Integrate analysis of the prognostic values of COL4As to human gastric cancer

Xiaojia Zheng
Hebei University of Chinese Medicine
Pingping Chen
Hebei University of Chinese Medicine
Yang Liu
Hebei University of Chinese Medicine
Bin Wang
Hebei University of Chinese Medicine
Qiquan Liu (liuqq56@163.com)
The First Affiliated Hospital of Hebei University of Chinese Medicine https://orcid.org/0000-0001-5283-8006

Research Article

Keywords: gastric cancer, function, COL4A, bioinformatics

Posted Date: August 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-834294/v1

Title page

Integrate analysis of the prognostic values of COL4As to human gastric cancer

Xiaojia Zheng[1], Pingping Chen[1],Yang Liu[1],Bin Wang[1],Qiquan Liu[2*]

1 Hebei University of Chinese Medicine, Shijiazhuang, 050200, China;

2The First Affiliated Hospital of Hebei University of Chinese Medicine, Shijiazhuang, 050011, China.

*Correspondence to Qiquan Liu; Email: liuqq56@163.com.

Integrate analysis of the prognostic values of COL4As to human gastric cancer

Abstract

Collagen type IV (Col IV) is the main constituent of the basement membrane. Under physiological conditions, Col IV plays an important role in maintaining epithelial integrity and stabilizing epithelial function of the gastric mucosa. Under pathological conditions, Col IV can be free from the basement membrane under the influence of tumor cells and play a pro-metastatic role. Although there is a increasing number of investigations on Col IV, no studies to date have directly uncovered the prognostic role and potential regulatory role of the six isoforms of Col IV in gastric cancer. In the present experiment, we aimed to analyze the role of COL4A family genes in gastric cancer. COL4A1/2/3/4 was significantly overexpressed, while COL4A5/6 was decreased in gastric cancer tissues according to TCGA data and our immunohistochemical staining results. And COL4A1 had a positive correlation with tumor stage, while COL4A5/6 had negative correlations with tumor stage. COL4A1/2/4/5/6 can be considered as a diagnostic indicator of gastric cancer. High levels of COL4A1/2/4/5/6 expression may be predictive of a poor prognosis of gastric cancer. The percentages of genetic alterations in COL4As for stomach cancer varied from 3 to 17% based on the TCGA data (COL4A1, 10%; COL4A2, 8%; COL4A3, 3%; COL4A4, 5%; COL4A5, 17%; COL4A6, 17%). Besides, COL4As may modulate tumor progression by participating in classical cancer pathways: COL4A1/2/3/4/5/6 can activate the EMT process; COL4A2/3/4/6 can inhibit apoptosis and cell cycle; COL4A3/4/5 can activate the PI3K/AKT signaling pathway. This study implied that COL4As have diagnostic and prognostic value for gastric cancer.

Keywords

gastric cancer; function; COL4A; bioinformatics

Introduction

Gastric cancer (GC) is a malignant tumor originating from the epithelial cells of the gastric mucosa. And GC is the third most deadly disease globally [1]. Most gastric cancer patients have no obvious symptoms in the early stage, and most of them are in the progressive stage or with multiple metastases throughout the body at the time of initial diagnosis, thus losing the opportunity for radical surgery. Moreover, patients with GC who suffered from lymph node or distant metastasis of cancer cells often had a reduced 5-year survival rate of 7%-34% [2-4]. Although clinicians have improved treatment plans, the therapeutic effect of patients with progressive gastric cancer is not satisfactory.

The tumor microenvironment is a sophisticated ecological environment that, to a certain extent, contains important factors that determine the malignancy of gastric cancer cells, such as immune cells and extracellular matrix (ECM) [5]. There is increasing evidence of a close regulatory relationship between the tumor microenvironment and tumor cells in gastric cancer [5]. Collagen type IV (Col IV) is a major component of the ECM and is involved in forming the basement membrane of various tissues. Moreover, the adhesion capacity of human cells can be regulated by Col IV. Mak et al stated that Col IV can be identified as a valid marker of fibrosis and cirrhosis [6]. Moreover, Col IV can be recognized as a biomarker for primary and metastatic hepatoma [7]. Zhou et al pointed out that multiple forms of mutations in COL4A occurred in cervical cancer and that these mutations can suppress immune infiltration [8]. These suggest that Col IV may have a regulatory effect on cancers. The

aim of this study was to investigate the expression of COL4As in normal and gastric cancer tissues by performing bioinformatics analysis and IHC to elucidate its potential prognostic and diagnostic role in the progression of gastric cancer.

Materials and methods

Clinical tissue specimens

The tissue samples involved in this experiment were obtained from the First Affiliated Hospital of Hebei University of Chinese Medicine with 30 gastric cancer patients. All subjects provided informed consent.

TCGA database

The expression data of COL4As were obtained from TCGA database [9]. And, the ROC of COL4As were assessed using GraphPad Prism 7 software according to TCGA database .

UALCAN analysis and Oncomine database analysis

We compared the COL4As mRNA expression between normal and tumor groups by UALCAN platform [10] and Oncomine database [11]. Besides, the relationship between COL4As expression and specific clinical characteristics was also analyzed by using UALCAN platform.

TIMER database analysis and GSCALite analysis

In this experiment, the correlations of COL4As expression status with immune infiltration level were assessed using the gene module and the relevant results were re-visualized with R [12]. Then, drug targets and pathway activity of COL4A family genes were analyzed using GSCALite tool [13].

Overall survival analysis and cBioPortal analysis

The overall survival information of COL4As was analyzed by K-M plotter [14, 15]. The cBio Cancer Genomics Portal is a multi-omics database for research and analysis of cancer-related genetic data [16]. The genetic alterations of COL4As were analyzed by cBioPortal tool and methylation CpG site of OS for COL4As were analyzed by MethSurv.

Functional enrichment analysis of COL4As

To explore the linkage between COL4As, a PPI network was constructed [17]. GO and KEGG of COL4As and their top-30 related genes were performed using DAVID tool [18].

Immunohistochemistry

Immunostaining of COL4A1-6 was conducted using mouse polyclonal antibodies against COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, COL4A6 (Santa Cruz Biotechnology, dilution:1:500). The detailed criteria for defining the frequency and extent of SLC30A1 and SLC30A10 expression was scored as follows: 0 (0-5%); 1 (6-25%); 2 (26-50%); 3 (51-75%); and 4 (76-100%). Staining intensity was scored as follows: 0 (negative); 1-2 (weak); and >2 (strong).

Statistical analysis

All statistical analysis was performed using SPSS 21.0 software and R software. $P < 0.05$ indicated a statistically significant difference.

Results

The differential expression of COL4As in pan-cancers

To determine the expression levels of COL4As, the ONCOMINE database was used to compare levels of COL4As transcripts in cancer and

normal tissue. We revealed that the six COL4As were overexpressed in most tumors (Fig. 1A). In addition, with the GEPIA platform, the prognostic value of COL4As in pan-cancers were also evaluated. As shown in Fig. 1B, we found that high levels of COL4A1-6 expression may be predictive of a poor prognosis of most tumors, while acted as protection factors in cholangiocarcinoma, kidney renal clear cell carcinoma.

Transcriptional levels of COL4As in gastric cancer

As shown in Fig. 2A-F that COL4A1/2/3/4 was significantly overexpressed, while COL4A5/6 was decreased in gastric cancer tissues. And COL4A1 had a positive correlation with tumor stage, while COL4A5/6 had negative correlations with tumor stage (Fig. 2G-I). COL4A1/2/3/4 was significantly overexpressed, while COL4A5/6 was decreased in gastric cancer patients with a positive lymph node status compared to those with a negative lymph node status (Fig.3A-F). Besides, COL4A1/2 gene expressions had significant correlations with H. pylori infection status, while COL4A5/6 had negative correlations with H. pylori infection status in gastric cancer patients (Fig. 3G-L).

Prognostic and diagnostic value of COL4As

The KM plotter tool was used to investigate the prognostic ability of COL4A1/2/3/4/5/6 expression in gastric cancer. As shown in Fig. 4A-F, increased expression of COL4A1/2/4/5/6 tended to be related to poor OS. And, the diagnostic values of COL4As were assessed using ROC curves. As shown in Fig. 4G-L, COL4A1/2/4/5/6 can effectively distinguish gastric cancer patients from healthy individuals. Meanwhile, we again validated the prognostic value of COL4As in gastric cancer using the GSE62254 dataset (Table 1). And the prognostic value of COL4As

expression with different clinical parameters of gastric cancer patients were explored according to GSE62254 dataset (Table 2-4). In addition, the effects of methylated CpG sites on COL4As'OS in thyroid cancer patients were summarized in Table 5.

