Original Article

Collagen family genes and related genes might be associated with prognosis of patients with gastric cancer: an integrated bioinformatics analysis and experimental validation

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Background: Gastric cancer (GC) is disease with a high morbidity. The purpose of this study was to identify genes essential to GC development in patients and to reveal the underlying mechanisms of progression.

Methods: Bioinformatics analysis is an effective tool for discovering essential genes of different disease states. We used the Gene Expression Omnibus (GEO) database to identify differentially expressed genes (DEGs), the DAVID online tool to perform Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of DEGs, the STRING database to construct the protein-protein interaction (PPI) network of DEGs, the Oncomine and the Cancer Genome Atlas-Stomach Adenocarcinoma (TCGA-STAD) databases to analyze the gene expression differences, the Human pan-Cancer Methylation database (MethHC) to compare the DNA methylation of genes, and the Kaplan-Meier plotter to show the survival analysis of DEGs. We performed Real-Time quantitative PCR (RT-qPCR) experiment to confirm our analysis results.

Results: After the integration of four Gene Expression Series (GSEs), we identified 407 DEGs. GO and KEGG pathway analysis indicated that the upregulated DEGs were significantly enriched in Extracellular Matrix (ECM) related functions and pathways. The main DEGs were collagens (COLs). Moreover, the downregulated DEGs were enriched in ethanol oxidation. Several groups of DEGs, such as insulin-like growth factor binding protein (IGFBP), collagen (COL) and serpin peptidase inhibitors (SERPIN) gene families, constituted several PPI networks. In the Oncomine database, all of the collagen genes were highly expressed in breast cancer, esophageal cancer, GC, head and neck cancer and pancreatic cancer, compared with normal tissues. Consistently, from the TCGA-STAD database, most of the collagens (COLs) were highly expressed and exhibited methylated variation in GC patients. In GC patients, some of these collagen (COL) genes related to worse prognosis, as evidenced by the results from the Kaplan-Meier plotter database analysis. Our RT-qPCR results showed that collagen type III α1 chain (COL3A1) was highly expressed in GC cells. Collagen type V α1 chain (COL5A1) was highly expressed, except in AGS cells, which was consistent with our analysis.

Conclusions: Collagen (COL) family genes might serve as progression and prognosis markers of GC.

Keywords: Gastric cancer (GC); bioinformatics analysis; collagens; prognosis; experimental validation

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Introduction

Data from the GLOBOCAN database indicates that, globally, there are more than 1,000,000 new cases of gastric cancer (GC) each year, causing an estimated 783,000 deaths in 2018, making it the fifth most frequently diagnosed cancer and the third leading cause of cancer deaths (1). While new treatment strategies and drug developments have made significant progress, due to the low early detection rate of GC, the survival rate of GC patients remains low (2,3). In addition to the existing primary treatments, targeted therapy is expected to be an essential supplementary treatment for advanced GCs (4). Therefore, it is necessary to explore new molecular targets as well as new, highly sensitive and specific biomarkers to elucidate the molecular mechanisms of GC and improve the prognosis of patients with GC.

Recently, bioinformatics analyses (5) have become increasingly popular for analyzing gene expression changes of the in the progression and development of diseases. For example, the online GEO database (http://www. ncbi. nlm. nih. gov/geo) is a public functional genomics tool that can be utilized to analyze experimental gene expression data uploaded by researchers to identify differentially expressed genes (DEGs) of import to disease. The DAVID online database (http://david.ncifcrf.gov) holds information related to proteins and genes, and can be used to mine data for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of these genes. Similarly, differences in gene expression between tumor and normal tissues can be obtained from the TCGA database. STRING (http://string-db.org) is an online database for use in analyzing PPI networks. These online databases assist in experimental data integration and identification of important genes. In the present study, using GO enrichment analysis, we found several DEGs in GC patients, including collagens (COLs), alcohol dehydrogenases (ADHs), N-acetyl galactosyltransferases (GALNTs). Combining the KEGG, GO, and PPI network analysis results, we selected COLs for more in-depth analysis.

