Clinical Significance of COL11A1 and Its Effect on Immune Infiltration in Colorectal Cancer

Yibin Wu
Shanghai Cancer Hospital: Fudan University Shanghai Cancer Center
Yuankun Cai
Fifth People's Hospital of Shanghai Fudan University
Wenjie Chen
Fifth People's Hospital of Shanghai Fudan University
Tao Ye
Fifth People's Hospital of Shanghai Fudan University
Ye Xu
Shanghai Cancer Hospital: Fudan University Shanghai Cancer Center
Huipeng Wang (✉ 20111290007@fudan.edu.cn)
Fifth People's Hospital of Shanghai Fudan University https://orcid.org/0000-0001-6760-9572

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Abstract

Background

Collagen type XI alpha 1 chain (COL11A1) is an extracellular matrix (ECM) protein and plays a crucial role in tumors. However, the correlations of COL11A1 to prognosis and tumor-infiltrating lymphocytes in colorectal cancer (CRC) remain unclear.

Methods

Here, we used bioinformatics methods with experimental exploration to evaluate the prognostic value of COL11A1 in CRC, while the alteration of the gene and its function in CRC was explored by using cBioPortal and DAVID. Furthermore, the correlations between COL11A1 expression and cancer immune infiltrates, gene marker sets of immune infiltrates were analyzed by TIMER, GEPIA and human samples.

Results

Increased COL11A1 expression was found in CRC and positive correlated with tumor stage, node metastasis and histological type. Patients with higher COL11A1 expression had shorter survival data. Function network revealed that COL11A1 mainly regulated pathways involving in focal adhesion, PI3K-AKT signaling pathways and ECM-receptor interaction. Furthermore, COL11A1 expression was positively correlated with infiltrating levels of CD4 + T and CD8 + T cells, macrophages, neutrophils, and dendritic cells (DCs) in colon adenocarcinoma (COAD). COL11A1 expression showed strong correlations with diverse immune marker sets in COAD. In addition, COL11A1 expression potentially contributes to regulation of tumor-associated macrophages (TAMs), DCs, T cell exhaustion and Tregs in COAD.

Conclusions

Our data identify COL11A1 as a novel prognostic biomarker for determining prognosis and immune infiltration in COAD, which might optimize therapeutic strategies for CRC patients.

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide[1]. In advanced CRC, over half of postoperative patients need chemotherapy to shrink tumor size, reduce tumor growth and inhibit tumor metastasis. The majority of patients with advanced CRC are initially responsive to the combined chemotherapy[2]. However, the patients eventually experience tumor recurrence due to drug resistance, and the 5 years survival rate is lower than 10% in advanced CRC[3]. Importantly, immunotherapy involves drugs that block a mechanism called checkpoint, allowing immune cells to restore their capability of fighting cancer is widely studied and gradually used in

clinic[4]. Certain of patients with poor curative effect on chemotherapy are found to be well responsive to the immune checkpoint therapy[5]. Many studies have reported that the tumor-infiltrating lymphocytes, such as tumor-associated macrophages (TAMs) and tumor-infiltrating neutrophils (TINs), affect the prognosis and efficacy of chemotherapy and immunotherapy[6; 7]. Thus, it is of paramount importance to explore the immunophenotypes of tumor-immune interactions and identification of novel immune-related therapeutic targets in CRC.

Collagen type XI alpha 1 chain (COL11A1), a gene encoding a minor fibrillary collagen of the extracellular matrix, plays a critical role in regulating the development and progression of malignant tumors[8; 9]. Studies have found that COL11A1 could bind to α1β1 integrin and activate DDR2-Src-PI3K/Akt/NFkB-IAP signaling axis to induce cisplatin resistance in ovarian cancer[10]. In pancreatic cancer, COL11A1 was upregulated in cancer tissues and predicted poor prognosis[11; 12]. Other study found that COL12A1 was also overexpressed in gastric cancer tissues and inhibited proliferation, migration and invasion of HGC-27 cells in vitro[13]. Increased evidences have revealed the significant effects of COL11A1 on regulating the carcinogenicity of colorectal cancer. In general, elevated COL11A1 expression was observed in colorectal cancer tissues compared to the normal tissues, which suggested COL11A1 as an oncogene in CRC[14–16]. However, the prognostic value and function of COL11A1 in CRC is still not clear. Another aspect of studies have reported COL11A1 is an important ECM protein which constitutes the scaffold of tumor microenvironment (TME) and regulates cancer behavior[17]. TME comprises various cells (endothelial cells, fibroblasts, immune cells, etc.) and extracellular components (cytokines, growth factors, hormones, extracellular matrix, etc.) that are surrounding tumor cells and nourished by a vascular network. It is well known that immune cells can vary their activation status and location within the TME to affect therapeutic efficacy. Owing to its interaction with immune system, TME not only plays a pivotal role during tumor initiation, progression, and metastasis but also has profound effects on therapeutic efficacy[18]. But whether COL11A1 could regulate the immune-related mechanisms to influence the progression of CRC remains unknown.

In this present study, we comprehensively analyzed COL11A1 expression and correlation with prognosis of CRC patients though bioinformatics methods and clinical data. In addition, we explored the function network of COL11A1 in CRC by analyzed DAVIAD. Moreover, we investigated the correlation of COL11A1 with tumor-infiltrating immune cells in the different tumor microenvironments via Tumor Immune Estimation Resource (TIMER). The findings in this work suggested the crucial role of COL11A1 in CRC as well as provide a potential association and an underlying mechanism between COL11A1 and tumor-immune interactions.

Materials And Methods

Human Samples

With the approval of the Ethnic Committee of Fudan University Shanghai Cancer Center. 118 cases cDNA of cancer tissues with matched normal tissues from CRC patients were used to examine the expression

level of COL11A1 mRNA in CRC.

