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Expression, prognostic value and potential immunotherapeutic target of COL1A1 in colon cancer

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ABSTRACT

This study aimed to investigate the link between COL1A1 production and colorectal carcinoma and assess the value of prognosis and immunotherapy. For this purpose, the transcriptional level of COL1A1 was analyzed. The clinicopathological information and gene expression profile were analyzed to reveal the link between COL1A1 and clinicopathological characteristics. For bioinformatics examination, GSEA and GSVA were utilized. Correlation analysis was implemented to study the causal relationship between COL1A1 and immune checkpoint molecules and inflammation immune cell infiltration. Results showed that in colorectal cancer, COL1A1 was highly expressed and linked with a few clinicopathological characteristics, inflammation and immunological response, tumor immune cell infiltration, and immune checkpoint markers. COL1A1 might likely indicate a bad prognosis and serve as a target of immunotherapy for colon cancer.

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Introduction

Among the malignant tumors of the digestive system, colon cancer is very common (1). More recently, because of the variation in lifestyle and diet construction, the number of colon cancer patients has increased year by year. The clinical signs of colon cancer are usually not obvious in the early stage. When the symptoms are visible, the tumor frequently spreads, which is the primary factor contributing to the high fatality rate. Therefore, finding promising biomarkers is crucial for colon cancer early diagnosis and prognosis prediction.

The study of immune checkpoint inhibitors (ICIs) has become more and more popular in recent years. ICIs have good treatment effects on solid tumors, like melanoma (2), non-small cell pulmonary cancer (3) and renal cell carcinoma (4). Whereas, for colon cancer, except for a small number of microsatellite unstable (MSI) tumors, other types of colon cancer have no good effect on ICI immunotherapy (5). Therefore, the need for new immunotherapeutic targets in the treatment of colon cancer is essential.

Type I collagen α1 (COL1A1) encoding type I collagen pro-α1 chain, which is a triple helix composed of an α1 chain and an α2 chain (6,7) Recent studies show that the high COL1A1 expression is related to several tumorigeneses, namely gastric cancer (8), carcinoma of the lungs (9), liver cancer (10) and renal carcinoma (11). However, there are few reports of a connection between COL1A1 and colon cancer. This investigation aims to use bioinformatics to find how colon cancer prognosis and survival are affected by COL1A1 expression, as well as to identify the link between COL1A1 and the colon cancer immune microenvironment, in order to propose a fresh approach to the identification and treatment of colon cancer.

Materials and Methods

Download research materials

Download The Cancer Genome Atlas (TCGA) clinical data for tissues with colon cancer and normal tissue, as well as the date of RNA sequencing (HTSeq-FPKM).

Bioinformatics analysis

Gene set enrichment analysis (GSEA) was utilized to recognize the biotic route that contributed to the differential enrichment between the groups that produced high levels and low levels of COL1A1. In addition, the gene sets, converted from the immune-related genes in the TCGA database, were scored by gene set variation analysis (GSVA). For evaluating the relationship between COL1A1 and immuno-related genes in colon cancer, correlation analysis was used. The results were visualized.

Statistical analysis

In this research, R software 3.6.0, GraphPad Prism7.0, and SPSS25.0 were utilized for statistical analyses. The Shapiro-Wilk normality test was performed to gauge the variable distribution. Student's test was utilized to test information that obeys normal distribution, while the Mann-Whitney U test was used for other data. The clinical information and gene expression data in TCGA Informix were examined using a descriptive methodology. To discuss the link between COL1A1 and clinicopathological features, logistic regression was utilized. Kaplan-Meier survival analysis and univariate and multivariate Cox regression analysis were executed to examine the consequence of

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COL1A1 production and other issues on overall survival. To investigate the causal link between COL1A1 production and immunological response, immune cell, and immune checkpoint molecule, correlation analysis was utilized. For data visualization, the R program generates Heat maps, Circos, and Corrgram maps. Results were considered significant for $p<0.05$.

Results

COL1A1 production in colon cancer and non-tumor specimens

We noticed that COL1A1 was synthesized at greater levels in colon cancer sections than in healthy tissues after checking RNA sequencing data from colon tumour and non-tumor specimens (Figure 1A). Additionally, the ROC curve was formed depending on the differential expression of COL1A1 in normal and malignant tissues. As shown in Figure 1B, the area under the curve of the TCGA data set (AUC) reached 88.9%. The results indicate that COL1A1 could become a potential biomarker of colon cancer.