From the HUGO Gene Nomenclature Committee database (https://www.genenames.org/), we know that collagen-encoded proteins contain one or more collagen-like domains. Found in vertebrates, this fibrin is a significant component of skin, bones, tendons, cartilage, blood vessels and teeth. Moreover, it is a substantial component of the tumor microenvironment and is involved in cancer fibrosis (6,7). Cancer cells can regulate collagen biosynthesis through mutant genes (8), transcription factors (9,10), signaling pathways and receptors (11,12). Furthermore, collagen can affect tumor cell behavior through tyrosine kinase receptors, integrins, domain receptors, discoidin and some signaling pathways. In GC, collagen type IV α3 chain (COL4A3) has been identified as a potential prognostic factor (13), but few articles have discussed the relationship between collagen genes and GC (14). Therefore, we performed an in-depth study of the COL gene family's role in GC in order to expose progression mechanisms and to identify prognostic and progression markers.

We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/tcr-20-1726).

Methods

Microarray data and Identification of DEGs.

We downloaded four gene expression series (GSE79973, GSE26899, GSE54129 and GSE29272) from the GEO database and screened the DEGs of each series between GC and normal samples by GEO2R (http://www. ncbi. nlm. nih. gov/geo/geo2r). Genes with more than one probe set or probe sets without corresponding gene symbols were removed or averaged, respectively. Adjusted P value <0.01 and |$\log_2$Fold Change| >1 were considered statistically significant. Venn diagram of the differentially upregulated and downregulated genes were created (http://bioinfogp.cnb.csic.es/tools/venny/index.html). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

KEGG and GO enrichment analyses of DEGs

We used the DAVID (http://david.ncifcrf.gov) online database (version 6.8) to analyze the function of identified DEGs and P<0.05 was considered statistically significant.

PPI network construction and module analysis

In the present study, we used STRING (http://string-db. org) (version 11.0) to construct the PPI network of the DEGs, where a combined score >0.9 was considered statistically significant. We utilized Cytoscape (version 3.7.2) to analyze the molecular interaction networks and MCODE, a Cytoscape app for finding densely connected regions in a given network, was used to identify the most significant modules in the PPI networks. The criteria for selection were as follows: node score cut-off =0.1, degree cut-off =2, k-score =2 and Max depth =100. The genes in the module were analyzed by GO and KEGG using DAVID.

COLs Gene Expression between normal and tumor samples

We utilized Oncomine (https://www.oncomine.org/) to investigate the mRNA levels of COLs in normal and tumor tissues. We retrieved twelve members of COL family genes from the Oncomine database. In our study, the P values of comparison were generated from the student's *t*-test. The fold change and cut-off P value were defined as 2 and 0.01, respectively. The expression of COL genes in normal and gastric tumor tissues was also studied using the TCGA-STAD database (http://ualcan.path.uab.edu/index.html).

COL gene methylation between normal versus tumor tissues

We compared the DNA methylation of COL genes between normal and GC tissues using the Human Pan-cancer Methylation database, MethHC (http://methhc. mbc. nctu. edu. tw/). The correlation between COL mRNA expression and the methylation in GC patients was analyzed. In our study, the average value was used as a method for evaluating methylation levels and promoter regions selected for analysis.

Prognostic values of COL members in GC patients

The Kaplan-Meier plotter online database (http://kmplot.com) was used to analyze the relationship between COL expression and the overall survival (OS), first progression (FP), and post-progression survival (PPS) in GC patients. The median COL expression was used as the cut-off. Log-rank P value and hazard ratios, with 95% CI, were calculated.

Cell culture, RNA extraction and real-time quantitative PCR

Human GC cells (AGS, MKN45, HGC27, SGC7901) and human gastric mucosal epithelial cells (GES-1) brought from ATCC were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were maintained at 37 ℃ in a 5% CO_2 atmosphere. Total RNA was extracted from cell samples using an Animal Total RNA Isolation Kit (Foregene, China). After quality control, total RNA was reverse transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). A SYBR Green ITM premix Ex TaqTM II reagent kit (Takara Biomedical Technology, Guangzhou, China) was employed to amplify and quantify the cDNA templates. All PCR reaction systems and conditions were conducted according to the manufacturer's instructions. Primers for COL3A1 were 5'-GAAAGAGGATCTGAGGGCTCC-3' (forward) and 5'-AAACCGCCAGCTTTTTCACC-3' (reverse) and those for COL5A1 were 5'-CTGACAAGAAGTCCGAAGGGG-3' (forward) and 5'-CGTCCACATAGGAGAGCAGTTT-3' (reverse). Primers for β-actin were 5'-AACTGGGACGACATGGAGAAAA-3' (forward) and 5'-GGATAGCACAGCCTGGATAGCA-3' (reverse). The $2^{-\Delta\Delta Ct}$ method was used to calculate expression levels of target genes.