Oncomine Database Analysis

The expression level of COL11A1 mRNA in different kinds of cancers was identified in Oncomine database (https://www.oncomine.org) [19]. We used the data to explore the expression level of COL11A1 mRNA in various cancers including colorectal, gastric, breast, pancreatic cancers ect.

UNLCAN Database Analysis

UALCAN is publicly available at http://ualcan.path.uab.edu. It provides easy access to publicly available cancer OMICS data (TCGA and MET500) from 31 cancer types, which contains graphs and plots depicting gene expression and patient survival information based on gene expression[20]. We used this database to analyze the expression of COL12A1 expression in normal tissues and cancer tissues based on patients' individual cancer stages, node metastasis status and histological subtypes.

qRT-PCR

cDNAs from colorectal cancer tissues and the normal tissues were amplified by PCR using Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan) in the Applied Bio-systems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The thermal cycler protocols included 3 min at 95◦C and 40 cycles of 5s at 95◦C and 30s at 60◦C. The primers sequence of COL11A1 was: Forward: 5'-GACTATCCCCTCTTCAGAACTGTTAAC-3'; Reverse: 5'- CTTCTATCAAGTGGTTTCGTGGTTT-3'). GAPDH was served as an internal gene.

cBioPortal Database Analysis

The cBioPortal (http://cbioportal.org), currently containing 225 cancer studies, is an open-access resource for interactive exploration of multidimensional cancer genomics datasets[21]. We used cBioPortal to analyze COL11A1 alterations in the TCGA CRC dataset, which containing 526 samples. Cancer Type Summary shows an overview of COL11A1 alteration in CRC subtype such as mucinous adenocarcinoma colorectal cancer, colon adenocarcinoma and rectal adenocarcinoma. Network tab reflects the biological functional network of COL11A1 interacting with neighboring genes acquired from public pathway databases, with filter options and color-coding based on the frequency of genomic alterations in each gene. Next, we chose COL11A1 along with its top 50 significant neighboring genes to reveal the GO and KEGG pathways in CRC via DAVIA analysis.

GO and KEGG Pathways Analysis

Functions of COL11A1 mutations and the top 50 significant neighboring genes were chosen for GO and KEGG pathways analysis though DAVIA (https://david.ncifcrf.gov/) [21]. GO enrichment analysis can predict the functional roles of COL11A1 mutations and chosen neighboring genes on the basis of three aspects, including biological processes(BP), cellular components (CC), and molecular functions (MF), while KEGG analysis can define the pathways regulated by COL11A1 in CRC. This suggests that COL11A1 may act as an oncogene for CRC.

TIMER Database Analysis

TIMER is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types (https://cistrome.shinyapps.io/timer/). It includes 10,897 samples across 32 cancer types from The Cancer Genome Atlas (TCGA) to estimate the abundance of immune infiltrates[22]. We used TIMER to investigate the correlations between COL11A1 expression with the abundance of immune infiltrates, including B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells, via gene modules. In addition, we also analyzed the correlations between COL11A1 expression and gene markers of the different tumor infiltrating immune cells. The relative gene markers are ever reported in literature.

Gene Correlation Analysis in GEPIA

GEPIA (http://gepia.cancer-pku.cn/) is an online tool for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the Cancer Genome Atlas and the Genotype Tissue Expression (GTEx) projects[23]. In this study, we used it to further confirm the significant genes in TIMER. GEPIA was also performed to generate survival curves, including OS and DFS, based on gene expression with the log-rank test and the Mantel Cox test in 33 different types of cancer. The Spearman method was used to determine the correlation coefficient.

Statistical Analysis

All statistical analysis were performed using SPSS 17.0 (Chicago, IL, USA). Graphpad Prism 8.3 (La Jolla, CA, USA) was used for graphs. Student t test was used to compare the expression level of COL11A1 mRNA in colorectal cancer tissues with the normal tissues. Chi-square test was used to analyze the correlations between COL11A1 expression and clinicopathological features. Cumulative survival time was calculated by the Kaplan-Meier method and analyzed by the log-rank test. Logistic regression analysis was performed to assess the effects of COL11A1 on patients' survival. The strength of the correlation was determined using the following guide for the absolute value: 0.00–0.19 "very weak," 0.20–0.39 "weak," 0.40–0.59 "moderate," 0.60–0.79 "strong," 0.80–1.0 "very strong." *P*-values < 0.05 were considered statistically significant.

Results

Clinical Value of COL11A1 Expression in Colorectal Cancer

To examine the potential relationship between COL11A1 expression and CRC prognosis, we compared the expression of COL11A1 by analyzed two databases from Oncomine and TCGA, and found that COL11A1 mRNA was highly expressed in colorectal cancer tissues as compared to the normal tissues (Figure 1A-B). We further quantified the expression level of COL11A1 in 118 CRC tissues from patients with matched normal tissues. In agreement with our data in Figure 1A-B, the expression of COL11A1 mRNA was higher in cancer tissues than that in normal tissues (Figure 1C). This suggests COL11A1 correlates with CRC occurrence.

We next evaluated the relationship between COL11A1 expression and different clinicopathological features by using UNCLA database, expression of COL11A1 was positively associated with tumor stage (Figure 1D), node metastasis status (Figure 1E) and histological subtypes (Figure 1F). A high expression of COL11A1 was strongly related with overall survival (OS) (Figure 1G) and disease free survival (DFS) (Figure 1H). To further validate if COL11A1 could affect CRC patients' survival, we re-analyzed the clinical data from 118 CRC patients. The five-year OS was significantly shorter in the COL11A1-high group than the COL11A1-low group (Figure 1I). In addition, univariate and multiple Cox regression analyses in Figure 1J-K revealed that COL11A1 expression was an independent risk predictor for CRC patients' survival. Hence, our data indicate that COL11A1 is clinically associated with CRC patient outcome.

