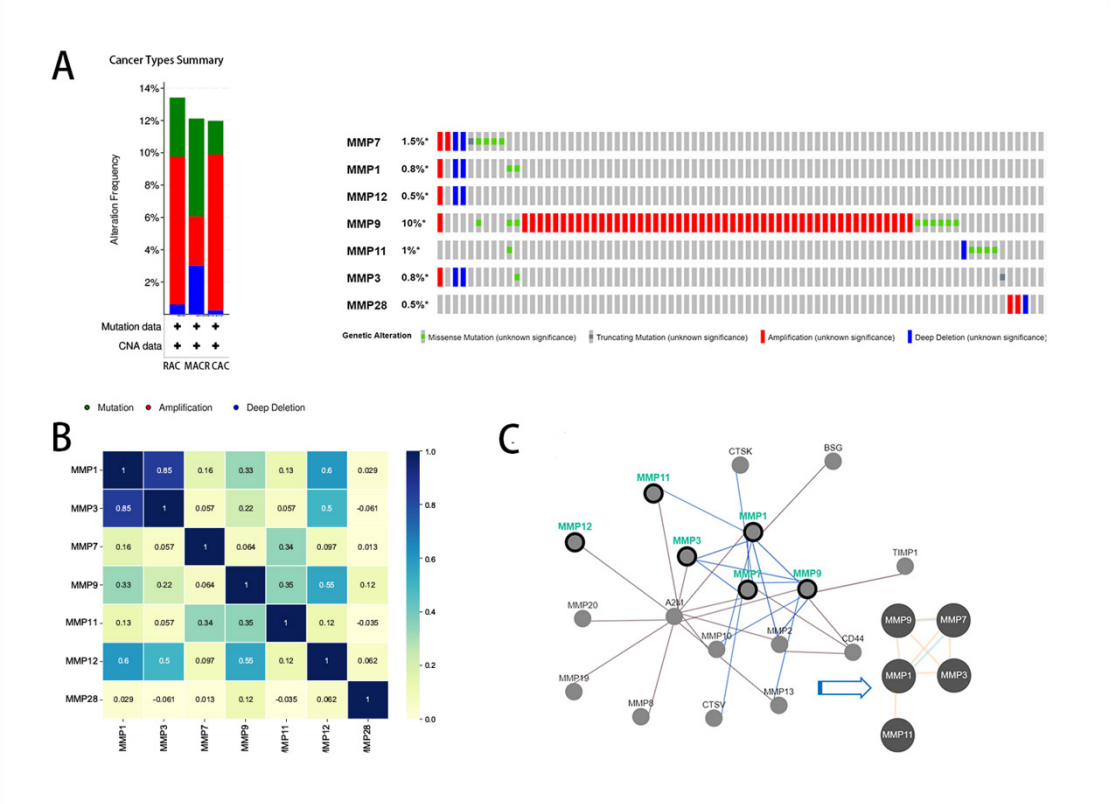

Figure 6. (A): Genomics research includes the general display of the proportion of genomic changes in MMPs families and the display of single genomic changes; (B): the correlation between gene expression; (C): the interaction map of top adjacent genes closely related to gene changes.

Further, genes in mRNA expression (RNA sequencing V2 RSEM, 382 samples) that are correlated with MMPs in mRNA expression were identified (RNA sequencing V2 RSEM, 382 samples) using the cBioPortal webserver using COAD dataset (The Cancer Genome Atlas, Provisional), and Pearson's correction analysis was performed. Analysis showed significant and positive correlations in the following MMPs: MMP7 with MMP11, MMP2, and MMP1; MMP1 with MMP3, MMP12, MMP9, MMP7 and MMP11; MMP12 with MMP1, MMP9, MMP3 and MMP11; MMP9 with MMP12, MMP11, MMP1, MMP3 and MMP28; MMP11 with MMP9, MMP7, MMP1 and MMP12; MMP3 with MMP1, MMP12, and MMP9 (Figure 6B).

