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Article

COL5A3: A prognostic biomarker and potential therapeutic target in pancreatic cancer

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Abstract: Pancreatic cancer is a malignant tumor of the digestive system with a high mortality rate and a poor prognosis. While type V collagen 3 (COL5A3) is extensively expressed in many tumor tissues, its prognostic significance and immune infiltration in pancreatic cancer remain unknown. As a result, we investigated COL5A3's predictive function in pancreatic cancer and its relationship to immune infiltration. COL5A3 is significantly expressed in pancreatic cancer tissues compared to normal tissues. High COL5A3 expression is associated with poor clinicopathological characteristics and a worse prognosis of pancreatic cancer. The Kaplan-Meier survival analysis revealed that pancreatic cancer patients with high COL5A3 expression had a poorer prognosis than those with low COL5A3 expression. According to the ROC curve, COL5A3 has high sensitivity and specificity in the detection of pancreatic cancer. Correlation studies revealed that COL5A3 mRNA expression is associated with immune cell infiltration. This work indicates for the first time that COL5A3 may be a novel predictive biomarker linked to immune infiltration, providing a new target for pancreatic cancer detection and therapy.

Keywords: COL5A3; pancreatic cancer; prognosis; immune infiltration; biomarker

1. Background

Pancreatic cancer is a highly malignant tumor disease of the digestive system that has become more common in recent years. The incidence of fatalities from pancreatic cancer is rising in the United States. Given of its dismal prognosis, its 5-year survival rate is around 10%, making it a prevalent cause of cancer mortality [1]. According to the International Agency for Research on Cancer's 2018 global cancer morbidity and mortality estimates, there are 458,918 new cases of pancreatic cancer and 432,242 deaths globally, accounting for 2.5% of newly diagnosed malignancies and 4.5% of all cancer deaths that year [2]. Clinical diagnosis and therapy are difficult due to the clinical features of patients with pancreatic cancer, such as insidious onset, challenging early detection, rapid progression, low resection rates, and a high likelihood of recurrence and metastasis after surgery. Pancreatic cancer treatment is evolving as novel surgical procedures and pharmacological therapies, including laparoscopy and neoadjuvant radiation and chemotherapy, are introduced. Nevertheless, there has been relatively little progress in improving the therapeutic outcomes for patients [3]. Immunotherapy has shown significant efficacy in the

treatment of a variety of malignant tumors in recent years [4–6], its application in pancreatic cancer is on the horizon; however, it is not suitable for all patients [7]. Moreover, pancreatic cancer patients' access to targeted therapies has improved significantly, which is expected to usher in a new era of precision oncology [8]. The identification of early pancreatic cancer biomarkers is crucial for the study of the disease, given the limitations of current therapies, the effectiveness of treatment outcomes, and the lack of understanding regarding its potential etiology.

The collagen gene family is the principal component of the extracellular matrix, accounting for 30% of mammalian total protein mass, whereas type V collagen alpha3 (COL5A3) is a member of the collagen family and is important in controlling fiber synthesis in the extracellular matrix [9]. Nowadays, COL5A3-related research is infrequently mentioned in the literature. The expression of the collagen gene COL5A3 has been shown to have a crucial function in bone development [10]. COL5A3 has been linked to the onset and progression of a number of malignancies, including breast cancer [11], uveal melanoma [12], prostate cancer [13], renal cell carcinoma [14], and others. Although high-throughput sequencing has revealed that COL5A3 is substantially expressed in numerous cancers, there is no evidence of a link between COL5A3 expression and prognosis in pancreatic cancer.

As a result, in this work, we analyzed clinical indicators and survival data from numerous online databases, including The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), and Clinical Proteomic Tumor Analysis Consortium (CPTAC), to assess the importance of COL5A3 expression in patients with pancreatic cancer. We also investigated the relationship between COL5A3 mRNA levels and tumor-infiltrating immune cells. In conclusion, our study found a strong relationship between COL5A3 overexpression and a poor survival rate in pancreatic cancer.

2. Materials and methods

2.1. TCGA datasets

TCGA is a breakthrough cancer genomics study that includes 33 cancer types and over 20,000 samples. We obtained the transcriptional expression data and clinical information for COL5A3 from the TCGA website. In addition, we investigated the expression level of the COL5A3 gene in cancer and paracancerous tissues using data from the TCGA and GTEx databases. After performing log2 transformation, RNAseq data in TPM (transcripts per million reads) format were examined and compared. Lastly, we analyzed the expression of COL5A3 in normal and malignant tissues in the GEO dataset (GSE16515).

2.2. RNA-sequencing data of COL5A3 in pancreatic cancer

The RNA-Seq expression data of COL5A3 in pancreatic cancer may be downloaded from the TCGA website. Because there are few pancreatic cancer normal tissue samples in the TCGA database, we combined the pancreatic cancer normal tissue samples in the GTEx database and eventually kept the data of 171 samples of nearby normal tissue and 179 cases of tumor tissue. COL5A3 gene

expression data and relevant clinical information, such as patient sex, age, smoking and drinking history, tumor TNM stage, and first treatment result, are included in the selected clinical samples.