Genomic Alteration of COL11A1 in CRC

Genomic alteration is one of important factor for the occurrence of CRC. To determine the frequency and type of COL11A1 alteration in CRC, we used TCGA data with 562 CRC cases from cBioPortal database to examine the genetic alteration. COL11A1 was altered in 54 (10%) of 526 patients with CRC (Figure 2A) and genetic mutation was the most common form (Figure 2B). In addition, we next wanted to study the biological interaction network of COL11A1 in CRC. Though cBioPortal analyses, we showed COL11A1-neighboring genes that were altered at frequencies >5% (Figure 2C). The 20 most frequently altered neighbor genes were further used to explore the biological function network. Analysis of significantly enriched gene ontology (GO) terms indicated that these genes encoded proteins mainly localized to extracellular region, plasma membrane and extracellular exosome. These proteins primarily involved in extracellular matrix organization and cell adhesion, while they also served as metal ion binding, structural molecule activity and identical protein binding (Figure 3A-C). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed enrichment in focal adhesion, PI3K-AKT signaling pathways and ECM-receptor interaction (Figure 3D).

COL11A1 Expression Is Correlated With Immune Infiltration Level in Colon Cancer

Tumor-infiltrating lymphocytes are an independent predictor of sentinel lymph node status and survival in cancers[24]. Tumor purity is an important factor that influences the analysis of immune infiltration in clinical tumor samples by genomic approaches[25], and TIMER and GEPIA have most of the homologous data from TCGA[26; 23]. By analyzing data from TIMER, we found that COL11A1 had a negative correlation with tumor purity in CRC. And COL11A1 expression level also correlated with poorer prognosis and high infiltration in COAD, as the results showed that COL11A1 had positive related with B cell (r=0.025, p=2.07e-10), CD8+ T cell (r=0.213, p=1.54e-5), CD4+ T cell (r=0.345, p=1.13e-12), macrophage (r=0.54, p=5.98e-32), neutrophil (r=0.422, p=9.93e-19) and dendritic cell (r=0.44, p=1.9e-20) (Figure 4). This finding strongly indicates COL11A1 plays a crucial role in immune infiltration in COAD, especially in those of macrophages, neutrophil and dendritic cell.

Correlation Analysis between COL11A1 Expression and Immune Marker Sets

In order to analyze the correlation between COL11A1 and the diverse immune infiltrating cells, we further investigate the relationships between COL11A1 and immune marker sets of various immune cells of COAD in the GEPIA and TIMER databases. We studied the associations between COL11A1 expression and immune marker genes of different immune cells, such as CD8+ T cells, T cells (general), B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells and DCs in COAD, and the different functional T cells included Th1 cells, Th2 cells, Tfh cells, Th17 cells, Tregs and exhausted T cells. The results showed that expression of COL11A1 was significantly correlated with most immune marker sets of various immune cells and different T cells in COAD (Table 1 uploaded in the supplementary material.).

Interestingly, we also found that COL11A1 expression had strong positive correlations with the expression levels of most marker sets of monocytes (CD86, CSF1R), TAMs (CCL2, CD68 and IL10), M2 macrophages (CD163, VSIG4 and MS4A4A) in COAD ($p<0.0001$) (Figure 5 and Table 2). We also analyzed the correlations though GEPIA analyses, and the results between COL11A1 and markers of monocytes and TAMs are similar to those in TIMER (Table 2, uploaded in the supplementary material). In addition to bioinformatics analysis, we further used clinical data to examine the relationships between COL11A1 expression and the expression levels of marker sets of monocytes (CD86, CSF1R), TAMs (CCL2, CD68 and IL10), M1 (COX2 and IRF5) and M2 macrophages (CD163, VSIG4 and MS4A4A) in COAD, and the results were in agreement with that in TIMER and GEPIA (Figure 6A-D). These findings suggest that COL11A1may regulate macrophage polarization in COAD.

In addition, we also found significant positive correlations between COL11A1 and the marker genes of Treg (FOXP3, CCR8, STAT5B, and TGF-) and T cell exhaustion (PD-1, CTLA4, LAG3, and TIM-3) (Table 1). FOXP3 plays an important role in Treg cells which leads to the suppression of cytotoxic T cells attacking tumor cells. For PD-1, as a crucial immunosuppressive molecule that regulates T cell exhaustion[27], has a positive association with COL11A1 expression, suggesting that high COL11A1 expression plays an important role in PD-1 mediating T cell exhaustion. Therefore, these results further confirm the findings that COL11A1 is specifically correlated with immune infiltrating cells in COAD which suggests that COL11A1 plays a vital role in immune escape in the colon cancer microenvironment.

Discussion

In this study we aimed to reveal the clinical relevance by correlation with survival and immune correlation of COL11A1 expression level in CRC. Using mRNA expression data from TCGA and clinical samples from an independent cohort, we have showed the following: (1) COL11A1 was highly expressed in cancer tissues compared to the normal tissues; (2) High COL11A1 expression was correlated with poor prognosis; (3) Relatively high mutation rate of COL11A1 gene was observed in CRC. In addition, we also identify the mutation rate of COL11A1 occurred in CRC, and predicted the potential function networks. Furthermore, our analyses show that immune infiltration levels and diverse immune marker sets are correlated with levels of COL11A1 expression. Therefore, our work provides insights in understanding the potential role of COL11A1 in tumor immunology and its value as a prognostic biomarker in CRC.