Further, a protein interaction network for MMPs was constructed and interactions between MMPs were visualized using the Pathway Common online tool (Pathway Commons: A Resource for Biological Pathway Analysis). Analysis showed that MMP members were closely interrelated, for instance, MMP1, 7, 3, and 9 showed significant interactions. Notably, Wnt-signaling cascade genes, including A2M, CTSK, and BSG were significantly correlated with MMPs alterations (Figure 6C).

Functions of significantly related genes of MMPs were predicted using GO (gene ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses using the DAVID (Database for Annotation, Visualization and Integrated Discovery) webserver (https://david.ncifcrf.gov/summary.jsp).

GO enrichment analysis was used to explore the functional roles of target host genes based on biological processes, cellular components (CC), and molecular functions (MF). Analysis showed that GO: 0030574 (collagen catabolic process), GO: 0022617 (extracellular matrix disassembly), GO: 0006508 (proteolysis), GO: 0032461 (positive modulation of protein oligomerization), and GO: 0050900 (leukocyte migration) were significantly correlated with the MMP expressions in COAD (Figure 7A). In addition, GO:0005578 (proteinaceous extracellular matrix), GO:0005576 (extracellular region), GO:0031012 (extracellular matrix), and GO:0005615 (extracellular space) were significantly correlated with MMPs expressions in the CC category (Figure 7B). Moreover, GO:0004222

(metalloendopeptidase activity), O:0004252 (serine-type endopeptidase activity), GO:0008270 (zinc ion binding), GO:0004175 (endopeptidase activity), GO:0005509 (calcium ion binding), and GO:0008237 (metallopeptidase activity) were significantly correlated with MMPs expressions in MF category. KEGG analysis was used to explore pathways associated with MMP expressions and significantly altered neighboring genes. A total of12 pathways associated with the roles of MMP alterations in COAD were identified using KEGG analysis (Figure 8). Out of these pathways, TNF signaling pathways, TGF-beta signaling pathways, pathways in cancer, MicroRNAs in cancer, PPAR signaling pathway, and Wnt signaling pathway are implicated in tumorigenesis and pathogenesis of COAD (Figures 9A and 9B).

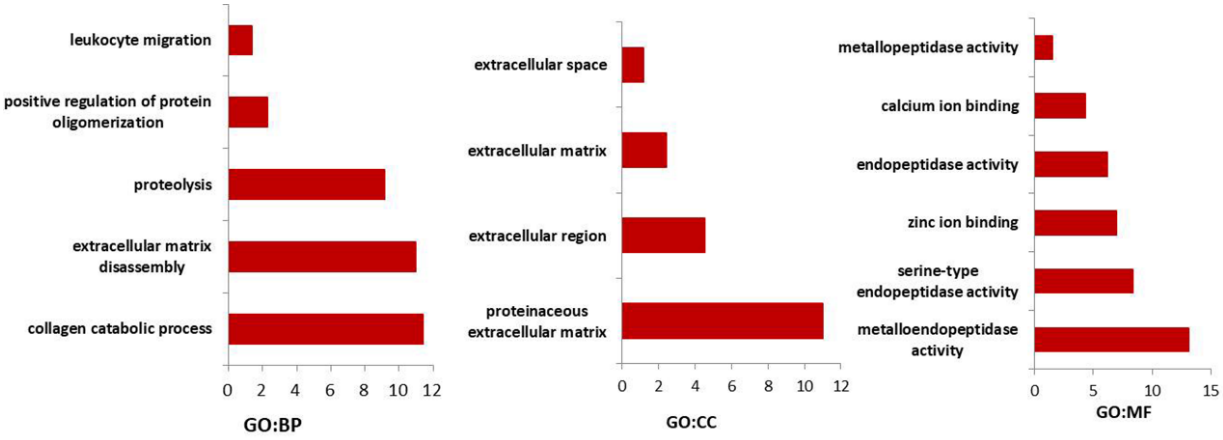

Figure 7. Functions of MMPs and genes significantly associated with MMPs alterations. The functions of MMPs and genes significantly associated with MMP alterations were predicted using gene ontology (GO) by DAVID tool (Database for Annotation, Visualization and Integrated Discovery) (https://david.ncifcrf.gov/summary.jsp). GO enrichment analysis predicted the functional roles of target host genes based on three aspects, including (A) biological processes, (B) cellular components, and (C) molecular functions.