2.3. The UALCAN website analyzes CPTAC data

UALCAN (https://ualcan.path.uab.edu/index.html) is a comprehensive cancer genomics data analysis website that provides access to publicly available cancer genomics data (TCGA, MET500, CPTAC, and CBTTC). CPTAC is a protein expression database that allows for the analysis of target protein expression patterns in different tumors through the UALCAN website.

2.4. Analysis of prognostic indexes

To investigate the clinical significance of the COL5A3 gene in the prognosis of patients with pancreatic cancer, we examined longevity indices such as overall survival (OS) and disease specific survival (DSS). The Kaplan-Meier curve was used to show the RNA sequencing data and clinical details of pancreatic cancer cases from the TCGA database. The COL5A3 expression was split into two groups: low expression and high expression, and the P value was determined using the Log-rank test and Cox regression analysis.

2.5. Protein-protein interaction (PPI) networks and functional enrichment analysis

STRING (https://www.string-db.org/) is a widely used database for finding and forecasting known protein-protein interactions. It is possible to mine central regulatory genes by analyzing protein-protein interaction networks. In this research, we used the STRING database to find the top ten COL5A3-associated genes and created a PPI network diagram. We examined the chosen data using GO and KEGG enrichment analysis, the ggplot2 package (version 3.3.3) for visualization, and the clusterProfiler package (version 3.14.3).

2.6. Tumor immune estimation resource database (TIMER)

TIMER (https://cistrome.shinyapps.io/timer/) detects immune cell invasion in tumor tissues using RNA-Seq expression profile data. At the moment, it primarily offers the invasion of six types of immune cells. We used the TIMER database to identify the expression of COL5A3 and tumor purity, as well as the relationship between six types of immune infiltrating cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) in this research. In addition, we also calculated the stromal and immune scores using the R package - estimate [1.0.13], and the statistical method used was the Welch t-test.

2.7. Tumor immune system interaction database (TISIDB)

TISIDB (http://cis.hku.hk/TISIDB) is a website dedicated to researching the interplay of cancer and the immune system. It includes several tumor-related datasets. In this research, we found the expression of COL5A3 and 28 distinct types of tumor infiltrating lymphocytes (TILs) in various human cancers, and we used

TISIDB to establish a connection between the expression of COL5A3 and the abundance of different TILs.

2.8. Statistical analysis

All of our data is statistically analyzed and visualized in R (version 3.6.3), with the primary R tool involved: ggplot2 (version 3.3.3) being used for visual analysis. The Mann-Whitney U test was used to distinguish between pancreatic cancer cells and nearby normal tissue. To assess the impact of COL5A3 expression on survival, we used Kaplan-Meier and log-rank tests for the statistical analysis of survival data, and the survminer software (version 0.4.9) for visualization. The ROC curve was examined using the pROC program (version 1.17.0.1).

3. Results

3.1. Expression pattern of COL5A3 in pan-cancer perspective

We studied a dataset of 33 cancer types from the TCGA combined GTEx database to assess COL5A3 mRNA expression in various cancer types. As demonstrated in **Figure 1A**, COL5A3 expression was substantially altered in 28 of the 33 cancer types compared to normal tissues. In PAAD, expression was considerably considerably elevated.