Previous studies identified COL11A1 as an oncogene in CRC though using bioinformatics methods to reveal its overexpression in tumor tissues[15; 14]. However, the result was not ever further confirmed by the experimental data. In our work, we examined the expression level of COL11A1 and systematic prognostic landscape in CRC by comprehensively analyzing clinical data combined with integrated bioinformatics methods. Based on the results of Oncomine, TCGA and 118 cases of human samples, we demonstrated that COL11A1, compared to the normal tissues, was highly expressed in colorectal cancer tissues. The correlations between COL11A1 expression and clinicopathological parameters were also observed in TCGA data and our clinical data. Patients with higher tumor stage or tumor metastasis tended to express higher COL11A1 level. Similarity in ovarian, gastric and breast cancers, elevated COL11A1 expression also increased the risk of nodes and tumor distant metastasis in CRC. Thus, these findings suggested the characteristics of COL11A1 in tumorigenesis and metastasis, indicating it as a poor prognostic factor in CRC. Furthermore, analysis of data from TCGA and human samples, Kaplan-Meier Plotter showed that high level of COL11A1 expression was correlated with poor prognosis and could be used as an independent poor prognostic factor in CRC. Taking together, our work strongly suggest that COL11A1 is a prognostic biomarker in CRC.

Genetic mutation as regarded as one of important factors to in cancer initiation and progression[28; 29]. Alterations in chromosomal structure could cause the altered COL11A1 expression and COL11A1 dysfunction in CRC. In this study, 10% mutation rate of COL11A1 was observed in CRC, which suggested COL11A1 as a susceptibility gene in CRC. In addition, we used the top 50 COL11A1 neighborhood genes in CRC to explore the potential function network though analyzing DAVID database. As to the results, the function network of COL11A1 in CRC was mostly involved in focal adhesion, PI3K-AKT signaling pathways and ECM-receptor interaction owing to its property of being as a part of extracellular matrix. Thus, function network analysis suggest that COL11A1 might regulate proliferation, metastasis and invasion of colorectal cancer cells via pathways involving in focal adhesion, PI3K-AKT signaling pathways and ECM-receptor interaction.

COL11A1 is well known to be a major ECM protein which plays an important role in TME[17]. Moreover, TME is also found to affect the therapeutic efficacy of cancers via interacting with immune system[18]. It can be speculated that COL11A1 may be a novel immune checkpoint for the treatment of CRC. In this study, we found that COL11A1 expression was related with diverse immune infiltration levels in CRC. Our results demonstrated that there was a strong positive relationship between COL11A1 expression level and macrophages, neutrophil and DCs, and significantly positive correlations between B cells, CD8 + cells and CD4 + cells and COL11A1 expression level in CRC. Moreover, the correlation between COL11A1 expression and the marker genes of immune cells implicated the role of COL11A1 in regulating tumor immunology in CRC. First, gene markers of M1 macrophages such as COX2 and IRF5 showed weak correlations with COL11A1 expression, whereas M2 macrophage markers such as CD163, VSIG4, and MS4A4A showed strong correlation. These findings suggested the potential regulating role of COL11A1in polarization of tumor-associated macrophages (TAM). In addition, our results indicated that COL11A1 has the potential to activate Tregs and induce T cell exhaustion. The increase in COL11A1 expression positively correlates with the expression of Treg and T cell exhaustion markers (FOXP3, CCR8, STAT5B, TIM-3, PD-1, CTLA4,

and LAG3 in COAD. TIM-3, a crucial surface protein on exhausted T cells[30], is highly correlated with COL11A1 expression in COAD. Furthermore, significant correlations can be found between COL11A1 expression and the regulation of several markers of T helper cells (Th1, Th2, Tfh, and Th17) in COAD. These correlations could be indicative of a potential mechanism where COL11A1 regulates T cell functions in COAD. Together these findings suggest that the COL11A1 plays an important role in recruitment and regulation of immune infiltrating cells in COAD.

In conclusions, our work may be relevant in clinical management of CRC patients. As COL11A1 overexpression is associated with the risk of poor survival, the measurement of postoperative patients with high COL11A1 expression may consider to add adjuvant treatment to reduce tumor metastasis and recurrence. In addition to the clinical importance, our work also revealed the function network of COL11A1 in CRC involved in focal adhesion, PI3K-AKT signaling pathways and ECM-receptor interaction, indicating the potential mechanisms for further biological analysis. Moreover, we found that COL11A1 expression was related with diverse immune infiltration levels in CRC, which may affect the therapeutic efficacy in CRC. These findings suggest that COL11A1 can be used as a prognostic biomarker for determining prognosis and immune infiltration in COAD.

Declarations

Ethics approval and consent to participate: Human samples of this study approved by the ethical committee of The Fifth People's Hospital of Shanghai, Fudan University , and Fudan University Shanghai Cancer Center.

Consent for publication: Not applicable

Availability of data and material: All data generated or analysed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Yibin Wu and Yuankun Cai analyzed the data regarding the Colorectal Cancer disease and COL11A1. Wenjie Chen and Tao Ye collected the data. Ye Xu and Huipeng Wang were major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Correlation analysis between COL11A1 and related genes and markers of immune cells.

		COL11A1			
Description	Gene markers	None		Purity	
		Correlation	P	Correlation	P
CD8+ T cell	CD8A	0.231	***	0.137	***
	CD8B	0.081	***	0.026	***
T cell	CD3D	0.145	***	0.024	***
	CD3E	0.244	***	0.133	***
	CD2	0.254	***	0.163	***
B cell	CD19	0.113	***	0.003	***
	CD79A	0.214	***	0.097	***
Monocyte	CD86	0.577	***	0.536	***
	CD115 (CSF1R)	0.525	***	0.465	***
TAM	CCL2	0.56	***	0.498	***
	CD68	0.381	***	0.332	***
	IL10	0.372	***	0.332	***
M1 Macrophage	INOS (NOS2)	-0.225	***	-0.26	***
	IRF5	0.237	***	0.236	***
	COX2(PTGS2)	0.144	***	0.079	***
M2 Macrophage	CD163	0.608	***	0.568	***
	VSIG4	0.57	***	0.518	***
	MS4A4A	0.547	***	0.501	***
Neutrophils	CD66b (CEACAM8)	-0.214	***	-0.205	***
	CD11b (ITGAM)	0.696	***	0.634	***
	CCR7	0.199	***	0.085	***
Natural killer cell	KIR2DL1	0.126	***	0.078	***
	KIR2DL3	0.1	***	0.069	***
	KIR2DL4	0.106	***	0.023	***
	KIR3DL1	0.184	***	0.129	***
	KIR3DL2	0.146	***	0.093	***