Then, integrated prognostic value of COL4As was also conducted by SurvExpress online platform. Prognostic risk scores of COL4As were calculated based on the SurvExpress online platform's unique algorithm. Compared with low-risk group, the high-risk group of COL4As displayed a significantly unfavorable OS outcome (HR = 1.55, 95% CI = 1.09-2.21, P = 0.01359). And ROC curves of COL4As were showed in different survival time points in patients with gastric cancer. The expression of COL4A1/2/4/5/6 in the high-risk group was significantly higher than in low-risk group (Fig.5A-D). Besides, the relationships between COL4As expression and clinicopathological characteristics in patients with gastric cancer were shown in Supplementary Table 1-6. For instance, COL4A2 was associated with M stage with gastric cancer paitents; COL4A3 was linked to the histological type of gastric cancer; COL4A4 was correlated with pathologic stage of gastric cancer, etc.

Mutations of COL4As

Genetic mutations of COL4As were analyzed through cBioPortal database. 739 patients from five studies of stomach adenocarcinoma were analyzed. As showed in Fig. 6A, mutations in COL4As were present in almost all gastric cancer subtypes. And COL4As were altered in 232 samples of 739 patients with stomach cancer (31%) (Fig. 6B). The details of all mutations of COL4As in stomach cancer are summarized: COL4A1 had 9 truncating mutation, 3 splice mutation and 31 missense mutations; COL4A2 had 9 truncating mutation and 19 missense mutations; COL4A3

had 3 truncating mutation and 16 missense mutations; COL4A4 had 6 truncating mutation, 5 splice mutation and 22 missense mutations; COL4A5 had 2 truncating mutation, 3 splice mutation and 14 missense mutations; COL4A6 had 1 truncating mutation, 3 splice mutation and 14 missense mutations. The percentages of genetic alterations in COL4As for stomach cancer varied from 3 to 17% based on the TCGA data (COL4A1, 10%; COL4A2, 8%; COL4A3, 3%; COL4A4, 5%; COL4A5, 17%; COL4A6, 17%) (Fig. 6B).

Function enrichment of COL4As

To explore the linkage between COL4As, a PPI network was constructed (Fig. 7A). We also computed the association of COL4As with each other by calculating COL4As' mRNA expression. We found a strong and positive correlation in the following COL4As: COL4A1 with COL4A2; COL4A3 with COL4A4; COL4A5 with COL4A6 (Fig. 7B). Besides, DAVID was used to perform GO and KEGG analyses to find the functional enrichment of COL4As. As shown in Fig. 7C, biological processes included extracellular matrix organization, collagen catabolic process, cell adhesion, etc. Cellular components analysis indicated that these proteins localized mainly to extracellular region, extracellular matrix, collagen type IV trimer,integrin complex, basement membrane, extracellular exosome and collagen type V trimer (Fig. 7D). Molecular function analysis found that these proteins were primarily involved in matrix structural constituent, extracellular matrix constituent, collagen binding, etc (Fig. 7E). As shown in Fig. 7F, KEGG analysis found that these proteins were primarily involved in ECM-receptor interaction, PI3K-Akt signaling pathway, pathways in cancer, etc. As shown in Fig. 8A, COL4As may modulate tumor progression by participating in classical cancer pathways: COL4A1/2/3/4/5/6 can activate the EMT

process; COL4A2/3/4/6 can inhibit apoptosis and cell cycle; COL4A3/4/5 can activate the PI3K/AKT signaling pathway. And the drug targets of COL4As were also analyzed (Fig. 8B). As shown in Table 6-11, these results were the kinase, miRNA and transcription factor targets that can regulate the expression of COL4As by using the LinkedOmics database.

Immune infiltration of COL4As

Then, immune infiltration levels of COL4As were explored (Fig. 9). Dendritic cells were correlated with COL4A1 (Cor = 0.203, p = 8.34e-05), COL4A2 (Cor = 0.227, p = 1.02e-05), COL4A3 (Cor = 0.332, p = 5.67e-11), COL4A4 (Cor =0375, p = 7.84e-14). CD4+ T cells were correlated with COL4A1 (Cor = 0.281, p = 4.60e-08), COL4A2 (Cor =0.402, p=1.20e-15), COL4A3 (Cor = 0.539, p = 5.17e-29), COL4A4 (Cor =0.336, p=4.24e-11), COL4A5 (Cor =0.508, p=1.97e-25) and COL4A6 (Cor =0.382, p= 3.67e-14). Macrophages were correlated with COL4A1 (Cor =0.369, p=2.30e-13), COL4A2 (Cor =0.401, p=9.97e-16), COL4A3 (Cor =0.448, p=1.19e-19), COL4A4 (Cor =0.241, p=2.87e-06), COL4A5 (Cor =0.494, p=3.36e-24) and COL4A6 (Cor =0.249, p=1.29e-06). And neutrophils were correlated with COL4A1(Cor =0.133, p=1.05e-02), COL4A3 (Cor =0.181, p=4.50e-04), COL4A4 (Cor =0.194, p=1.72e-04). These results suggested that the immune cells most associated with COL4A in gastric cancer were macrophages and CD4+ T cells. And IHC results showed that COL4A1/2/3/4 staining was notably dominant in the cytoplasm of gastric cancer tissues, while COL4A5/6 was decreased in gastric cancer tissues (Fig.10).

Discussion

Malignant tumors have become the primary risk factor for human health [19]. Stomach cancer is the third most deadly disease globally [20, 21]. There is substantial evidence that rapid proliferation, abnormal differentiation, distant metastasis and immune infiltration of human cells are important features of cancer development and are strongly associated with poor prognosis of patients with malignancies [22-24]. Although the usage of gastroscopy has improved the diagnosis rate of gastric cancer. However, there are still a large proportion of patients who are already in the advanced stage of stomach cancer at the time of diagnosis [25]. Therefore, searching for sensitive and specifically altered genes in gastric carcinogenesis and exploring the molecular mechanism of their roles in depth are of great theoretical and practical significance for the clinical diagnosis and treatment of gastric cancer as well as providing new targets for the research of anti-cancer drugs, which meet the current urgent need for individualized treatment of gastric cancer.

The tumor microenvironment (TME) is composed of cellular and non-cellular components [26]. For example, cellular components include stromal cells, macrophages, and immune cells; non-cellular components include a large number of extracellular matrix, cytokines, growth factors, chemokines, etc [26]. During the progression of gastric cancer, tumor cells are regulating the formation of TME by continuously secreting specific factors; on the other hand, TME continuously promotes cancer progression in response to changing environmental conditions, leading to abnormal cancer growth, angiogenesis, metastasis and drug resistance [27-30]. It is well documented that abnormal expression of COL IV is associated with liver disease [31], breast cancer [32], and colon cancer [33]. Besideds, different histological patterns of V-type collagen levels have prognostic values for a variety of solid tumors [34]. To investigate

the different roles of each COL IV isoform in gastric carcinogenesis, GC expression profiles were analyzed using public sequencing data from the TCGA database.