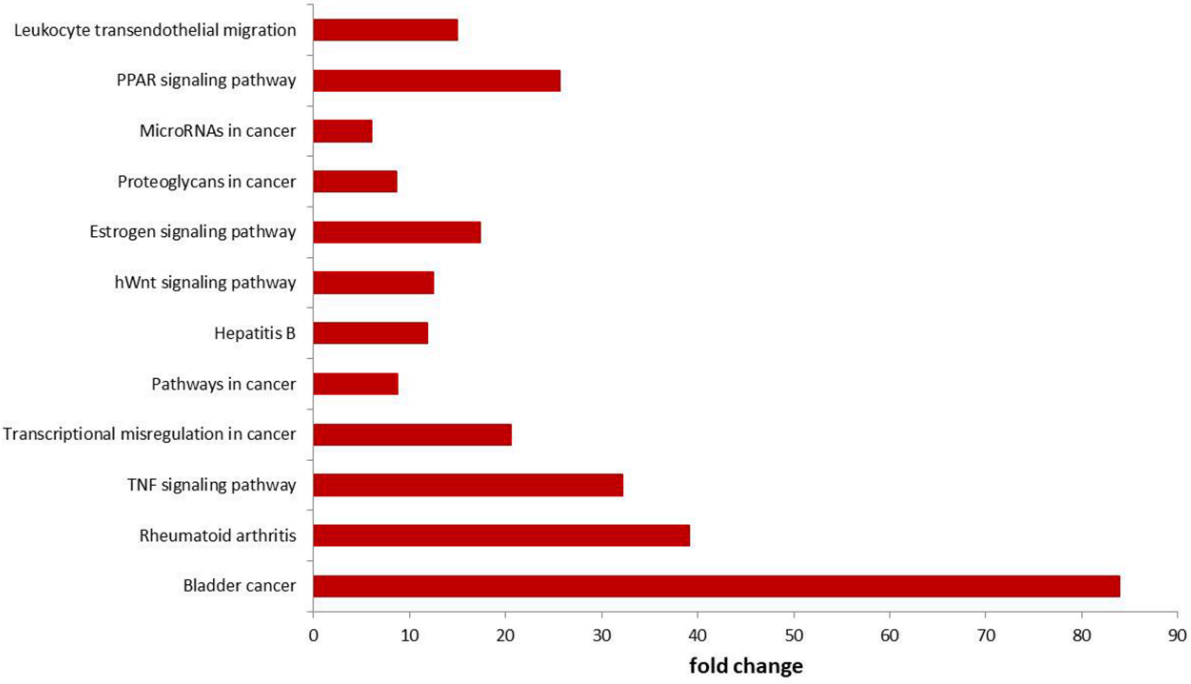

Figure 8. Functions of MMPs and genes significantly associated with MMP alterations. Functions of MMPs and genes significantly associated with MMP alterations were predicted by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis using DAVID tools (https://david.ncifcrf.gov/summary.jsp).