Clinical information on patients with colorectal carcinoma

The clinical details on COL1A1 of 447 individuals with colorectal cancer were obtained from TCGA Informix (Table 1). Among the 447 cases, the median age was 69 years (range 31 to 90), 212 females (44.4%) and 235 males (52.6%). The tumor stages I, II, III and IV were 75 (17.2%), 176 (40.4%), 124 (28.4%) and 61 (14.0%), respectively. T stages T1, T2, T3 and T4 were 10 (2.2%), 76 (17.0%), 304 (68.2%) and 56 (12.6%), respectively. N stages N0, N1 and N2 accounted for 186 (41.6%), 182 (40.7%) and 79 (17.7%), respectively. M stages M0 and M1 were 330 (84.4%) and 61 (15.6%), respectively. The pathological types were adenocarcinoma and mucinous adenocarcinoma, which were 381 (86.2%) and 61 (13.8%), respectively. After the operation, the patients reexamined the tumor-free state and tumor-carrying state, which were 168 (37.6%) and 172 (38.5%), respectively.

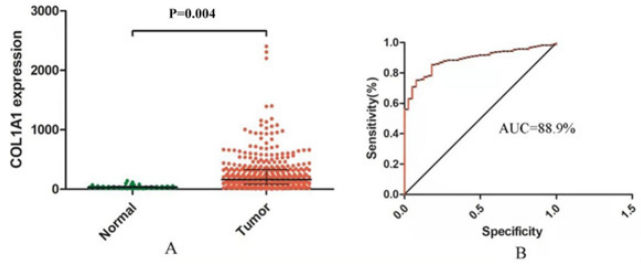

Figure 1. A: COL1A1 production in colon cancer and non-tumor specimens, B: ROC curve analysis.

Table 1. Clinical information of patients with colorectal carcinoma.

Clinicopathological features		No. Of patients (%)
Age at diagnosis	Median (range)	67.1 (31-90)
Gender	Women	212 (47.4%)
	Men	235 (52.6%)
Tumor Stage	Stage I	75 (17.2%)
	Stage II	176 (40.4%)
	Stage III	124 (28.4%)
	Stage IV	61 (14.0%)
T Stage	T1	10 (2.2%)
	T2	76 (17.0%)
	T3	304 (68.2%)
	T4	56 (12.6%)
N Stage	N0	186 (41.6%)
	N1	182 (40.7%)
	N2	79 (17.7%)
M Stage	M0	330 (84.4%)
	M1	61 (15.6%)
Tumor Status	Tumor free	168 (37.6%)
	With tumor	172 (38.5%)

The link between COL1A1 production and clinicopathological features in colon cancer

Mann-Whitney statistical analysis was performed between COL1A1 production and clinicopathological features. As revealed in Figure 2, the production of COL1A1 was meaningfully related to tumor stage ($p<0.001$), T stage ($p<0.001$), N stage ($p<0.001$), M stage ($p<0.001$) and tumor status ($p=0.032$) (Table 2).

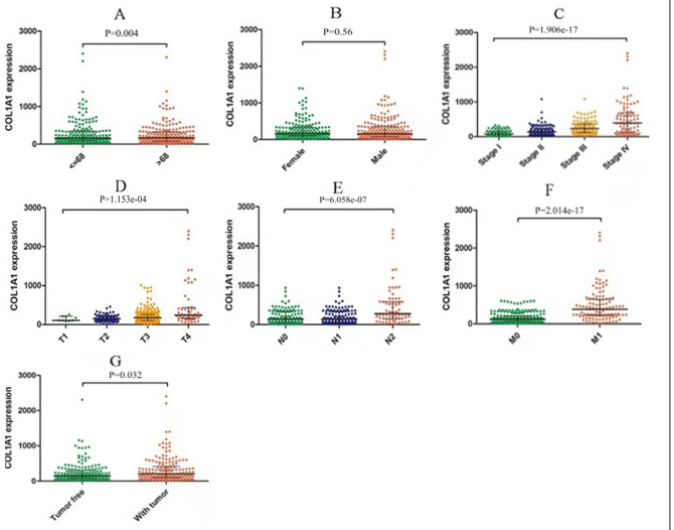

Figure 2. The link between COL1A1 production and clinicopathological features in colon cancer. A: Age, B: Gender, C: Tumor stage, D: T stage, E: N stage, F: M stage, G: Tumor status.