It has been documented that COL4As are frequently aberrantly expressed in cancers. Li et al showed that COL4A1 was overexpressed in gastric cancer and had a diagnostic and prognostic value for gastric cancer by performing bioinformatics analysis according to GSE27342, GSE29272, and GSE33335 data [35]. In glioma and glioblastoma, upregulated SLC30A1 and SLC30A2 genes may promote carcinogenesis by regulating PI3K-Akt pathway-related proteins [36]. COL4A1 may play a critical function in the proliferation and colony formation of breast cancer cells. In particular, recent experimental results indicated that silencing COL4A1 expression in hepatocellular carcinoma HepG2 cells significantly inhibited the capacity for proliferation, migration and invasion [37]. Inhibition of COL4A2 gene expression in hepatocellular carcinoma cells attenuated the proliferation and migration ability of hepatocellular carcinoma cells [37]. COL4A2 can be regarded as a core gene in the pathogenesis of breast cancer and has a prognostic value in breast cancer [38]. Jiang et al suggested that overexpressed crosstalk genes, including COL1A1 and COL1A2, may be involved in the progression and poor prognosis of low-grade gliomas by regulating tumor microenvironment interactions [39]. And COL4A2 expression had the most significantly positive correlations with the existence of macrophages and dendritic cell in cervical cell carcinoma [8]. COL4A3 is up-regulated in ovarian cancer [40]. And COL4A3 was an angiogenesis related gene of kidney renal clear cell carcinoma [41]. Besides, COL4A3 was a prognostic indicator of gastric carcinomas [42]. Li et al indicated COL4A4 was notably down-regulated in esophageal squamous cell

carcinoma [43]. COL4A3 and COL4A4 may have a cancer-promoting effect on Wilms tumor and can influence the evolution of Wilms tumor [44]. Peng pointed out that the COL4A5 gene can be methylated in gastric cancer and that methylation is strongly associated with cancer recurrence [45]. COL4A5 was involved in regulating the formation of hemangiomas [46]. Down-regulation of COL4A6 expression enhances the invasiveness of prostate cancer cells (PC-3 and DU145), and the methylation status of COL4A6 can be used as an important marker of prostate cancer prognosis [47]. In this experiment, we first compared the levels of COL4A family gene transcripts in multiple cancers and corresponding normal tissues. Then, we concretely evaluated the expression levels of six COL4As. COL4A1/2/3/4 was significantly overexpressed, while COL4A5/6 was decreased in gastric cancer tissues. Consistent with previous results, we found that COL4As had prognostic value for gastric cancer, except for COL4A3 gene. And COL4A1, COL4A2, COL4A4, COL4A5 and COL4A6 can effectively distinguish gastric cancer patients from healthy individuals.

To date, genetic changes of the COL4A family gene have been identified in glomerulosclerosis, familial microhematuria, and some cancers [8, 48-50]. Consistent with previous research, GO analysis displayed that COL4A family genes contributed to cell adhesion, endodermal cell differentiation, cell-matrix adhesion. Alterations in cell-cell adhesion have the potential to directly initiate and exacerbate the ability of cancer cells to metastasize. And KEGG pathway enrichment analysis showed that the functions of COL4As were riched in some cancer-related pathways. Besides, we found that the immune cells most associated with COL4A in gastric cancer were macrophages and CD4+ T cells. And multiple forms of mutations in COL4A occurred in cervical cancer and

that these mutations can suppress immune infiltration [8]. Therefore, these results imply that COL4As may contribute to the evolution of gastric cancer in cancer by regulating specific immune cells, such as macrophages and CD4+ T cells.

Conclusion

The results of different expression, ROC curves, survival curve and immune cell infiltration of COL4A family genes showed that COL4A family genes have diagnostic and prognostic value for gastric cancer.

Funding

This project is funded by The 2018 National Famous Traditional Chinese Medicine Inheritance Studio Construction Project of the China Administration of Traditional Chinese Medicine ([2018] 119).

Authors' contributions

This article was written by Xiaojia Zheng. Pingping Chen,Yang Liu,Bin Wang,Qiquan Liu conducted the statistical analysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

Data Availability

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

References

[1] Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019; 144 : 1941-1953.
[2] Asplund J, Kauppila JH, Mattsson F, et al. Survival Trends in Gastric Adenocarcinoma: A Population-Based Study in Sweden. Ann Surg Oncol 2018; 25 : 2693-2702.
[3] Iwasaki K, Suda T, Takano Y, et al. Postoperative outcomes of gastric carcinoma with lymphoid stroma. World J Surg Oncol 2020; 18 : 102.
[4] Jeong O, Jung MR, Ryu SY. Clinicopathological features and prognostic impact of splenic hilar lymph node metastasis in proximal gastric carcinoma. Eur J Surg Oncol 2019; 45 : 432-438.
[5] Hinshaw DC, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Res 2019; 79 : 4557-4566.
[6] Mak KM, Chen LL, Lee TF. Codistribution of collagen type IV and laminin in liver fibrosis of elderly cadavers: immunohistochemical marker of perisinusoidal basement membrane formation. Anat Rec (Hoboken) 2013; 296 : 953-964.
[7] Kawai S, Kubo S, Tsukamoto T, et al. Serum concentration of type IV collagen 7S domain as a marker for increased risk of recurrence after liver resection for hepatocellular carcinoma. Dig Surg 2003; 20 : 201-208.
[8] Zhou C, Li C, Peng S, et al. Comprehensive Analysis of the Relationships Between Tumor Mutation Burden With Immune Infiltrates in Cervical Cell Carcinoma. Front Mol Biosci 2020; 7 : 582911.
[9] Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015; 19 : A68-77.
[10] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia 2017; 19 : 649-658.
[11] Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 2004; 6 : 1-6.
[12] Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res 2017; 77 : e108-e110.
[13] Liu CJ, Hu FF, Xia MX, et al. GSCALite: a web server for gene set cancer analysis. Bioinformatics 2018; 34 : 3771-3772.
[14] Gyorffy B, Lanczky A, Eklund AC, et al. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat 2010; 123 : 725-731.
[15] Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45 : W98-W102.
[16] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6 : pl1.
[17] Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019; 47 : D607-D613.
[18] Huang Da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4 : 44-57.
[19] Torre LA, Siegel RL, Ward EM, et al. Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiol Biomarkers Prev 2016; 25 : 16-27.

[20] Smyth EC, Nilsson M, Grabsch HI, et al. Gastric cancer. Lancet 2020; 396 : 635-648.
[21] Hanna TP, King WD, Thibodeau S, et al. Mortality due to cancer treatment delay: systematic review and meta-analysis. BMJ 2020; 371 : m4087.
[22] Golemis EA, Scheet P, Beck TN, et al. Molecular mechanisms of the preventable causes of cancer in the United States. Genes Dev 2018; 32 : 868-902.
[23] De Martel C, Georges D, Bray F, et al. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health 2020; 8 : e180-e190.
[24] Locy H, De Mey S, De Mey W, et al. Immunomodulation of the Tumor Microenvironment: Turn Foe Into Friend. Front Immunol 2018; 9 : 2909.
[25] Tan Z. Recent Advances in the Surgical Treatment of Advanced Gastric Cancer: A Review. Med Sci Monit 2019; 25 : 3537-3541.
[26] Oya Y, Hayakawa Y, Koike K. Tumor microenvironment in gastric cancers. Cancer Sci 2020; 111 : 2696-2707.
[27] Rojas A, Araya P, Gonzalez I, et al. Gastric Tumor Microenvironment. Adv Exp Med Biol 2020; 1226 : 23-35.
[28] Zeng D, Li M, Zhou R, et al. Tumor Microenvironment Characterization in Gastric Cancer Identifies Prognostic and Immunotherapeutically Relevant Gene Signatures. Cancer Immunol Res 2019; 7 : 737-750.
[29] Baj J, Korona-Glowniak I, Forma A, et al. Mechanisms of the Epithelial-Mesenchymal Transition and Tumor Microenvironment in Helicobacter pylori-Induced Gastric Cancer. Cells 2020; 9 :
[30] Jiang Y, Xie J, Huang W, et al. Tumor Immune Microenvironment and Chemosensitivity Signature for Predicting Response to Chemotherapy in Gastric Cancer. Cancer Immunol Res 2019; 7 : 2065-2073.
[31] Mak KM, Mei R. Basement Membrane Type IV Collagen and Laminin: An Overview of Their Biology and Value as Fibrosis Biomarkers of Liver Disease. Anat Rec (Hoboken) 2017; 300 : 1371-1390.
[32] Soran A, Ozmen V, Ozbas S, et al. Randomized Trial Comparing Resection of Primary Tumor with No Surgery in Stage IV Breast Cancer at Presentation: Protocol MF07-01. Ann Surg Oncol 2018; 25 : 3141-3149.
[33] Liang Y, Lv Z, Huang G, et al. Prognostic significance of abnormal matrix collagen remodeling in colorectal cancer based on histologic and bioinformatics analysis. Oncol Rep 2020; 44 : 1671-1685.
[34] Balancin ML, Teodoro WR, Baldavira CM, et al. Different histological patterns of type-V collagen levels confer a matrices-privileged tissue microenvironment for invasion in malignant tumors with prognostic value. Pathol Res Pract 2020; 216 : 153277.
[35] Li T, Gao X, Han L, et al. Identification of hub genes with prognostic values in gastric cancer by bioinformatics analysis. World J Surg Oncol 2018; 16 : 114.
[36] Vastrad B, Vastrad C, Godavarthi A, et al. Molecular mechanisms underlying gliomas and glioblastoma pathogenesis revealed by bioinformatics analysis of microarray data. Med Oncol 2017; 34 : 182.
[37] Wang T, Jin H, Hu J, et al. COL4A1 promotes the growth and metastasis of hepatocellular carcinoma cells by activating FAK-Src signaling. J Exp Clin Cancer Res 2020; 39 : 148.
[38] Yao Y, Zhang T, Qi L, et al. Integrated analysis of co-expression and ceRNA network identifies five lncRNAs as prognostic markers for breast cancer. J Cell Mol Med 2019; 23 : 8410-8419.