	KIR3DL3	0.014	***	0.009	***
	KIR2DS4	0.115	***	0.078	***
Dendritic cell	HLA-DPB1	0.417	***	0.346	***
	HLA-DQB1	0.239	***	0.162	***
	HLA-DRA	0.376	***	0.296	***
	HLA-DPA1	0.399	***	0.324	***
	BDCA-1(CD1C)	0.256	***	0.167	***
	BDCA-4(NRP1)	0.707	***	0.675	***
	CD11c (ITGAX)	0.613	***	0.572	***
Th1	T-bet (TBX21)	0.27	***	0.198	***
	STAT4	0.26	***	0.164	***
	STAT1	0.347	***	0.308	***
	IFN-γ (IFNG)	0.18	***	0.134	***
	TNF-α (TNF)	0.226	***	0.184	***
Th2	GATA3	0.332	***	0.272	***
	STAT6	-0.163	***	-0.165	***
	STAT5A	0.154	***	0.132	***
	IL13	0.218	***	0.157	***
Tfh	BCL6	0.423	***	0.858	***
	IL21	0.202	***	0.164	***
Th17	STAT3	0.174	***	0.115	***
	IL17A	-0.251	***	-0.262	***
Treg	FOXP3	0.435	***	0.362	***
	CCR8	0.482	***	0.425	***
	STAT5B	0.208	***	0.221	***
	TGFβ (TGFB1)	0.565	***	0.502	***
T cell exhaustion	PD-1 (PDCD1)	0.244	***	0.161	***
	CTLA4	0.297	***	0.216	***
	LAG3	0.213	***	0.126	***

	TIM-3 (HAVCR2)	0.603	***	0.571	***
	GZMB	0.022	***	-0.005	***

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. * p< 0.05; ** p< 0.01; *** p< 0.001; **** p< 0.0001.

Table 2. Correlation analysis between COL11A1 and relate genes and markers of monocyte and macrophages in GEPIA.

Description	Gene markers	COAD			
		Tumor		Normal	
		R	P	R	P
Monocyte	CD86	0.58	***	−0.16	0.33
	CD115 (CSF1R)	0.64	***	−0.11	0.48
TAM	CCL2	0.48	***	0.17	0.27
	CD68	0.48	***	−0.28	0.072
	IL10	0.41	***	0.0013	0.99
M1 Macrophage	INOS (NOS2)	-0.12	0.043	−0.0023	0.99
	IRF5	0.18	0.0034	-0.29	0.065
	COX2(PTGS2)	0.30	***	0.42	0.0065
M2 Macrophage	CD163	0.61	***	0.0089	0.96
	VSIG4	0.60	***	0.01	0.95
	MS4A4A	0.61	***	−0.16	0.33

CODA, colon adenocarcinoma; TAM, Tumor-associated macrophages. Tumor, correlation analysis in tumor tissue of TCGA. Normal, correlation analysis in normal tissue of TCGA. *P < 0.01; **P < 0.001; ***P < 0.0001.