Table 2. The link between COL1A1 production and clinicopathological features in colon cancer.

Clinicopathological features		OR (95% CI)	P
Age	>68 VS <=68	1.11 (0.74-1.66)	0.607
Gender	Men VS Women	0.81 (0.53-1.21)	0.304
Tumor Stage	Stage II VS Stage I	2.54 (1.31-5.11)	0.007
	Stage III VS Stage I	5.01 (2.52-10.43)	<0.001
	Stage IV VS Stage I	8.16 (3.97-17.59)	<0.001
T Stage	T2 VS T1	1.59 (0.99-2.58)	0.054
	T3 VS T1	2.15 (1.23-3.86)	0.009
	T4 VS T1	2.57 (1.78-3.77)	<0.001
N Stage	N1 VS N0	1.12 (0.71-1.77)	0.633
	N2 VS N0	1.67 (1.14-2.45)	0.008
M Stage	M1 VS M0	3.53 (1.14-2.45)	<0.001
Tumor status	With tumor vs. tumor-free	1.68 (1.10-2.59)	0.017

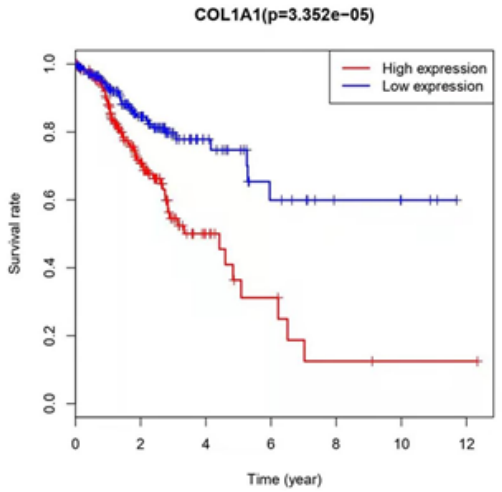

Figure 3. Survival analysis of COL1A1 in colon cancer.

The link between COL1A1 expression and the survival and prognosis in colon cancer

Kaplan-Meier curve was created using TCGA database data to examine the predictive significance of COL1A1 in colorectal carcinoma. As illustrated in Figure 3, those with higher COL1A1 production had a considerably lower survival rate than those with lower COL1A1 production. These outcomes showed that COL1A1 was an indicator of an unfavorable prognosis for colon cancer. In order to examine the predictive relevance of COL1A1 in colon cancer, both univariate and multivariate Cox regression analyses were used. Univariate COX regression analysis (Table 3) indicated tumor stage (HR=3.52,95% CI:1.78-5.38, p<0.001), T stage (HR=1.89,95% CI:1.05-3, 34, p=0.033), M stage (HR=1.54,95% CI:1.09-2.20, p=0.016), N stage (HR=2.52,95% CI:1.72-3.70, p<0.001), Tumor status (HR=1.68,95% CI:1.07-2.89 CI:1.41 p=0.038) and COL1A1 (95% CI:1.41-3.90 (p< 0.001) were related with prognosis and survival. Further multivariate Cox regression analysis (Table 4) revealed that tumor stage (HR=6.38,95% CI:1.88-28.36, p=0.03), M stage (HR=1.04,95% CI:1.01-1.17, p=0.009) and COL1A1 (HR=2.07,95% CI:1.39-3.78, p=0.004) were independently correlated with the prognosis of colorectal carcinoma. These results suggest that COL1A1 could become a novel independent molecular symbol for the prognosis of patients with colorectal carcinoma.

Related biological processes and signal pathways of COL1A1

To find out the biotic processes and signal pathways linked to elevated COL1A1 expression, gene set enrichment analysis (GSEA) was utilized. As revealed in Figure 4 and Table 5, patients with colorectal cancer who had an elevated level of COL1A1 production revealed enhancements in a few particular biotic processes and signaling networks. These pathways play a role in colorectal tumorigenesis and inflammatory immune response, such BCR (B-cell receptor) signaling pathway, TCR (T-cell receptor) signaling pathway, pathways of antigen processing and presentation, NK cell-mediated cytotoxicity pathway and

Table 3. Univariate Cox regression analysis of survival rate in colorectal carcinoma.