[39] Jiang Y, He J, Guo Y, et al. Identification of genes related to low-grade glioma progression and prognosis based on integrated transcriptome analysis. J Cell Biochem 2020; 121 : 3099-3111.
[40] Peng X, Yu M, Chen J. Transcriptome sequencing identifies genes associated with invasion of ovarian cancer. J Int Med Res 2020; 48 : 300060520950912.
[41] Zheng W, Zhang S, Guo H, et al. Multi-omics analysis of tumor angiogenesis characteristics and potential epigenetic regulation mechanisms in renal clear cell carcinoma. Cell Commun Signal 2021; 19 : 39.
[42] Nie XC, Wang JP, Zhu W, et al. COL4A3 expression correlates with pathogenesis, pathologic behaviors, and prognosis of gastric carcinomas. Hum Pathol 2013; 44 : 77-86.
[43] Li J, Wang X, Zheng K, et al. The clinical significance of collagen family gene expression in esophageal squamous cell carcinoma. PeerJ 2019; 7 : e7705.
[44] Guo C, Jiang X, Guo J, et al. Integrated bioinformatic analysis identifies COL4A3, COL4A4, and KCNJ1 as key biomarkers in Wilms tumor. Int J Clin Exp Pathol 2021; 14 : 196-208.
[45] Peng Y, Wu Q, Wang L, et al. A DNA methylation signature to improve survival prediction of gastric cancer. Clin Epigenetics 2020; 12 : 15.
[46] Panagopoulos I, Gorunova L, Lobmaier I, et al. Fusion of the COL4A5 Gene With NR2F2-AS1 in a Hemangioma Carrying a t(X;15)(q22;q26) Chromosomal Translocation. Cancer Genomics Proteomics 2020; 17 : 383-390.
[47] Ma JB, Bai JY, Zhang HB, et al. Downregulation of Collagen COL4A6 Is Associated with Prostate Cancer Progression and Metastasis. Genet Test Mol Biomarkers 2020; 24 : 399-408.
[48] Papazachariou L, Papagregoriou G, Hadjipanagi D, et al. Frequent COL4 mutations in familial microhematuria accompanied by later-onset Alport nephropathy due to focal segmental glomerulosclerosis. Clin Genet 2017; 92 : 517-527.
[49] Li Y, Wang Y, He Q, et al. Genetic mutational testing of Chinese children with familial hematuria with biopsyproven FSGS. Mol Med Rep 2018; 17 : 1513-1526.
[50] Gast C, Pengelly RJ, Lyon M, et al. Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. Nephrol Dial Transplant 2016; 31 : 961-970.

Figures

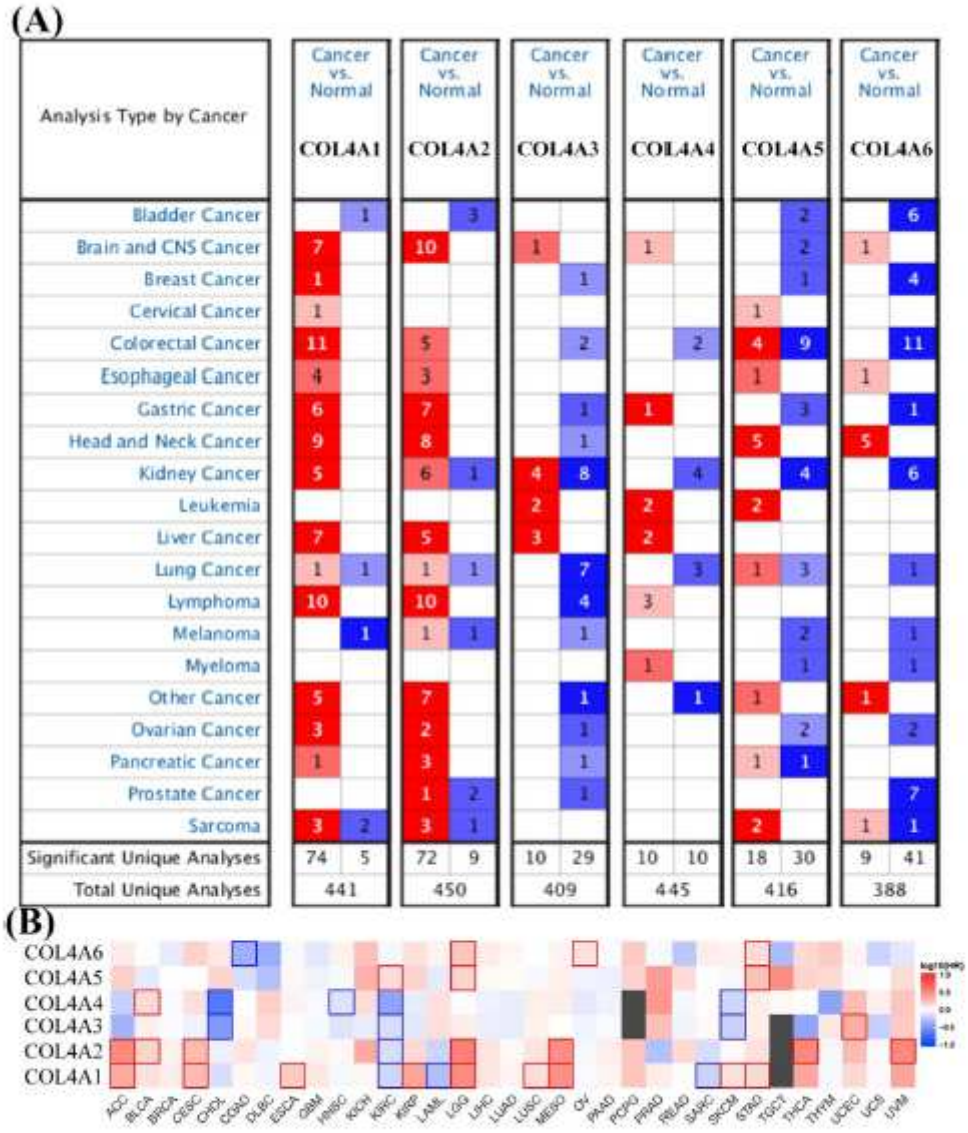

Figure 1

The transcription levels of COL4A family genes in different types of cancers (A) Oncomine data. (B) GEPIA data.