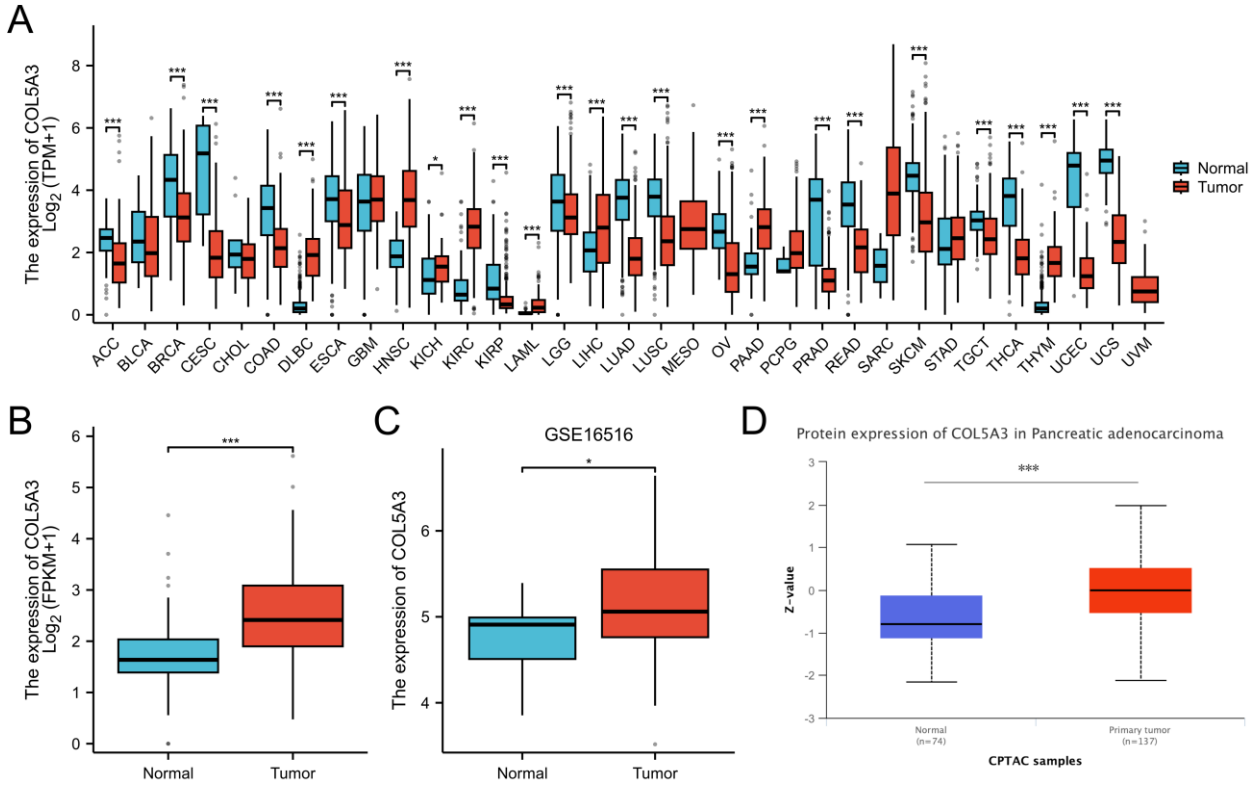

Figure 1. (A) Expression pattern of COL5A3 in Pan-cancer perspective; **(B)** The mRNA expression levels of COL5A3 in 179 pancreatic cancer samples and 171 normal samples; **(C)** The aberrant expression of COL5A3 based on the GEO database. COL5A3 mRNA levels in pancreatic cancer tumor tissues and normal tissues in the GSE16515 dataset; **(D)** Protein expression of COL5A3 in Pancreatic adenocarcinoma.

(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3.2. COL5A3 mRNA and protein expression was upregulated in patients with pancreatic cancer

To evaluate the level of COL5A3 mRNA and protein expression in pancreatic cancer, we examined COL5A3 expression data from TCGA and CPTAC database, and then further investigated and validated COL5A3 expression using the GEO database. **Figure 1B** shows that the expression of COL5A3 in pancreatic cancer is much greater than in normal tissues ($p < 0.01$). **Figure 1C** shows that the expression of COL5A3 is up-regulated in the GSE16515 data set when compared to normal tissues ($p < 0.05$). Finally, we analyzed the protein expression level of COL5A3 in pancreatic cancer using the UALCAN website based on the CPTAC database. **Figure 1D** shows that COL5A3 is still highly expressed in the tumor group compared to the normal group ($p < 0.001$). The findings revealed that COL5A3 mRNA and protein expression levels were increased in pancreatic cancer.

3.3. Univariate and multivariate Cox regression analysis of COL5A3 and clinicopathological parameters associated with OS

We used single and multivariate Cox regression to examine the relationship between COL5A3 expression and clinicopathological characteristics and OS, as shown in **Table 1** and **Figure 2**. A univariate COX regression analysis revealed that increased COL5A3 expression was linked with a lower overall survival in pancreatic adenocarcinoma (PAAD). The *T*-stage and N-stage were found to be linked with a poorer OS prognosis in advanced PAAD, whereas complete response (CR) was associated with an improved prognosis in primary treatment outcome. COL5A3 was identified as an independent risk factor for OS in a multivariate Cox regression analysis.

Table 1. Univariate and multivariate Cox regression analysis of the COL5A3 expression and overall survival in PAAD patients.

Characteristics	**Total (*N*)**	**Univariate analysis**		**Multivariate analysis**	
		Hazard ratio (95% CI)	***P* value**	**Hazard ratio (95% CI)**	***P* value**
T stage	175				
T1&T2	31	Reference			
T3&T4	144	2.040 (1.082–3.850)	0.028*	1.186 (0.593–2.369)	0.630
N stage	172				
N0	49	Reference			
N1	123	2.106 (1.254–3.539)	0.005**	1.730 (0.950–3.152)	0.043*
M stage	83				
M0	79	Reference			
M1	4	1.028 (0.246–4.297)	0.970		
Primary therapy outcome	139				

Table 1. (*Continued*).

Characteristics	Total (*N*)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	***P* value**	**Hazard ratio (95% CI)**	***P* value**
PD	49	Reference			
SD	9	0.544 (0.228–1.297)	0.170	0.652 (0.263–1.615)	0.355
PR	10	1.038 (0.408–2.645)	0.937	1.053 (0.405–2.738)	0.916
CR	71	0.335 (0.201–0.559)	<0.001***	0.364 (0.211–0.626)	<0.001***
COL5A3	177				
Low	88	Reference			
High	89	1.435 (0.950–2.167)	0.046*	1.411 (0.938–1.989)	0.048*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