Statistical analysis

Normally distributed data were expressed as mean ± standard deviation (x ± SD). To examine statistical differences between mRNA expression levels and DNA methylation levels of normal and tumor tissues in GC patients, a two-tailed unpaired Student's *t*-test was used, P<0.05 was considered to indicate a statistically significant difference. The RT-qPCR analysis was made by GraphPad Prism 7 software and the *t*-test was used.

Results

Identification of DEGs and GO enrichment and KEGG analyses

After integrating microarray results according to our standards, we identified several DEGs (3160 in GSE54129, 1581 in GSE79973, 428 in GSE26899 and 445 in GSE29272). The overlap among the four gene

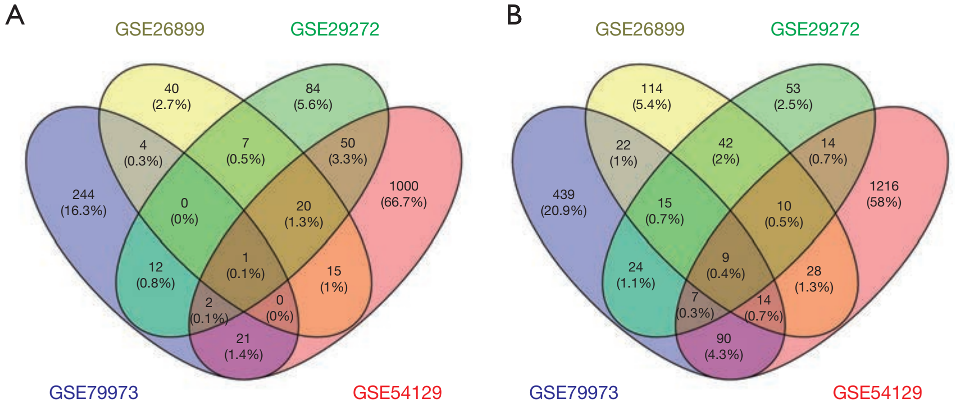


Figure 1 The distribution of differentially expressed genes between Gene Expression Series, GSE26899, GSE29272, GSE79973 and GSE54129. (A) The distribution of upregulated genes. (B) The distribution of down regulated genes.

expression series contained 407 genes, as shown in the Venn diagram (*Figure 1*), consisting of 275 downregulated genes (*Figure 1A*) and 132 upregulated genes (*Figure 1B*). Among the 407 overlapping genes, we used the DAVID online analysis tool to upload all genes that were upregulated and downregulated, thereby determining statistically rich GO terms and KEGG pathways. GO analysis results showed that upregulated DEGs were involved mainly in extracellular matrix (ECM), organization in biological processes (BP), the ECM in cell component (CC), and ECM structural constituent in molecular function (MF). Moreover, downregulated DEGs were involved mainly with ethanol oxidation in BP and ADH activity in MF (*Table 1*). The significantly enriched pathways of the DEGs analyzed by the KEGG database are shown in *Table 2*. Upregulated genes were enriched mainly in the ECM-receptor interaction, focal adhesion, protein digestion and absorption, amoebiasis and PI3K-Akt signaling pathway. Downregulated genes were enriched mainly in chemical carcinogenesis, retinol metabolism, glycolysis/gluconeogenesis, metabolism of xenobiotics by cytochrome P450 and drug metabolism-cytochrome P450.