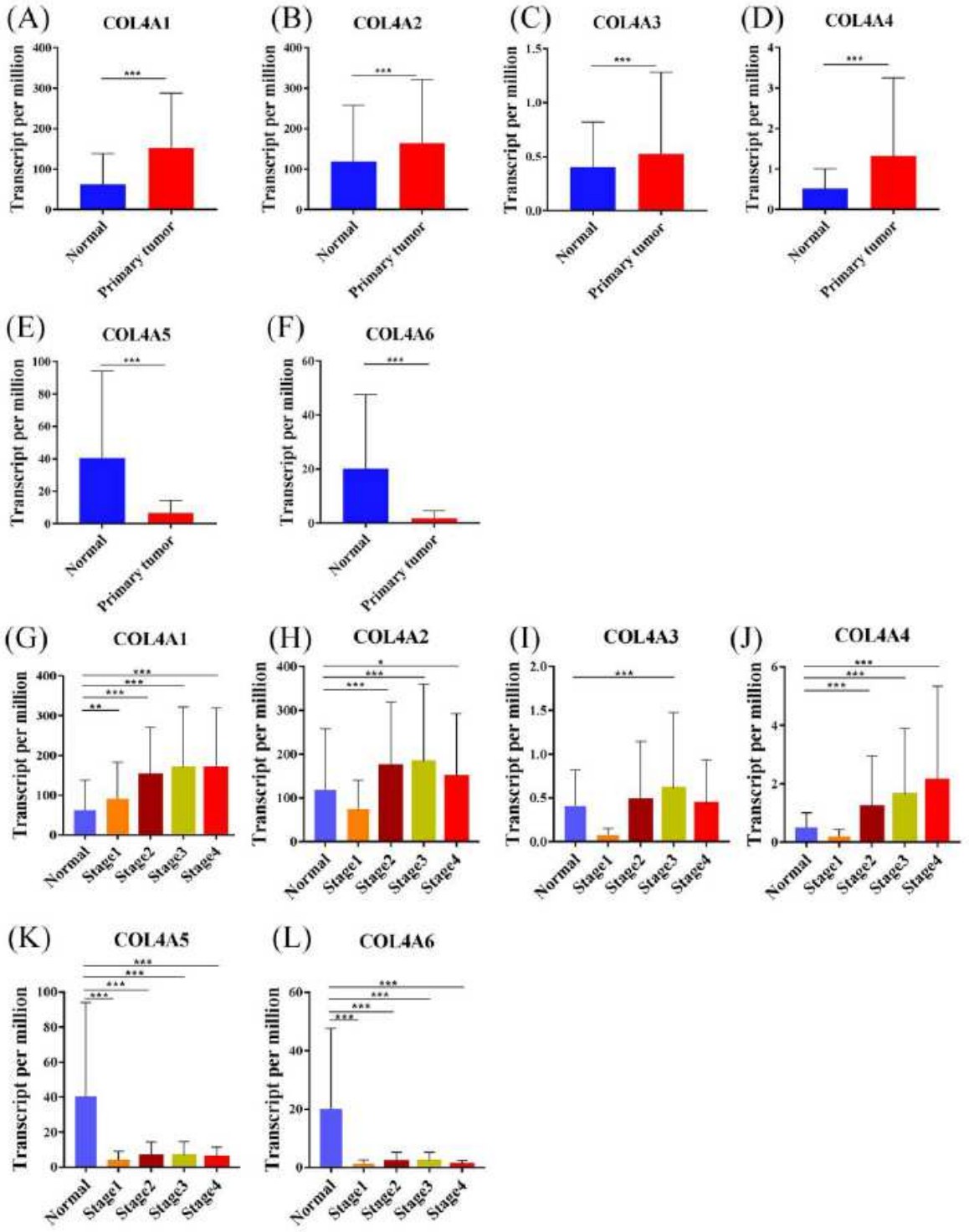

Figure 2

The expression level of COL4As (A-F) The expression of COL4As. (G-L) Correlation between expression of COL4As and tumor stages. *, P-value < 0.05, **, P-value < 0.01, ***, P-value < 0.001.

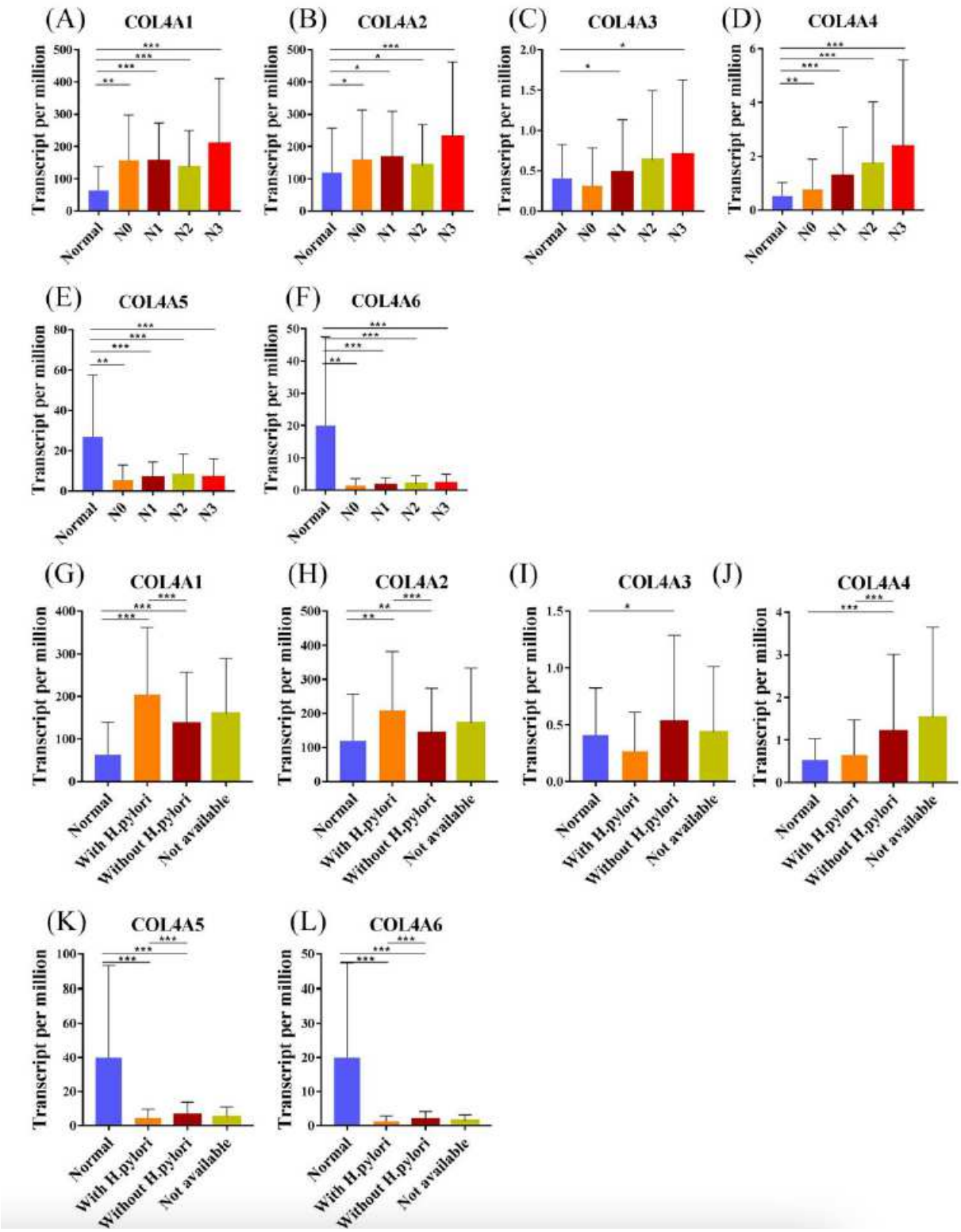

Figure 3

Relative expression and the correlation between COL4As with pathological parameter (A-F) Expression of COL4As based on nodal metastasis status. (G-L) Expression of COL4As based on H.pylori infection status.