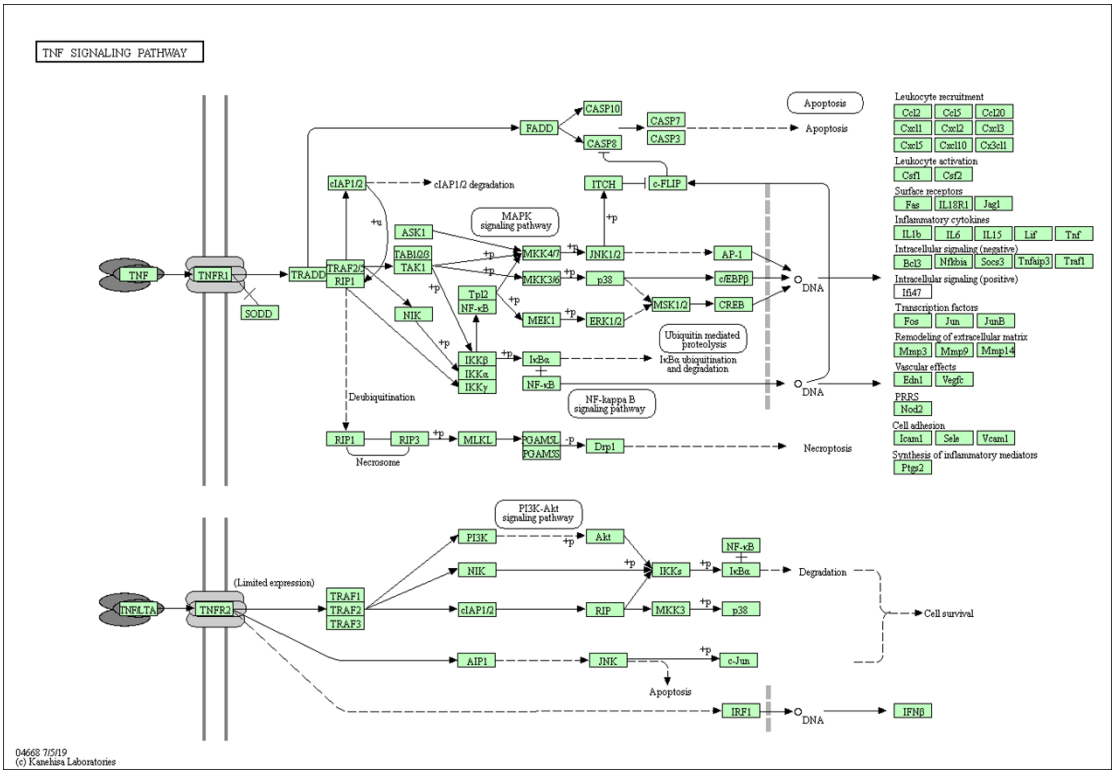

Figure 9A

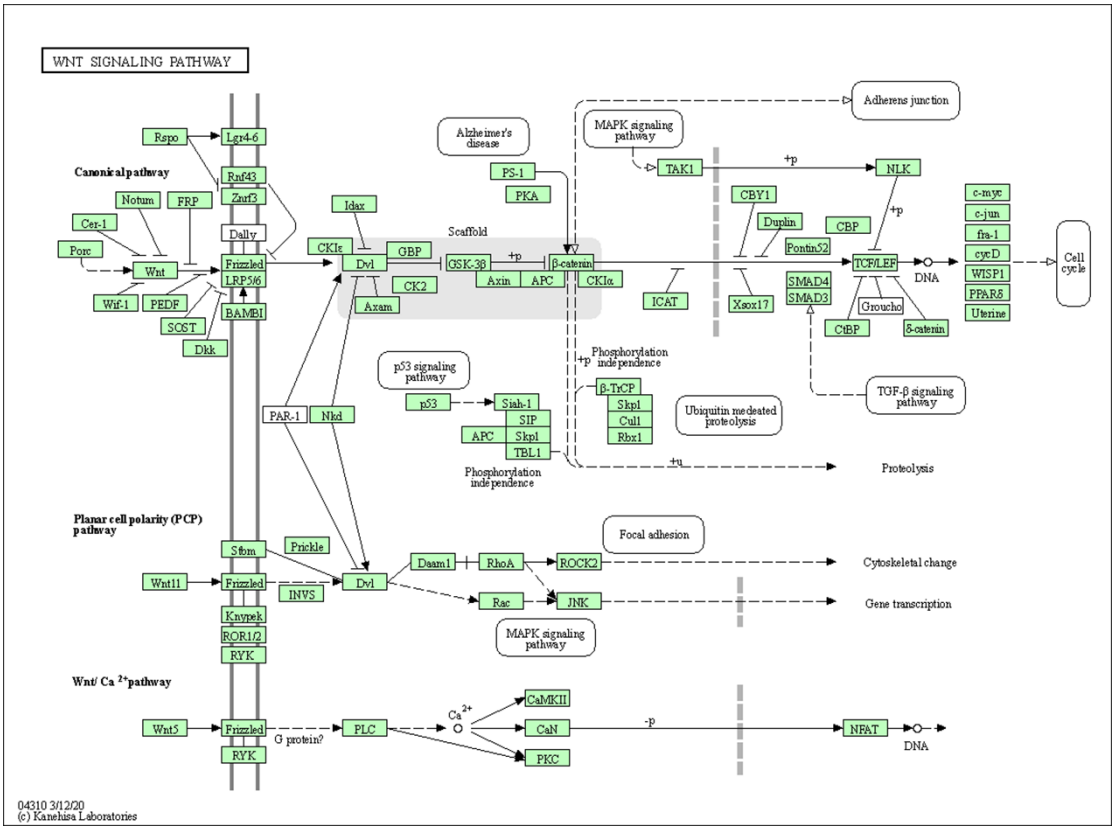

Figure 9B

Figure 9. TNF signaling pathway (A) and Wnt signaling pathway (B) regulated by the MMPs alteration in CRC.

4. Discussion

Aberrant expression of MMP factors has been reported in several cancer types. The roles of MMP activators in tumorigenesis and the prognosis of various cancer types have not been fully explored [21-25]. However, their role in CRC is not known. This study sought to explore transcript expression and the prognostic value (OS, and DFS) of various MMP factors in CRC using bioinformatics approaches. The findings of this study provide information on improving therapeutic developments and improving the effectiveness of the prognosis of individuals with CRC.

MMP1 is a direct functional target for STAT3. Furthermore, STAT3 and ReB form a minimal activator complex for positive modulation of MMP-1 in colon cancer [26]. Studies report that polymorphisms, rs1799750 in MMP-1 do not exhibit significant correlation with breast, colon and lung cancer risk among Polish patients [27]. In the current study, Oncomine analyses and CGAT showed that MMP1 levels were higher in CRC compared with the levels in non-malignant tissues, which was consistent with the research findings of Wang et al. [28]. However, their research showed that the high expression of MMP1 was significantly related to linear metastasis as well as TNM stage, which was inconsistent with our study. GEPIA analysis showed that increased MMP1 was significantly correlated with OS in each pathological stage. This was validated by Andreas et al.'s observation of colon cancer patients followed up for more than 10 years [29].

MMP3 is overexpressed in some tumors, such as breast cancer, and esophageal squamous cell carcinoma (ESCC) [30, 31]. High miR-519d expression level inhibits MCF-7 cell proliferation by targeting MMP3 in breast cancer [30]. Upregulation of CCAAT/enhancer binding protein β promotes tumor cell infiltration in an MMP3-dependent manner *in vitro* and is correlated with metastatic status in ESCC [31]. In the current study, MMP3 expression in CRC was higher compared with the level in non-malignant tissues. However, MMP3 mRNA expression was not correlated with tumor stage of CRC patients, and OS and RFS.