Clinicopathological features	HR (95% CI)	P
Age	1.02 (0.99-1.04)	0.136
Gender	1.10 (0.57-1.72)	0.708
Tumor Stage	3.52 (1.78-5.38)	<0.001
T Stage	1.89 (1.05-3,34)	0.033
N Stage	1.54 (1.09-2.20)	0.016
M Stage	2.52 (1.72-3.70)	<0.001
Tumor Status	1.68 (1.07-2.89)	0.038
COL1A1	2.33 (1.41-3.90)	<0.001

Table 4. Multivariate Cox regression analysis of survival rate in colorectal carcinoma.

Clinicopathological features	HR (95% CI)	P
Tumor Stage	6.38 (1.88-28.36)	0.003
T Stage	1.93 (0.58-6.39)	0.304
N Stage	0.40 (0.10-1.10)	0.068
M Stage	1.04 (1.01-1.17)	0.009
Tumor Status	1.59 (0.95-2.67)	0.079
COL1A1	2.07 (1.39-3.78)	0.004

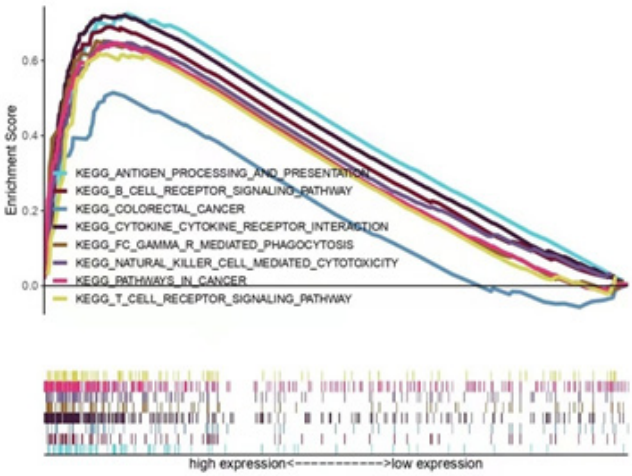

Figure 4. GSEA for COL1A1.

Table 5. Gene sets enriched in the high production group.

Name of gene set	Normalized enrichment score	Normalized P-value	False discovery rate q-value
Kegg_colorectal_cancer	1.9579843	<0.001	0.00386542
Kegg_pathways_in_cancer	2.6664305	<0.001	0
Kegg_b_cell_receptor_signaling_pathway	2.326231	<0.001	2.99E-05
Kegg_natural_killer_cell_mediated_cytotoxicity	2.3236187	<0.001	2.79E-05
Kegg_t_cell_receptor_signaling_pathway	2.236086	0.002	1.39E-04
Kegg_antigen_processing_and_presentation	2.1073139	<0.001	7.50E-04
Kegg_fc_gamma_r_mediated_phagocytosis	2.3238864	<0.001	2.89E-05

Fc gamma R-mediated phagocytosis pathway.

The correlation between COL1A1 and inflammatory and immune activity

In order to further understand the immune correlation with COL1A1, we used 7 gene clusters standing (12) for varying kinds of inflammation and immune activity to study. As revealed in Figure 5A, COL1A1 production definitely interacted with all gene clusters, namely Interferon, LCK, STAT-1, HCK, MHCI, MHCII and IgG. In order to confirm the truth of our analysis above, GSVA was used to turn the gene production data into the enrichment scores of gene clusters and created a correlation map to visualize the link of COL1A1 and 7 gene clusters, and these results were the same with our foregoing results (Figure 5B).

Correlation between COL1A1 and immune infiltrating cells

Numerous studies have revealed that tumor-infiltrating immune cells (TIICs) contribute to the regulation of the growth of tumors and outcomes (13,14). We studied the link between COL1A1 production and six common TIICs, like neutrophils, natural killer cells, regulatory T cells (Tregs), tumor-associated macrophages (TAMs), CD8+T cells and myeloid-derived suppressor cells (MDSCs). Supplementary file S2 shows the particular symbols for immune cells. Correlation analysis revealed that COL1A1 production was relative with six immune cell particular symbols, indicating that there were many TIICs in colon cancer patients with high COL1A1 expression (Figure 6A).