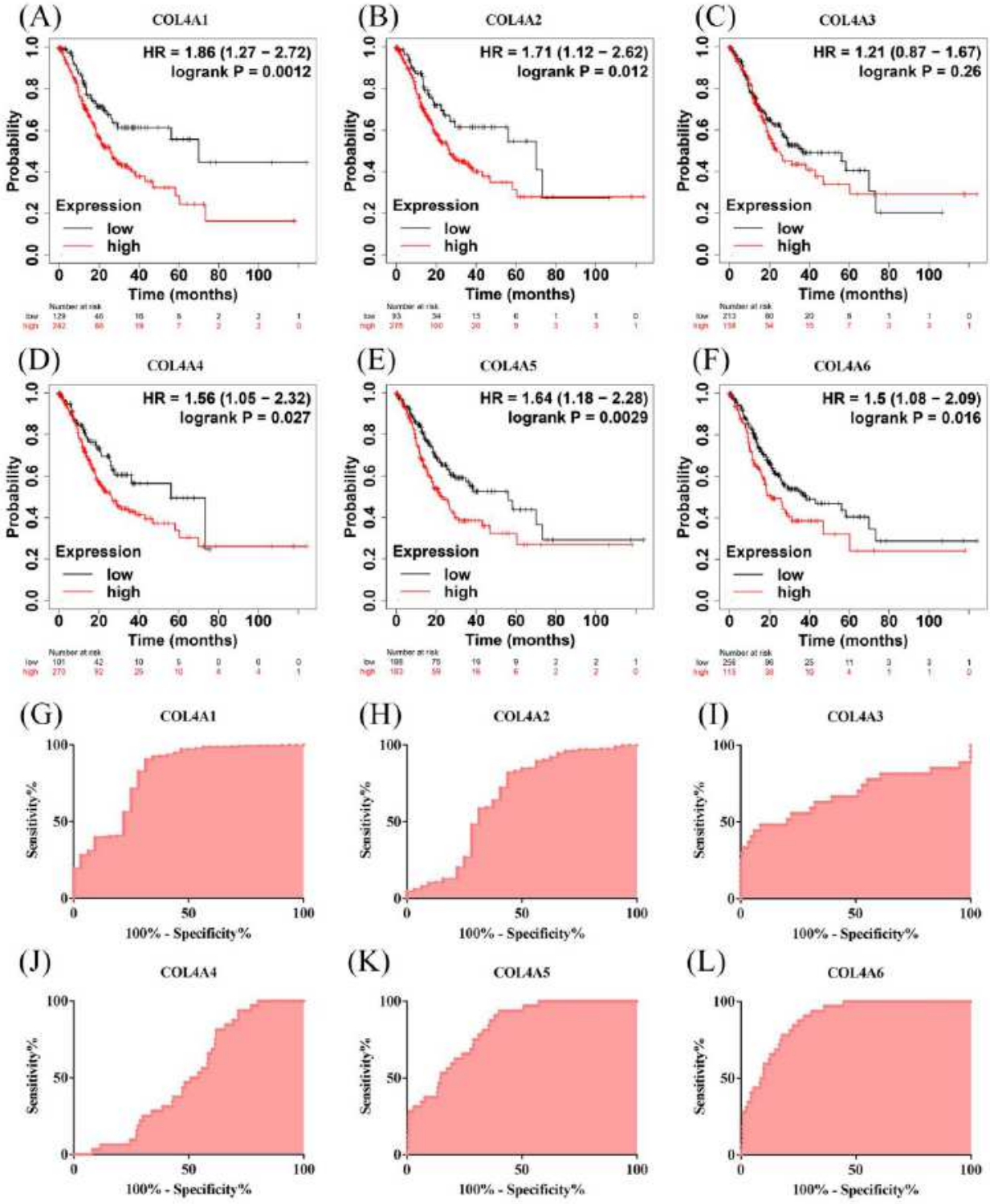

Figure 4

The prognostic value and ROC of individual COL4As (A-F) The OS of COL4As. (G-L) The ROC of COL4As.

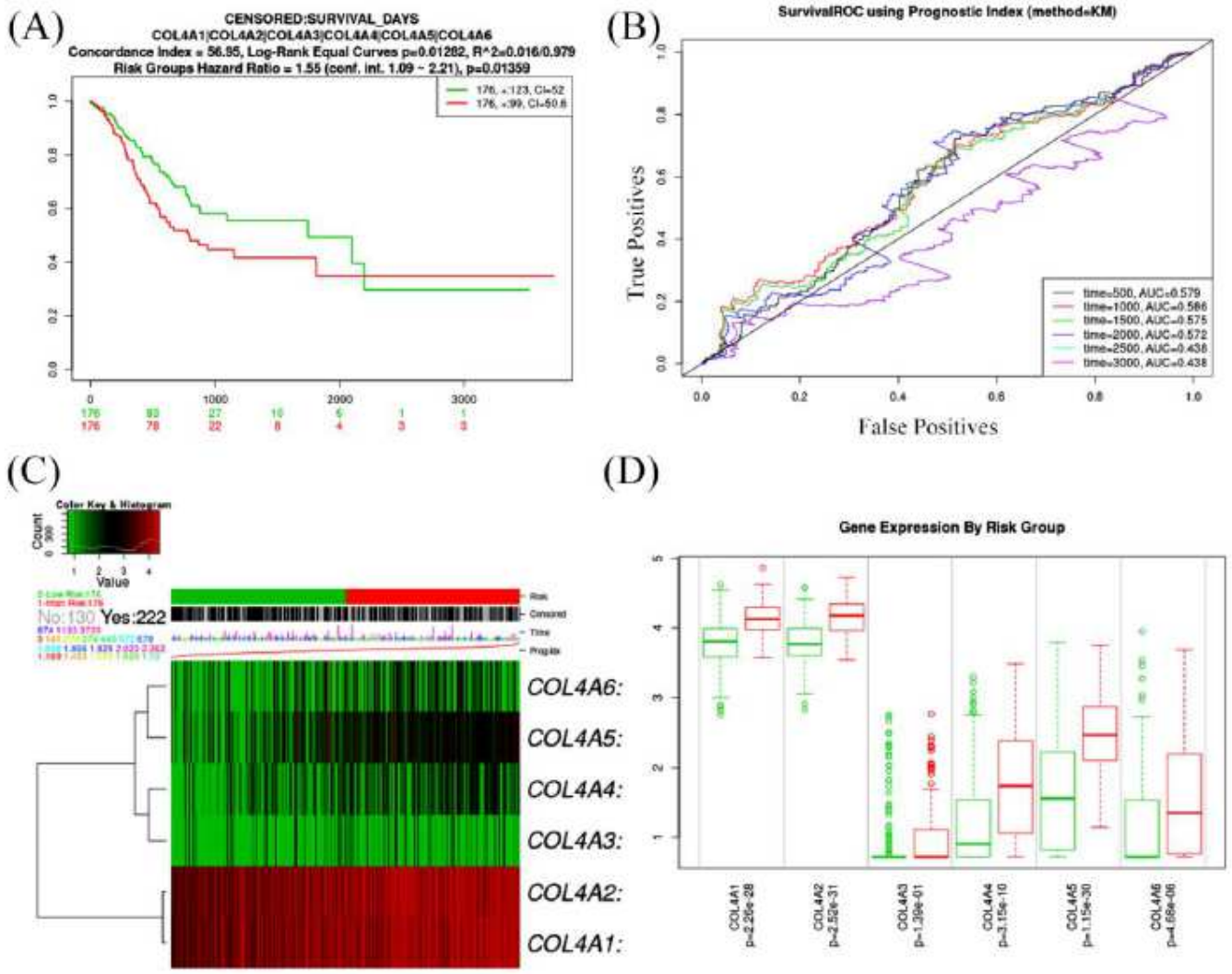

Figure 5

Integrate prognostic value of COL4As (A) The K-M plot of COL4As. (B) The ROC curve of COL4As showing the integrate the prognostic value of COL4As. (C) The heatmap of COL4As showing the different distribution of COL4As expression. (D) The bar plot of COL4As illustrating the mRNA level of COL4As in highand low-risk groups.