MMP7 and MMP11 are members of stromal dissolution factors. MMP7 promotes cell proliferation [32], however it has no significant effect on nasal cell proliferation [33]. MMP-11 induces cancer onset and progression by repressing apoptosis, and promoting migration and infiltration of cancer cells [34]. MMP9 functions as denatured collagen and type IV collagen. MMP9 is associated is implicated in several cancer pathology processes, including angiogenesis, apoptosis, migration and metastasis [35-37]. A previous study explored the value of MMP9 as a biomarker in several cancer types. For example, MMP9 expression significantly increases malignancy of CRC cell lines, through activation of the TGF-β/SMAD signaling cascade [38]. AXIN up-regulation in lymphoma cells causes significant decrease in MMP7 and MMP9 expression, therefore, it plays a role in repressing invasion and migration of lymphoma cells. In the current study, MMP7, 9, 11 were overexpressed in CRC tissue compared with non-malignant tissues, which was similar to the previous research [39, 40]. However, the relationship between MMPs and the prognosis of colorectal cancer varies greatly in different studies. In the current study, analysis of the different tumor stages showed that MMP7 and MMP9 were not significantly correlated with tumor stage, whereas MMP11 was significantly correlated with tumor stage. Expression levels of MMP7, MMP9 and MMP11 were not correlated with OS and RFS in CRC patients. However, MMP9 can be used as a biomarker for stage 2 rectum cancer patients as it was correlated with OS for patients at this stage. The studies of Barabás et al. and Mudatsir et al. showed that elevated serum MMP-7, and MMP-9 levels significantly correlated with advanced tumor stages [39, 41]. On the contrary, the studies of Tan et al. and Peltonen et al. suggest that MMP7 may be a protective factor for colon cancer [42], and high expression of MMP-9 in colorectal tumor tissue was associated with better disease-free survival [43]. This inconsistent result may be related to genetic polymorphism [44, 45].