DEG PPI network analyses

PPI networks involving 150 DEGs (consisting of 75 downregulated genes and 75 upregulated genes) were constructed (*Figure 2A*), excluding the DEGs which could not constitute a part of a network. With the cut-off criterion set as degrees ≥12, there were 26 genes selected as hub genes, including Quiescin sulfhydl oxidase-1 (QSOX1), Fibronectin-1 (FN1), Tissue inhibitor of metalloproteinases-1 (TIMP1), C3 complement, Collagen 18A1 (COL18A1), Mesothelin (MSLN) and Collagen 1A1 (COL1A1). Cytoscape was used to obtain the 5 most significant submodules (*Figure 2B,C,D,E,F*). In these submodules, we found several members of the insulin-like growth factor binding protein (IGFBP) gene family (first submodule), collagen (COL) gene family members (second submodule), and serpin peptidase inhibitors (SERPIN) gene family members (fourth submodule). These results suggested that IGFBP, COL and SERPIN family members play an essential role in the development of GC. Functional enrichment results of the second submodule, which involved collagen gene family members, revealed that the development of GC was associated with ECM organization in a biological process, similar to the GO analysis, platelet-derived growth factor binding in MF, and collagen trimer in the cellular component. Other submodules are detailed in *Table S1*.

Up-regulation of COLs in GC patients

In the GO and KEGG enrichment analysis, several members of the collagen gene family frequently were enriched and, in the PPI network analysis, several COL genes were involved in the second significant submodule. Therefore, COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A1, COL5A2, COL6A2, COL6A3, COL8A1, COL17A1 and COL18A1, which were in the

Table 1 The enriched Gene Ontology terms of up-regulated and down-regulated genes

Ontology	ID	Description	P value	Amount	Main gene
The enriched GO terms of upregulated genes					
BP	GO:0030198	Extracellular matrix organization	7.39E-20	23	*COL18A1, COL4A2, COL4A1, OLFML2B, COL3A1*
BP	GO:0030574	Collagen catabolic process	4.86E-14	13	*COL18A1, CTSL, COL4A2, COL4A1, COL3A1*
BP	GO:0030199	Collagen fibril organization	1.51E-08	8	*COL3A1, COL1A2, FOXC1, COL1A1, GREM1*
BP	GO:0071230	Cellular response to amino acid stimulus	0.00043	5	*COL4A1, COL3A1, COL1A2, COL1A1, COL5A2*
BP	GO:0030168	Platelet activation	0.0018	6	*PDPN, COL3A1, COL1A2, FCER1G, COL1A1*
BP	GO:0001568	Blood vessel development	0.0029	4	*SPHK1, COL1A2, COL1A1, COL5A1*
CC	GO:0031012	Extracellular matrix	3.53E-20	26	*ASPN, IGFBP7, COL3A1, SERPINE2, APOE*
CC	GO:0005581	Collagen trimer	4.41E-11	12	*COL18A1, CTHRC1, C1QB, COL3A1, COL6A3*
CC	GO:0005788	Endoplasmic reticulum lumen	1.083E-08	13	*COL18A1, COL4A2, COL4A1, COL3A1, COL6A3*
MF	GO:0005201	Extracellular matrix structural constituent	1.035E-09	10	*COL4A2, BGN, COL4A1, COL3A1, COL1A2*
MF	GO:0048407	Platelet-derived growth factor binding	7.71E-07	5	*COL4A1, COL3A1, COL1A2, COL1A1, COL5A1*
MF	GO:0046332	SMAD binding	0.0035	4	*COL3A1, COL1A2, COL5A2, FLNA*
MF	GO:0004252	Serine-type endopeptidase activity	0.0023	8	*CTSL, C1QB, BMP1, C3, FAP*
The enriched GO terms of downregulated genes					
BP	GO:0006069	Ethanol oxidation	0.00036	4	*ADH1C, ADH1B, ADH1A, ADH7*
BP	GO:0016266	Glycan processing	0.000087	7	*MUC1, GALNT10, GALNT6, GALNT5, GCNT1*
BP	GO:0071985	Multivesicular body sorting pathway	0.0089	3	*SYTL4, RAB27B, RAB27A*
MF	GO:0004024	Alcohol dehydrogenase activity, zinc-dependent	0.000036	4	*ADH1C, ADH1B, ADH1A, ADH7*
MF	GO:0016491	Oxidoreductase activity	0.000041	12	*FAR1, ERO1B, CYP2C9, EGLN3, ADH1C*
MF	GO:0004745	Retinol dehydrogenase activity	0.0013	4	*BMP2, ADH1C, ADH1A, ADH7*
MF	GO:0004022	Alcohol dehydrogenase (NAD) activity	0.0031	3	*ADH1C, ADH1A, ADH7*
MF	GO:0004653	Polypeptide N-acetylgalactosaminyltr ansferase activity	0.0018	4	*GALNT10, GALNT6, GALNT5, GALNT12*

GO, gene ontology; BP, biological process; CC, cell component; MF, molecular function.