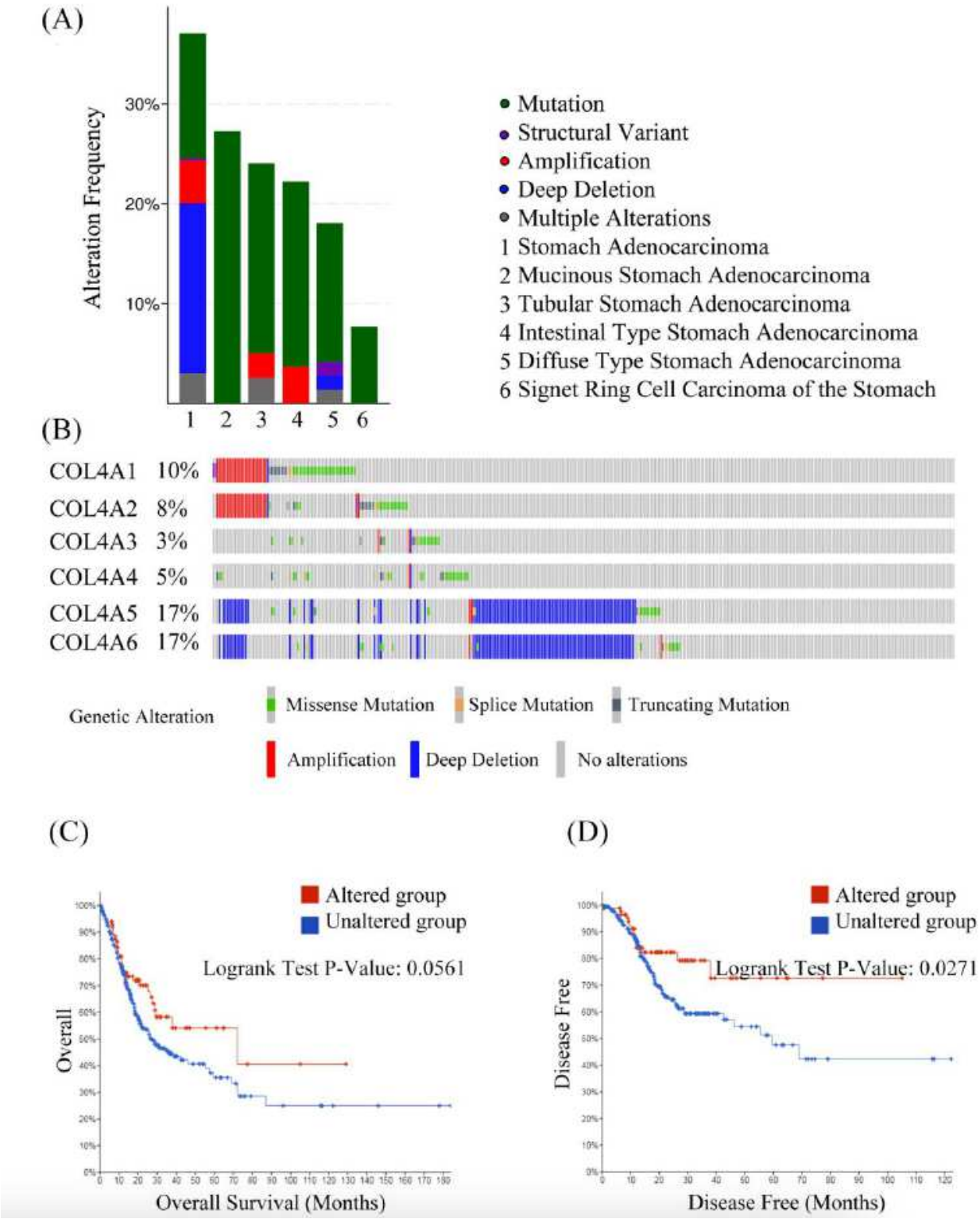

Figure 6

Oncoprint and alteration differences of COL4As in gastric cancer (A) A summary of alteration in COL4As. (B) The visual summary Oncoprint based on a query of the COL4As. (C) Kaplan–Meier plots comparing OS in cases with and without COL4As alterations. (D) KM plots comparing DFS in cases with and without COL4As alterations.

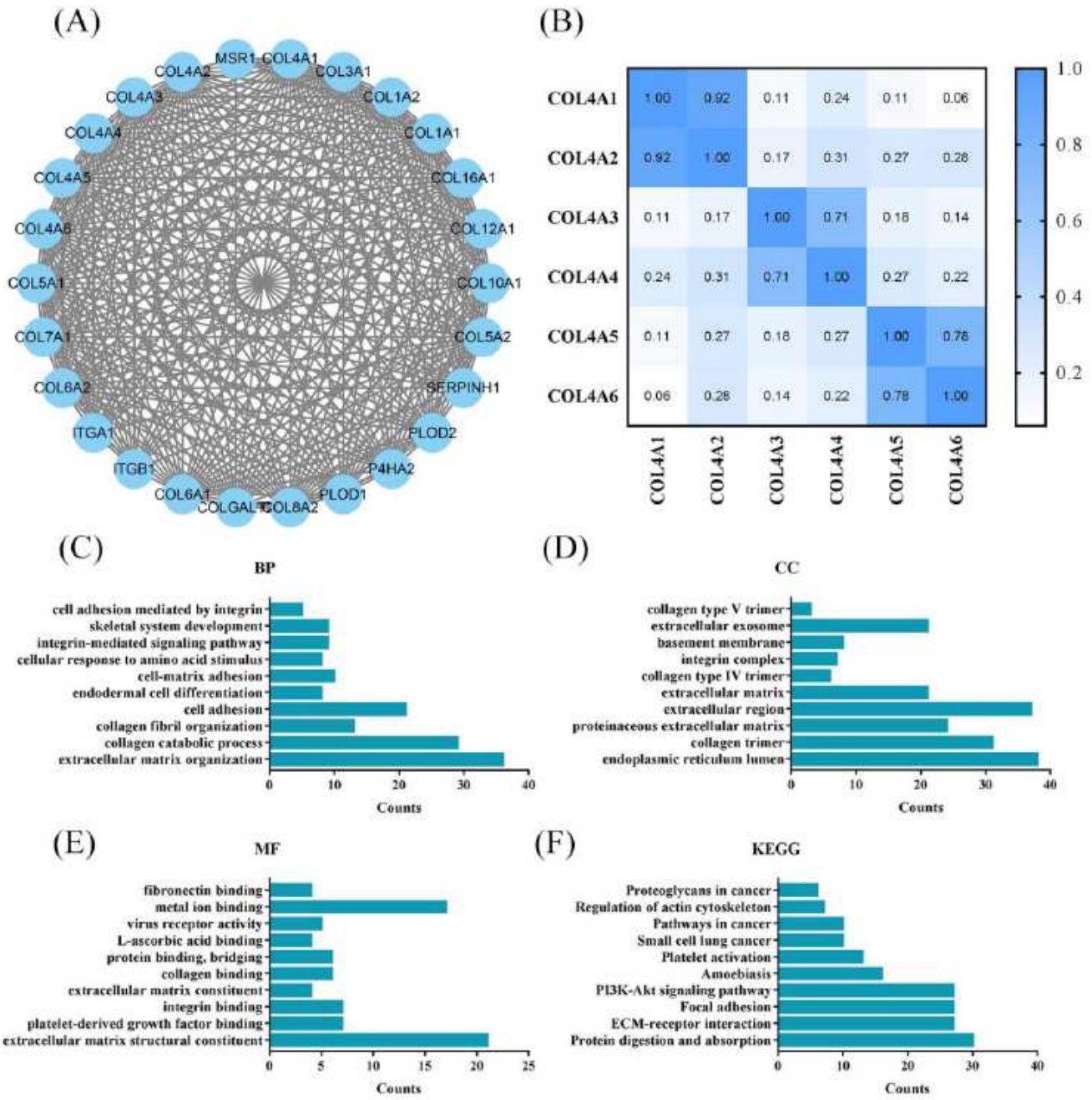

Figure 7

Correlation and functional enrichment analysis of COL4As (A) Protein–protein interaction network analysis using. (B) Pearson correlation analysis of individual among COL4As. (C) Biological process analysis; (D) cellular components; (E) molecular function. (F) KEGG analysis.