Figures

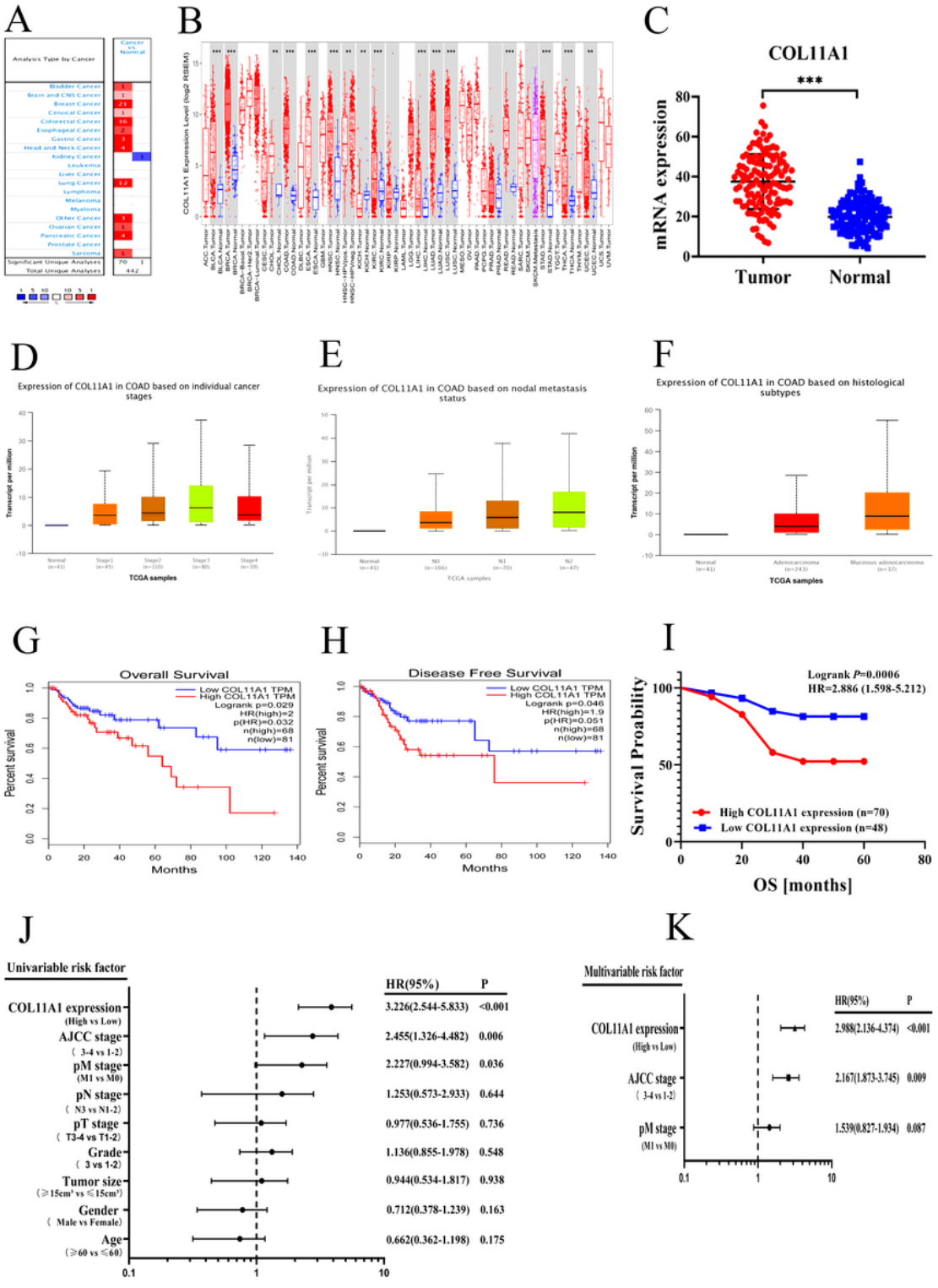

Figure 1

Clinical significance of COL11A1 in human CRC. (A) Expression levels of COL11A1 in data sets of different cancers compared with the normal tissues in the Oncomine database. (B) Increased or decreased COL11A1 expression in different tumors types from TCGA database by TIMER analysis. (C) Human samples from 118 cases of CRC tissues with the matched tissues were used to analyze the expression level of COL11A1 mRNA by qRT-PCR (*P < 0.05, **P <0.01, ***P < 0.001). (D-F) Results of

UNCLA analysis showed that increased COL11A1 was positive associated with some clinicopathological parameters (tumor stage, node metastasis status and histological types). (G-H) Survival curves of OS and DFS comparing the high and low expression of COL11A1 in CRC by GEPIA. (I) High COL11A1 expression was correlated with poor OS by analyzed the survival data of human samples. (J-K) Logical regression analysis identified COL11A1 as an independent prognostic factor for CRC.

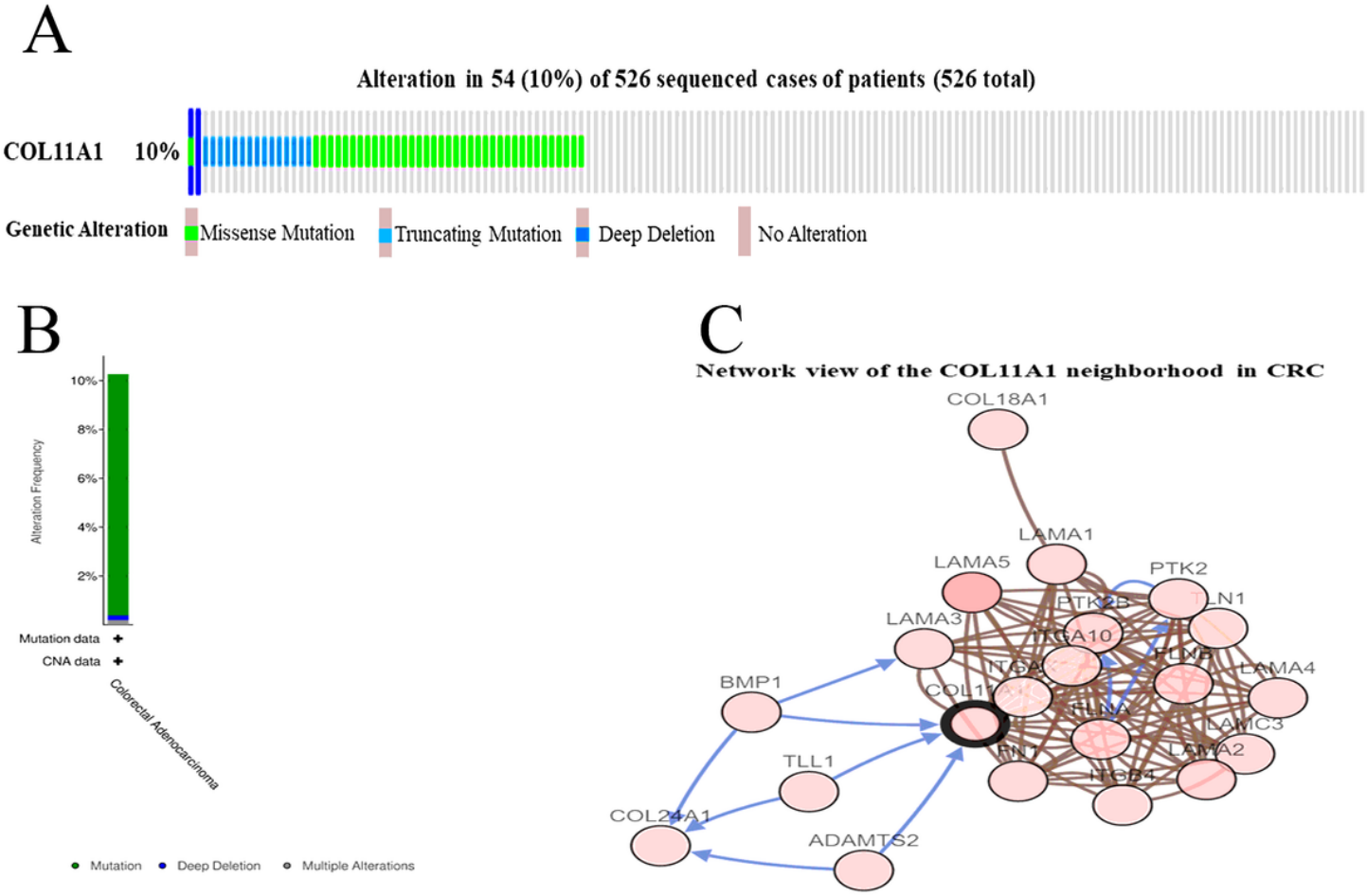

Figure 2

Genomic Alteration of COL11A1 in CRC. (A-B) 10% of COL11A1 alteration rate was observed in CRC and mutation was the most common type of alteration frequency. (C) Network view of COL11A1 neighbored genes in CRC. The blue connection indicated that the first protein controlled a reaction that changes the state of the second protein; the red connection suggested that the proteins belonged to members of the same complex.