COL gene family and involved in the DEGs, were chosen for more in-depth analysis. To understand better the potential relationship between GC and collagen genes, we used the Oncomine and TCGA-STAD databases to examine the mRNA expression levels of COL genes in normal and gastric tumor tissue. We assessed the expression differences of COLs in 20 cancer samples and their paired normal tissues in the Oncomine database. In these tumor datasets, COL isoforms were significantly upregulated in breast cancer, esophageal cancer, GC, head and neck cancer and pancreatic cancer (*Figure 3*) compared to matched normal tissues. As the Oncomine and TCGA-STAD databases showed, other COLs were significantly upregulated in tumor tissues (*Figures 3,4*), except for

Table 2 The enriched KEGG pathways of up-regulated and down-regulated genes

Ontology	ID	Description	P value	Counts	Gene
The enriched KEGG pathway of upregulated genes					
KEGGPATHWAY	hsa04512	ECM-receptor interaction	8.11E-13	14	*COL4A2, COL4A1, COL3A1, COL5A2, COL5A1*
KEGGPATHWAY	hsa04510	Focal adhesion	4.65E-10	16	*COL4A2, COL4A1, COL3A1, COL5A2, FLNA*
KEGGPATHWAY	hsa04974	Protein digestion and absorption	1.08E-07	10	*COL18A1, COL4A2, COL4A1, COL3A1, COL6A3*
KEGGPATHWAY	hsa05146	Amoebiasis	5.42E-07	10	*COL4A2, COL4A1, COL3A1, COL1A2, CXCL8*
KEGGPATHWAY	hsa04151	PI3K-Akt signaling pathway	1.55E-05	14	*COL4A2, COL4A1, COL3A1, COL5A2, COL5A1*
KEGGPATHWAY	hsa04611	Platelet activation	1.35E-03	7	*COL3A1, COL1A2, FCER1G, FCGR2A, COL1A1*
KEGGPATHWAY	hsa05133	Pertussis	6.81E-05	7	*C1QB, ITGA5, C3, CXCL8, SERPING1*
KEGGPATHWAY	hsa04610	Complement and coagulation cascades	4.55E-04	6	*C1QB, C3, SERPINE1, SERPING1, C2*
KEGGPATHWAY	hsa04610	Complement and coagulation cascades	4.55E-04	6	*C1QB, C3, SERPINE1, SERPING1, C2*
The enriched KEGG pathway of downregulated genes					
KEGGPATHWAY	hsa05204	Chemical carcinogenesis	1.36E-05	9	*CYP2C9, CYP2C18, SULT1A1, ADH1C, ADH1B*
KEGGPATHWAY	hsa00830	Retinol metabolism	2.68E-05	8	*CYP2C9, CYP2C18, ADH1C, ADH1B, ADH1A*
KEGGPATHWAY	hsa00010	Glycolysis / Gluconeogenesis	3.63E-05	8	*LDHB, ALDOB, ADH1C, ADH1B, ADH1A*
KEGGPATHWAY	hsa00980	Metabolism of xenobiotics by cytochrome P450	6.94E-05	8	*CYP2C9, ADH1C, ADH1B, ADH1A, ADH7*
KEGGPATHWAY	hsa00982	Drug metabolism - cytochrome P450	3.44E-04	7	*CYP2C9, ADH1C, ADH1B, ADH1A, ADH7*
KEGGPATHWAY	hsa00350	Tyrosine metabolism	0.001338831	5	*ADH1C, ADH1B, ADH1A, ADH7, ALDH3A1*
KEGGPATHWAY	hsa00512	Mucin type O-Glycan biosynthesis	8.40E-04	5	*GALNT10, GALNT6, GALNT5, GCNT1, GALNT12*

hsa, Homo sapiens.