Correlation between COL1A1 and immune checkpoint

Five kinds of immune checkpoint molecules were selected as immunotherapy, including PD-1, PD-L1, TIM-3, B7-H4, and B7-H3 (15-17). To examine the link between colon cancer COL1A1 and five immunological checkpoint molecules, the Pearson product-moment correlation coefficient was adopted. The visualization of the Circos map as shown in Figure 6B revealed a substantial positive connection between COL1A1 and B7-H3/TIM-3, indicating that it was closely related to T cell immunoglobulin and B7 family ligands (Figure 6B), and had a certain correlation

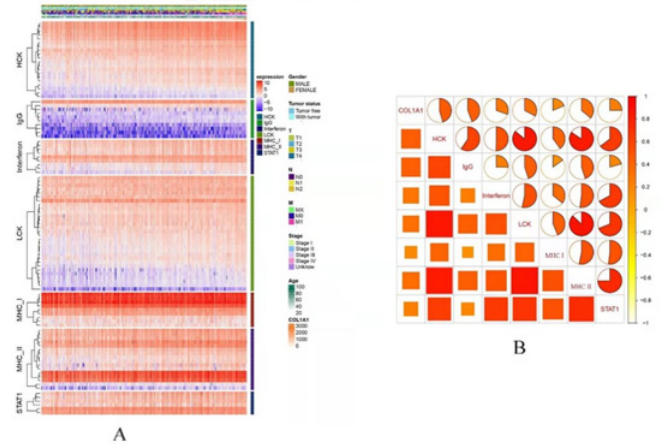

Figure 5. COL1A1-related inflammatory reaction. A: clinicopathological features, COL1A1 production, seven gene clusters. B: Correlograms were made on the ground of the link of COL1A1 production and seven gene clusters. The circles were colored with red clockwise for positive values and with yellow anticlockwise for negative.

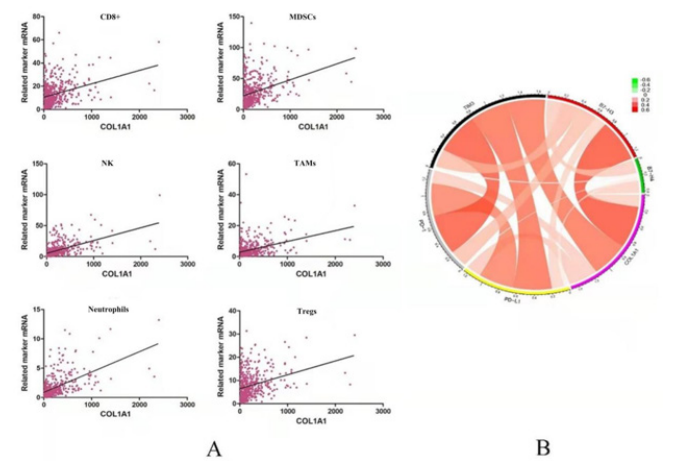

Figure 6. A: Correlation between COL1A1 and immune infiltrating cells. B: Correlation between COL1A1 and immune checkpoint.

with PD-1/L1 and COL1A1 expression. The above outcomes show that COL1A1 is closely associated with the common targets of immunotherapy, suggesting that COL1A1 can be a potential immunotherapy target.

Discussion

In this research, by studying the colon cancer gene expression data in TCGA Informix, we observed that COL1A1 production of colon cancer samples was up-regulated. We looked backward at the clinical data of 447 colorectal cancer patients from the TCGA RNAseq dataset and found that COL1A1 could forecast the colon cancer patients' overall survival. In addition, the level of COL1A1 expression increased with the grade of colon cancer and correlated with clinicopathological parameters (tumor grade, T stage, N stage, M stage, tumor status). Cox regression analysis revealed that COL1A1 might be an effective prognostic biological marker for colon cancer patients. The consequences above suggest that COL1A1 expression is decidedly correlated with the occurrence and malignant progression of colon cancer.