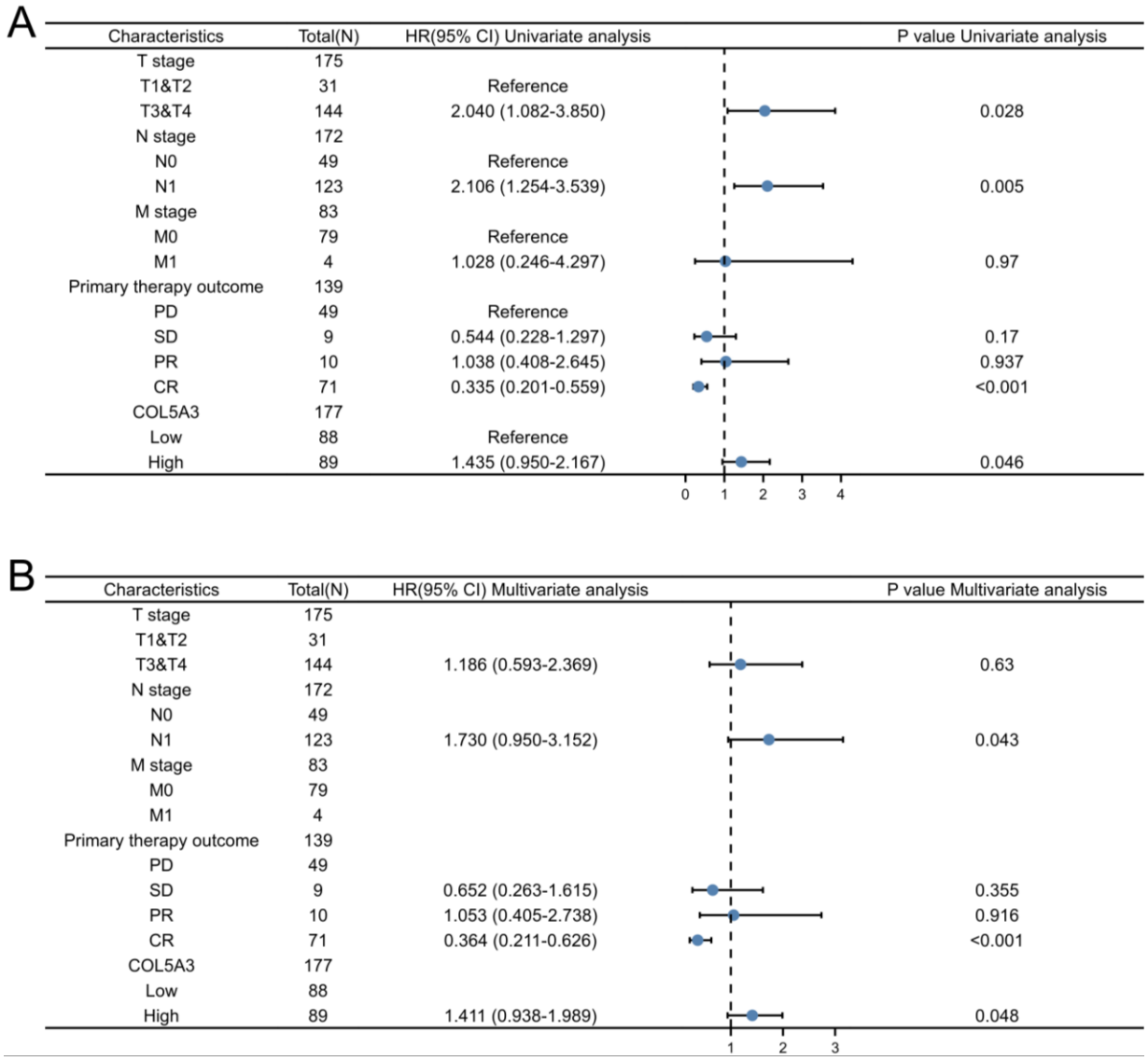

Figure 2. Association between clinicopathologic characteristics and survival outcome of PAAD patient through univariate and multivariate Cox regression analysis **(A)** Univariate Cox regression analysis of COL5A3 and clinicopathological parameters associated with OS; **(B)** Univariate Cox regression analysis of COL5A3 and clinicopathological parameters associated with OS.

3.4. Predictive value of COL5A3 for pancreatic cancer diagnosis and prognosis

We used K-M curve analysis to validate the forecast of clinical outcome of COL5A3 in order to assess its impact on the clinical prognosis of pancreatic cancer. As shown in **Figure 3A,B**, the OS survival rate in the high expression group was significantly lower than that in the low expression group of COL5A3 (p = 0.019), which was consistent with the DSS survival rate, which was also significantly lower in the high expression group (p = 0.026). Following that, we investigated the therapeutic advantages of COL5A3, using ROC curves to show its utility in the differential detection of pancreatic cancer. As shown in **Figure 3C**, the area under the curve (AUC) was 0.843, indicating that COL5A3 has good sensitivity and specificity in the detection of pancreatic cancer. The findings demonstrate that COL5A3 upregulation predicts a poor outcome and has a high diagnostic value in clinical diagnosis.