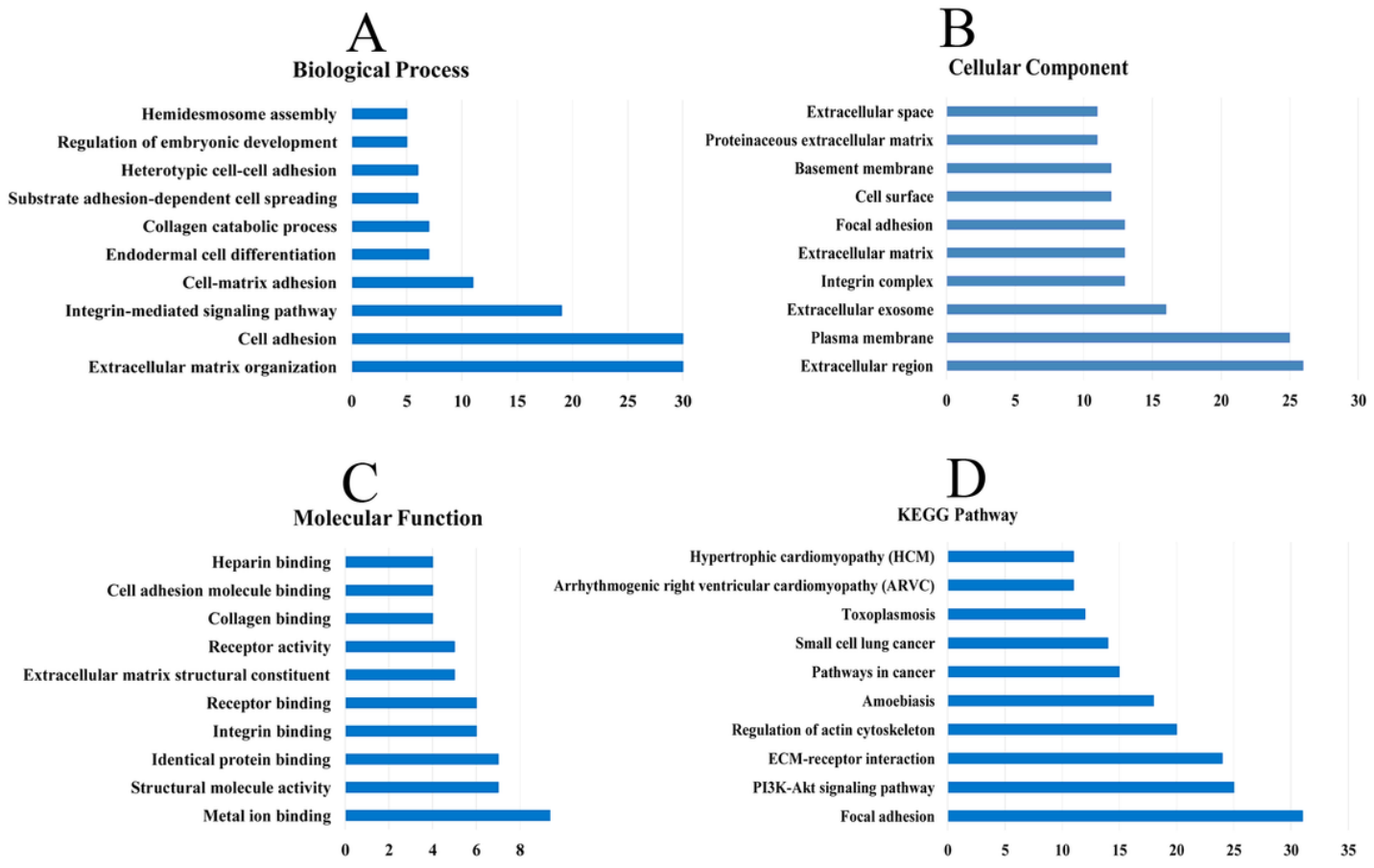

Figure 3

Enrichment analysis of the genes altered in the COL11A1 neighborhood in CRC. Functional networks of COL12A1 along with its top 50 frequently altered neighboring genes in CRC via analyzing DAVID. (A) Biological process. (B) Cellular component. (C) Molecular function. (D) KEGG pathway.

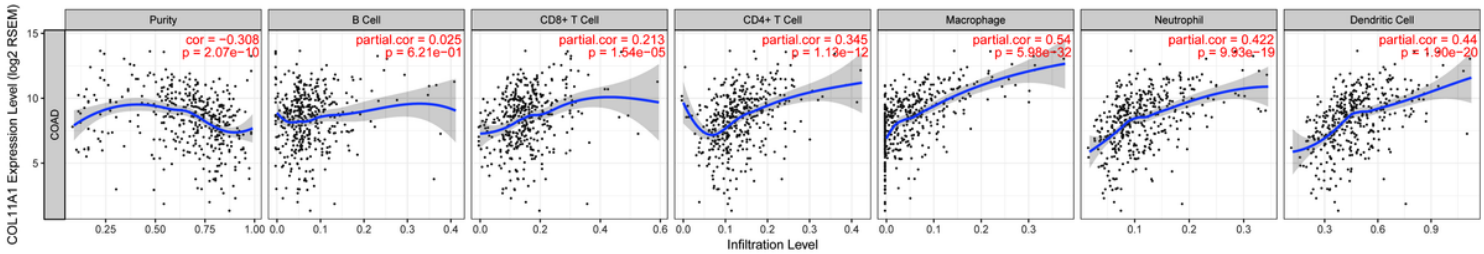

Figure 4

Correlation of COL11A1 expression with immune infiltration level in colon adenocarcinoma (COAD). LAYN expression is significantly negatively related to tumor purity and has significant positive correlations with infiltrating levels of CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in COAD, other than B cells (n=457).