By utilizing GSEA, we found that the over-expressed COL1A1 in colon cancer was enriched in the signal pathways related to colorectal tumorigenesis and inflammation and immune response. In order to better understand the inflammatory and immune activities related to COL1A1, we generated a heat map involving seven gene clusters and found that COL1A1 was decidedly related to HCK, IgG, Interferon, LCK, MHCI, MHCII and STAT1 gene clusters. The above results were verified by GSVA correlation map analysis. The production of particular marker genes of six significant immune cells, including innate immune cells (NK cells, Neutrophils, MDSCs, and TAMs) and adaptive immune cells (Tregs, CD8+T cells), was definitively correlated with the expression of the gene COL1A1. Additionally, the Circos map demonstrated that COL1A1 was clearly connected to alterations in a number of widespread immunological checkpoints. These findings suggest that COL1A1 is an essential component of the colon cancer immune microenvironment.

The traditional prognosis assessment of colon cancer patients mostly relies upon the TNM stage. Although the TNM stage has great value in predicting prognosis, it lacks dates of cellular and molecular level, which could lead to significant heterogeneity in the clinical prognosis

of colon cancer patients even with the same TNM stage. In this context, large numbers of studies have been trying to find a single biological marker that can be an effective indicator for colon cancer and other tumors (18,19). In this study, the COL1A1 we screened can well predict patients' overall survival rate and COL1A1 expression is decidedly correlated to tumor stage and TNM stage, which greatly improves the application value of COL1A1 as a biomarker in colon cancer.

More and more studies reveal that tumor immune cells are decidedly relative to the occurrence and development of cancers (20,21). The tumor microenvironment created by these non-tumor cells has important effects on cancer development, recurrence, reaction to therapeutic intervention and so on. Albeit the immune system can produce immune responses to tumor cells, the reactions are not sufficient to eliminate tumor cells mostly due to immunosuppression in the cancer microenvironment. Thus, beating the condition of immunosuppression is the key to improving the efficacy of anti-tumor immunotherapy. In our study, a new finding is that COL1A1 participates in the immune microenvironment of colon cancer, and COL1A1 transcription level is decidedly related to TIICs (including Treg, MDSCs and Neutrophils). These cells can show strong immunosuppressive activity and lead to unfortunate anticipation of cancer patients. Of course, future studies need to clarify the detailed interaction between COL1A1 and immunosuppressive cells.

In the past few years, tumor immunotherapy has made remarkable progress, largely due to the considerable success of ICIs in some kinds of cancers (22,23). Past research has revealed that ICIs have good effects on the type of colon cancer with microsatellite instability-high/deficient mismatch repair (MSI-H/dMMR). However, for the majority of colon cancer, microsatellite stable/mismatch repair proficient (MSS/pMMR), ICIs don't work effectively (24). Acting as a co-inhibitory receptor, TIM-3 can hinder T-cell work. Experiments revealed that TIM-3 blockers showed a positive effect on colon adenocarcinoma in mice (25,26). Research revealed that TIM-3 monoclonal antibody can raise the efficacy of chemotherapy (27). In this study, we found that there was a definite link between COL1A1 and TIM-3, indicating that they had a strong synergistic effect in regulating immune reactions in the cancer microenvironment. These findings present a brand-new opportunity for colon cancer combination treatment. The union of COL1A1 and immune checkpoint inhibitors would contribute to overcoming the restriction of using immune checkpoint inhibitors independently.

Finally, our research has a few limits. Firstly, the study data are incomplete since some patients' records are missing, therefore there weren't many patients included in the univariate and multivariate Cox analyses. Secondly, additional tests are required since fewer negative samples were utilized as controls. Thirdly, because the transcription process of tumor molecules can change, the level of mRNA isn't completely predictive of protein expression. Therefore, to further understand the important effect of COL1A1, it is necessary to additionally study the protein level of COL1A1 in colon cancer samples. Of course, other laboratory studies are expected to clarify the specific theory of COL1A1 overexpression in colon cancer and clarify its connection with immune regulation and unfortunate forecast in colorectal carcinoma.

To sum up, this study reveals that COL1A1 over-expresses in colon cancer. The high COL1A1 production is connected with the clinicopathological parameters and predicts the unfortunate forecast of colon cancer patients. COL1A1 is associated with inflammatory and immune reactions and is associated with immune checkpoint molecules. Thus, COL1A1 might be an indicator of unfortunate forecast and an effective target of immunotherapy for colorectal carcinoma.

Financial and competing interests disclosure

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