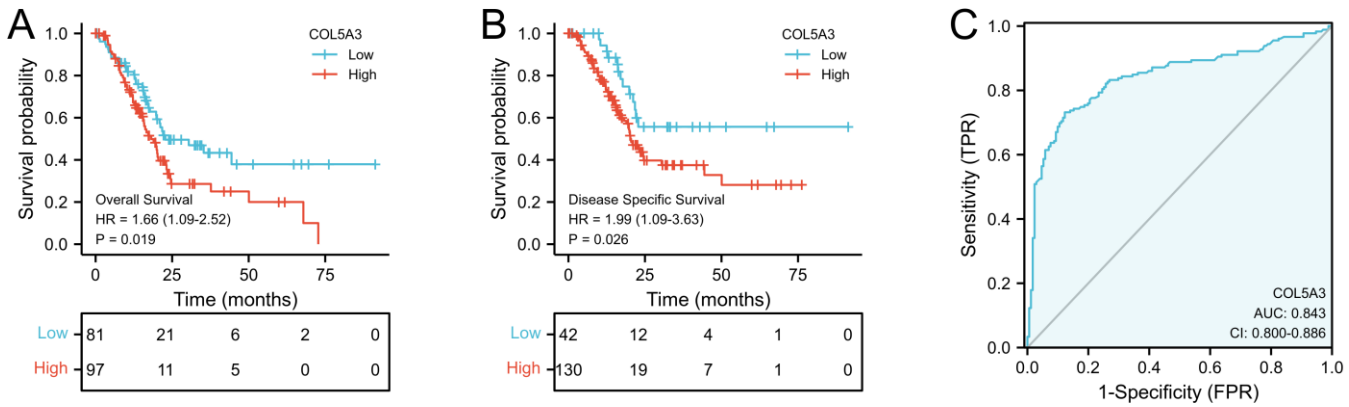

Figure 3. Kaplan-Meier and ROC curves for COL5A3. **(A,B)** Kaplan-Meier survival curves indicated that pancreatic cancer patients with high COL5A3 mRNA expression had a shorter OS (p = 0.019) and DSS (p = 0.026) than those with low-level of COL5A3; **(C)** ROC curve showed that COL5A3 had an AUC value of 0.843 to discriminate pancreatic cancer tissues rom healthy controls.

3.5. COL5A3-associated PPI network and functional enrichment

We used the STRING database, and the GO and KEGG databases to build COL5A3-associated PPI networks and functional descriptions. The PPI network reveals that some genes, such as ADAMTS14, ADAMTS2, ADAMTS3, BMP1, COL11A1, COL1A2, LUM, P4HA3, PCOLCE, and PCOLCE2, are closely linked to COL5A3. COL5A3-related genes were found to be involved in a variety of biological processes (BP), cellular components (CC), and molecular functions (MF), as shown in **Figure 4B**. The GO and KEGG enrichment analyses of COL5A3-related genes revealed that they were primarily engaged in extracellular structure organization, extracellular matrix organization, collagen fibril organization, and protein digestion and absorption. COL5A3 may support tumor growth, migration, and invasion by influencing the extracellular matrix, according to the GO and KEGG analyses. **Figure 5A–J** depicts an analysis of the association between COL5A3 expression and co-expression genes in pancreatic cancer patients using the TCGA database.

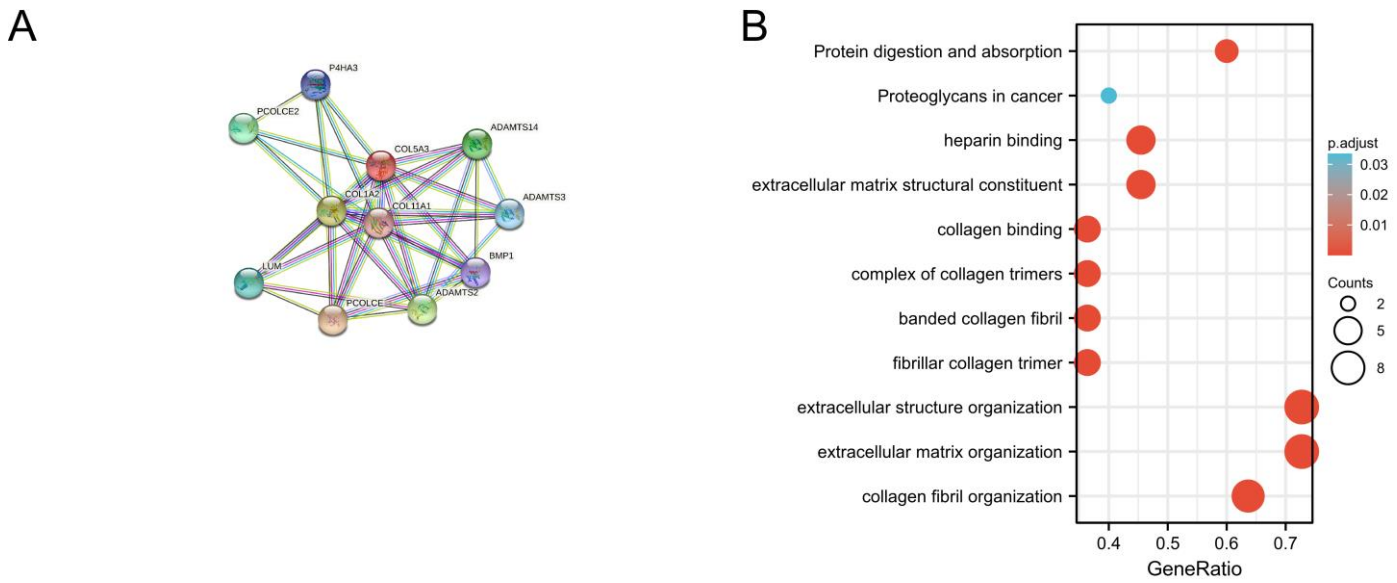

Figure 4. PPI networks and functional enrichment analysis. **(A)** A network of COL5A3 and its co-expression genes; **(B)** Functional enrichment analysis of 11 involved genes**.**