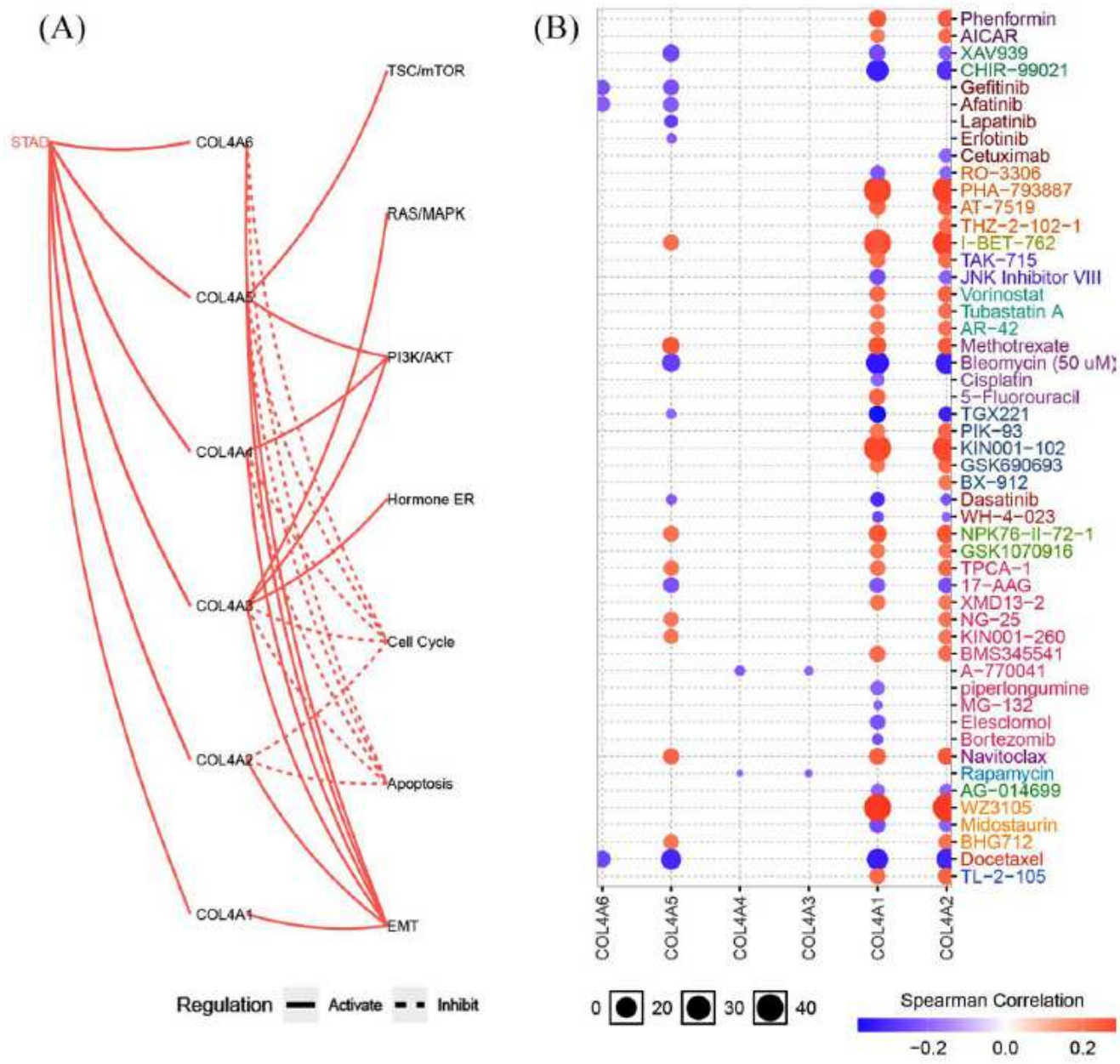

Figure 8

Analysis of the main cancer pathways and drug targets of COL4As (A) Cancer pathways of COL4As. (B) Drug targets of COL4As.

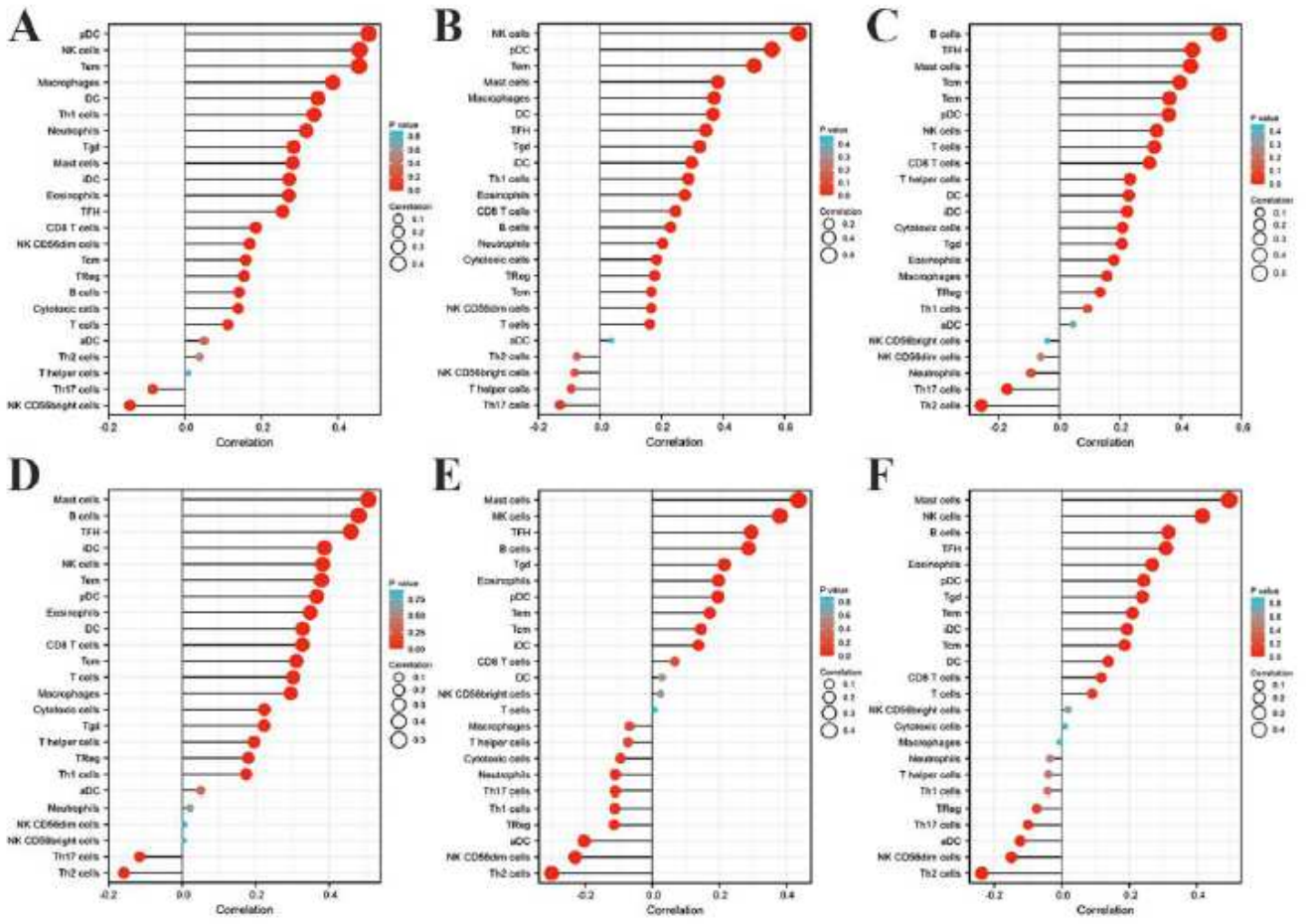

Figure 9

Correlations between expression of COL4As with immune infiltration level (A) COL4A1, (B) COL4A2, (C) COL4A3, (D) COL4A4, (E) COL4A5 and (F) COL4A6.

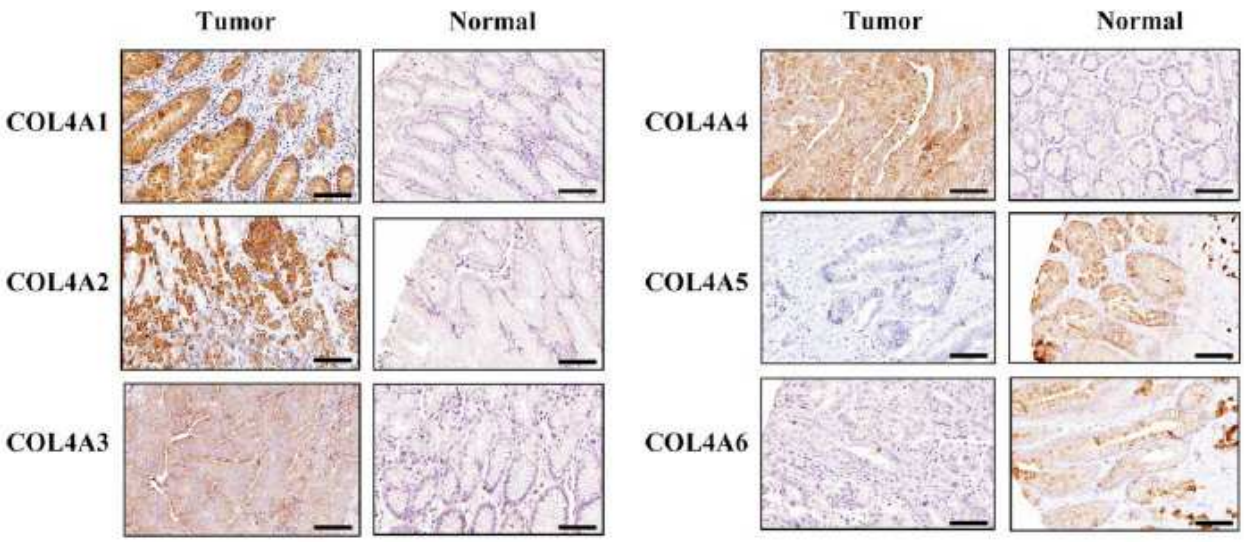

Figure 10

The IHC results of COL4As, bar=20μm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- table.pdf