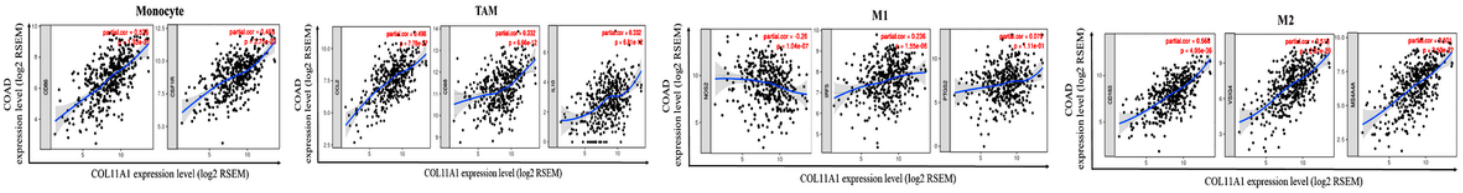

Figure 5

COL11A1 expression correlated with macrophage polarization in COAD. Markers include CD86 and CSF1R of monocytes; CCL2, CD68, and IL10 of tumor-associated macrophages (TAMs); NOS2, IRF5, and PTGS2 of M1 macrophages; and CD163, VSIG4, and MS4A4A of M2 macrophages (n=457).

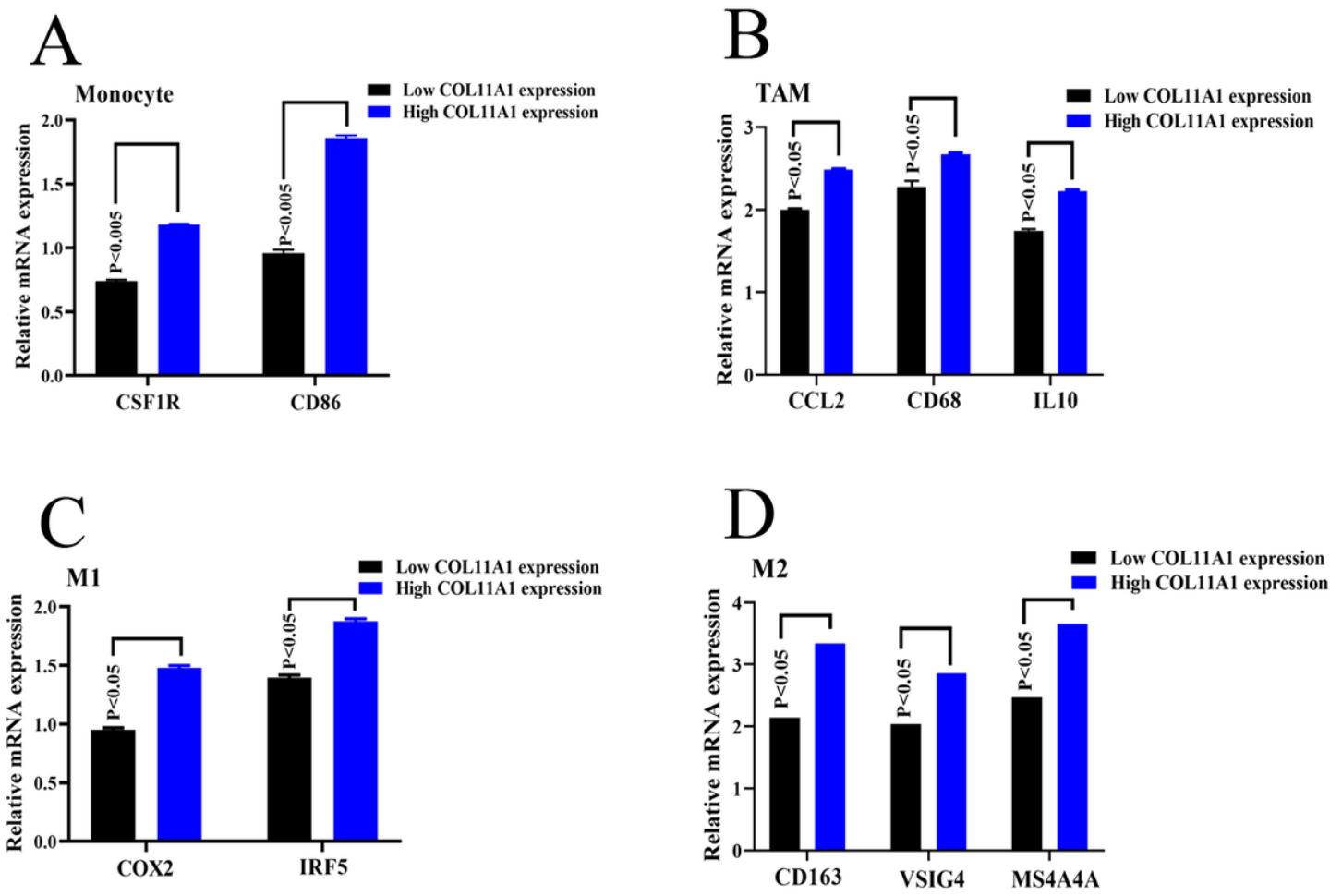

Figure 6

Human samples were used to further examine the correlations between COL11A1 expression and macrophage polarization by qRT-PCR. (A-D) Scatterplots of correlations between LAYN expression and gene markers of monocytes (A), TAMs (B), and M1 (C) and M2 macrophages (D) in COAD (n=118).