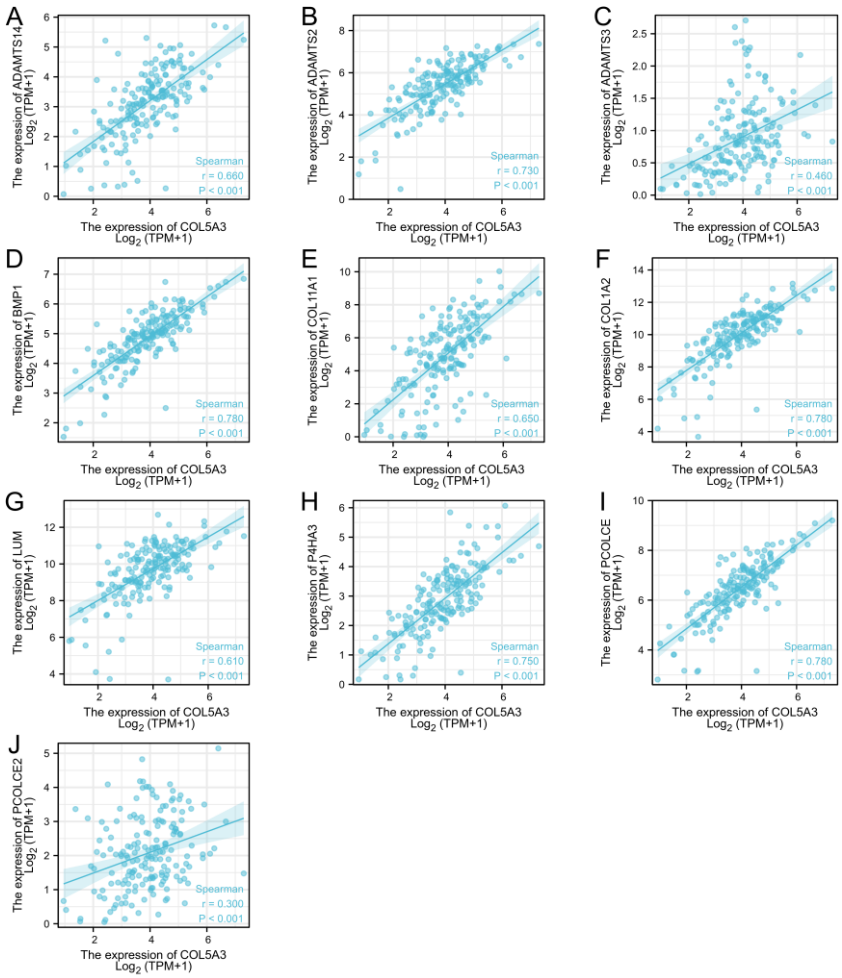

Figure 5. The correlation analysis between the expression of COL5A3 and co-expressed genes in pancreatic cancer. **(A–J)** Scatter plot of the correlation between the expressions of COL5A3 and ADAMTS14, ADAMTS2, ADAMTS3, BMP1, COL11A1, COL1A2, LUM, P4HA3, PCOLCE, PCOLCE2.

3.6. Analysis of the relationship between the expression of COL5A3 and the level of immune cell infiltration in pancreatic carcinoma