Although MMP12 and MMP28 are not grouped into any MMP group, several studies have explored their roles. For instance, silencing of MMP12 inhibits growth and infiltration of lung adenocarcinoma cells (LAC) and castration-resistant prostate cancer cells, and high MMP12 expression level is correlated with the pathological stage, and tumor metastasis in LAC patients, and the mechanisms involved in the promotion of cancer cell autophagy and the inhibition of lipid catabolism [46, 47]. Notably, studies have not explored the role of MMP28 in CRC. Pham et al [48] reported that specific inhibition of migration/invasion potential of BCMO1 mRNA expression by siRNA promoted increased MMP28 expression. Wang et al. [49] found that the upregulation of MMP-28 could be used as one of the effective indicators to diagnose bladder cancer and predict tumor progression. In the current study, expression of MMP12 was high whereas MMP28 level was low in CRC tissues compared with normal tissues. MMP12 mRNA expression level was correlated with CRC patients' tumor stage. However, mRNA expression level of MMP28 was not correlated with tumor stage. A high MMP12 expression was significantly correlated with poor RFS with follow-up period of 36 months, and with poor OS in stage 4 in 165 rectum carcinoma patients.

Cbioportal database is for genomic analysis which includes the general display of the proportion of genomic changes in MMP families and the display of single genomic changes (figure 6A). Furthermore, it also shows the correlation between MMPs expression (figure 6B) and the interaction map of top adjacent genes closely related to MMPs changes (figure 6C). String interaction and GO/KEGG analysis are to explore possible signaling pathways (figure 9). Finally, TNFs and Wnts pathway were focused on MMPs downstream gene analysis. TNF is enriched in the tumor microenvironment and reported to promote cancer growth. Wnt signaling pathway is an evolutionarily conserved signaling pathway, which plays an important role in controlling embryonic development, regulating cancer cell growth, migration and differentiation, et al. Its abnormal activation is closely related to the occurrence and development of many human tumors. It was reported that a new crosstalk mechanism of Hippo and Wnt signaling pathway, interacts directly with Tcf4, a downstream transcription factor of Wnt pathway, and regulates the expression of target gene promoter to affect cancer cell growth.

There are several limitations to this study. Our study did not conduct subgroup analysis on gender and race. Moreover, nucleotide polymorphism may affect the prognosis of tumors. Therefore, a multi-SNP analysis for MMPs will be investigated in our future work.

5. Conclusion

This study explored expression and prognostic value of MMPs in CRC. The findings of the study showed the heterogeneity and systemic molecular biological properties of MMPs in CRC. Upregulation of MMP1,3,7,9,11 and downregulation of MMP28 in CRC tissues may be implicated in CRC oncogenesis, and are potential therapeutic targets for CRC. Expression levels of MMP11 and 12 can be used as molecular biomarkers for identifying high risk stages of patients with improved CRC prognosis. The findings of this study show that MMP9 and 12 are potential markers for CRC survival.

List of Abbreviations

CC	cellular components
COAD	colon adenocarcinoma
CRC	colorectal cancer
ESCC	esophageal squamous cell carcinoma
LAC	lung adenocarcinoma cells
MF	molecular functions
MMP	matrix metalloproteinase
OS	overall survival
RFS	relapse free survival
TCGA	Cancer Genome Atlas

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Ethics Approval and Consent to Participate

All included patients gave their oral and written informed consent. The study was approved by the Ethics Committee from Affiliated Hospital of Chengde Medical University (reference number LL2021009).

Human and Animal Rights

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

Consent for Publication

A written informed consent was obtained from the patients for the publication of this report.

Availability of Data and Materials

(1) The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.
(2) All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Conflict of Interest

The authors declare no conflicts of interest, financial or otherwise.

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