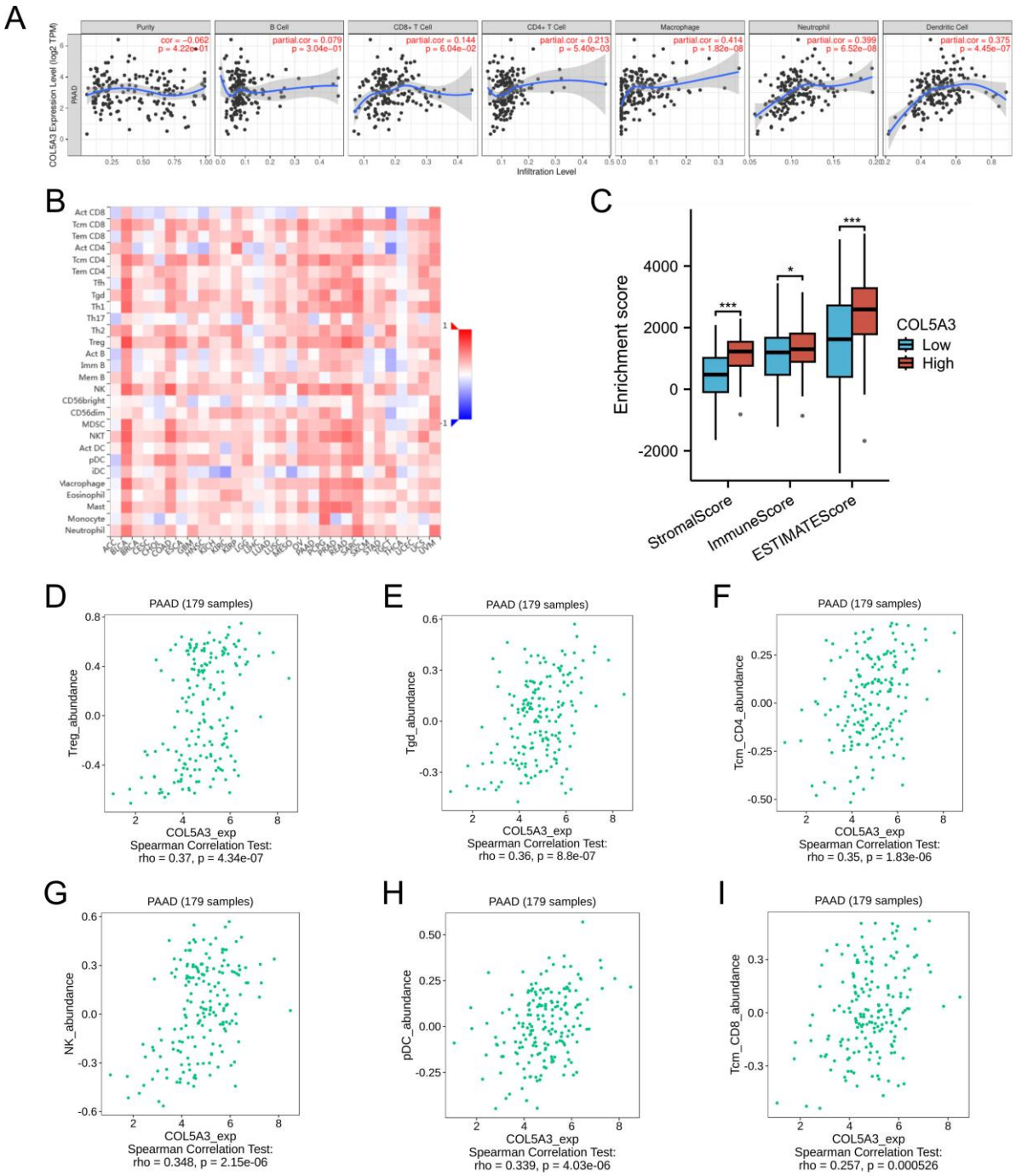

Figure 6. Correlations of COL5A3 expression with immune infiltration level. **(A)** COL5A3 expression has correlations with CD4+ T cell, macrophage, neutrophil, and dendritic cell in pancreatic cancer; **(B)** Relations between the expression of COL5A3 and 28 types of TILs across human cancers; **(C)** Differences in immune infiltration results between high and low expression groups of COL5A3; **(D–I)** COL5A3 was correlated with abundance of Treg cells, Tgd cells, Tcm CD4 cells, NK cells, pDC cells and Tcm CD8 cells.

We used the TIMER database to examine the relationship between COL5A3 expression and tumor purity, as well as the link between six types of invading immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and

dendritic cells). As shown in **Figure 6A**, the results displayed that the expression level of COL5A3 had an obviously positive correlation with infiltrating levels of CD4+ T cells (r = 0.213, p = 5.40e-03), macrophage cell (r = 0.414, p = 1.82e-08), neutrophils (r = 0.399, p = 6.52e-08), and dendritic cells (r = 0.375, p = 4.45e-07) in pancreatic cancer, but showed no association with tumor purity and CD8+ T cells. In human cancer, **Figure 6B** depicts the link between COL5A3 and the expression of 28 different types of tumor-infiltrating cells. **Figure 6C** shows that we analyzed the correlation between COL5A3 expression levels and Stromal Score, Immune Score, and ESTIMATE Score in the tumor microenvironment using box plots. The results indicate that the high expression group of COL5A3 has significantly higher Stromal Score, Immune Score, and ESTIMATE Score compared to the low expression group, with statistically significant differences (Stromal Score: $p < 0.001$, Immune Score: $p < 0.05$, ESTIMATE Score: $p < 0.001$). As demonstrated in **Figure 6D–I**, the expression of COL5A3 is connected to the quantity of Treg (r = 0.37, p = 4.43e-07), Tgd (r = 0.36, p = 8.8e-07), Tcm CD4 (r = 0.35, p = 1.83e-06), NK (r = 0.348, p = 2.15e-06), pDC (r = 0.339, p = 4.03e-06), and Tcm CD8 (r = 0.257, p = 0.000526).

4. Discussion

Despite ongoing medical advancements and the appearance of new technologies and therapies, the therapeutic effect on patients with pancreatic cancer remains quite limited. Pancreatic cancer is still a deadly malignant tumor with a high mortality rate [15]. As a result, the development of novel biomarkers for pancreatic cancer is critical for early detection, therapy, and prognosis.

In response to the lack of research on COL5A3 in tumors, we utilized bioinformatics to analyze its biological function in pancreatic cancer. Our analysis revealed that COL5A3 was significantly overexpressed in a range of tumor tissues, including pancreatic cancer ($p < 0.05$), compared to normal tissues. This overexpression was significantly associated with adverse clinicopathological characteristics, such as *T*-stage (p = 0.028) and N-stage (p = 0.043), suggesting a potential role in tumor progression.

The predictive effectiveness of COL5A3 was further evaluated using ROC curve analysis, which demonstrated a high AUC value of 0.843, indicating its potential as a reliable biomarker for early detection of pancreatic cancer. Kaplan-Meier curve analysis showed that patients with increased COL5A3 expression had significantly poorer OS and DSS rates (OS: p = 0.019; DSS: p = 0.026). These findings underscore the prognostic significance of COL5A3 in pancreatic cancer, suggesting its potential utility as a biomarker for identifying patients at higher risk of poor outcomes.

PPI network analysis implicates COL5A3 in significant interactions with genes involved in ECM organization, such as ADAMTS14 and COL11A1 [16,17]. This shows how vital COL5A3 is in the tumor microenvironment, potentially aiding cancer progression through modification of ECM. Defining the functions of these associated proteins in pancreatic cancer will enable the identification of new therapeutic targets and strategies to bias abnormal ECM signaling pathways [18].

Targeting COL5A3-related pathways may offer a promising avenue towards developing more effective treatments for pancreatic cancer.

To investigate the probable biological role of COL5A3, we performed enrichment analysis using GO function and KEGG pathway. Extracellular structure tissue, extracellular matrix tissue, collagen fiber tissue, protein digestion and absorption are all associated with COL5A3-related genes [16,17]. This suggests that COL5A3 could influence tumor growth and metastasis through its regulatory roles in these pathways. According to several research, type V collagen, particularly COL5A3, is extensively produced in the extracellular matrix of pancreatic cancer cells and promotes pancreatic cancer proliferation, migration, and metastasis through interacting to the α-2-β-1 integrin receptor [19]. In addition, COL5A3 upregulation in pancreatic cancer is a key hallmark of fibrosis and malignant tumor stroma [20]. Our findings imply that COL5A3 may influence the tumor microenvironment and regulate cell proliferation, migration, and metastasis. Future studies should aim to elucidate the impact of COL5A3 on the tumor microenvironment and explore its potential as a target for novel therapies aimed at disrupting tumor progression [18,21].

Tumor microenvironment (TME) is a complex biological milieu that includes lymphatic endothelial cells, vascular endothelial cells, mesenchymal cells, immune cells, extracellular matrix, and inflammatory matrix [22,23]. The dynamic and symbiotic interaction that develops between tumor cells and their surroundings during the early stages of malignant tumor formation influences tumor incidence and progression [24,25]. Various types of tumor patients have evident clinical effects following immunotherapy, however when all patients receive the same immunotherapy, the impact is frequently not optimal [26,27]. Due of the complexities of the tumor immune environment, it is critical to forecast and guide immunotherapy by studying the tumor's unique immune microenvironment. Nevertheless, no study has found a link between COL5A3 expression and immune cell infiltration in pancreatic cancer. Using the Timer and TISIDB databases, we discovered that COL5A3 expression in TME is favorably linked with numerous tumor infiltrating immune cells (Treg, Tgd, Tcm CD4, NK, pDC, and Tcm CD8). There is mounting evidence that innate immune cells (macrophages, neutrophils, dendritic cells, innate lymphoid cells, myeloid suppressor cells, and natural killer cells) and acquired immune cells (T cells and B cells) can promote carcinogenesis and development in TME. The interaction of cancer cells and proximal immune cells eventually results in an environment that favours tumor development and metastasis [28–30]. These findings point to a possible link between COL5A3 and immune infiltration in pancreatic cancer.

The following limitations remain in our study: we are relying on the mining of public database samples for statistical analysis, and we lack our own clinical samples for additional verification. Furthermore, the exact mechanism of COL5A3's influence on pancreatic cancer might be examined further by developing in vitro and in vivo research.

5. Conclusions

In conclusion, our research underscores the significant upregulation of COL5A3 in pancreatic cancer and its correlation with poor patient prognosis. The findings indicate that COL5A3 may be a promising biomarker for diagnosis and prognosis in pancreatic cancer and therapeutic strategies. Further research is suggested to incorporate ove a mechanistic study on COL5A3's role in tumor biology and other aboundant interactions in the tumor microenvironment to improve the understanding of its feasible application as a potential target for therapy.

Author contributions: Conceptualization, YL, MZ and CL; formal analysis, YL and TW; writing—original draft preparation, YL, CL and MZ; writing—review and editing, YL, CL, MZ, TW and FJ; visualization, YL and MZ; supervision, YL, CL, MZ and FJ; fund: CL. All authors have read and agreed to the published version of the manuscript.

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Ethics approval: Not applicable.

Conflict of interest: The authors declare no conflict of interest.

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