COL6A2 and COL17A1 (data not shown). The details of COL gene expression in all GC datasets in the Oncomine database are shown in *Table S2*.

DNA methylation of COL genes in GC patients

In order to explore the role of methylation in the regulation of COL expression in GC patients, the MethHC method was used to analyze the methylation level of the COL genes promoter regions, and the relationship between DNA methylation level and mRNA expression level. Among the COL members, the methylation levels between normal and cancer samples were statistically different (P<0.05, *Figure 5*),

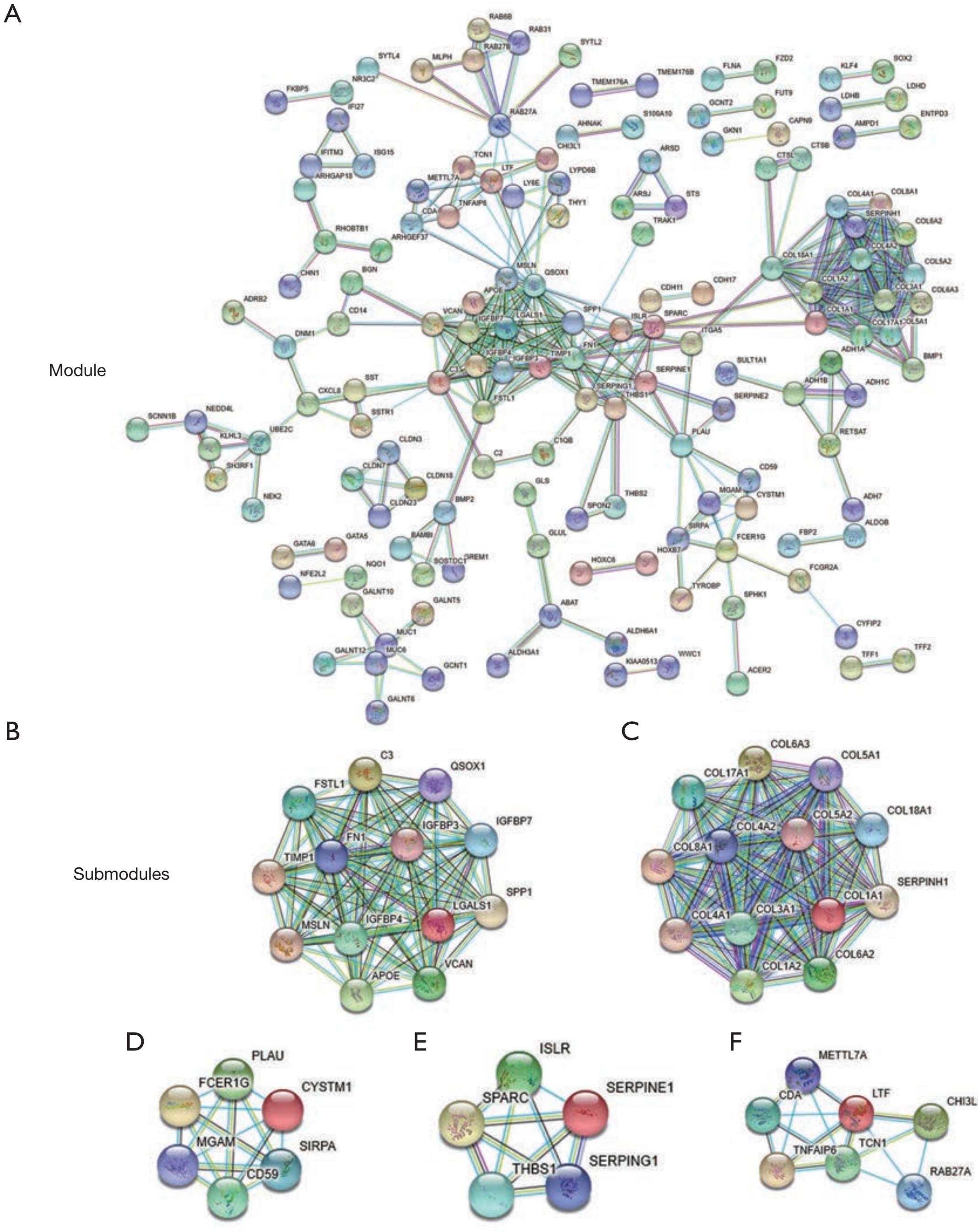
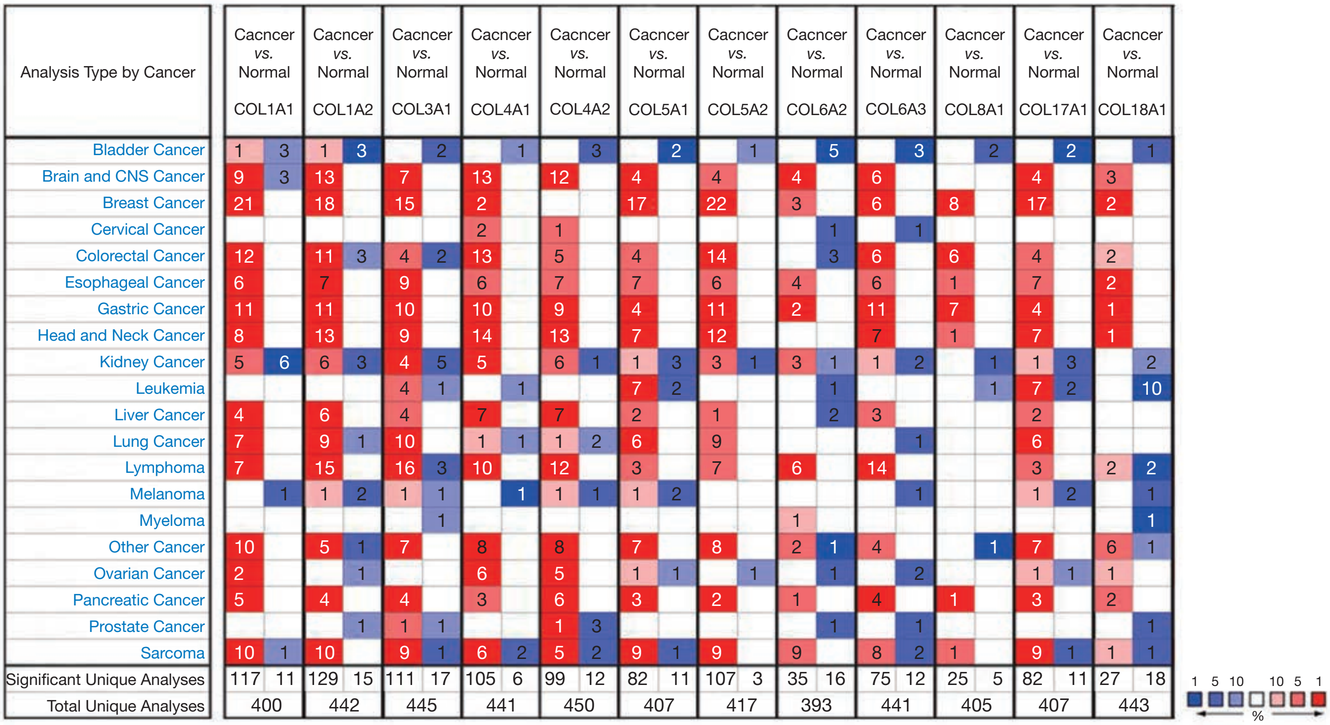


Figure 2 Module (A) and 5 submodules (B-F) of protein-protein interaction (PPI) network. Line color indicates the type of interaction evidence. The number of lines between two genes indicate the level of interaction between the two genes.

except for COL8A1. Notably, DNA methylation of most COLs (10/11) in GC was higher than in the matched normal tissue, except for COL5A2, which was lower than the normal tissue (*Figure 5*). The relationship between DNA methylation and mRNA expression of COL members in GC are listed in *Table S3*, although the R values did not prove the relationship between mRNA level and DNA methylation.



Analysis Type by Cancer	Cacncer *vs.* Normal COL1A1		Cacncer *vs.* Normal COL1A2		Cacncer *vs.* Normal COL3A1		Cacncer *vs.* Normal COL4A1		Cacncer *vs.* Normal COL4A2		Cacncer *vs.* Normal COL5A1		Cacncer *vs.* Normal COL5A2		Cacncer *vs.* Normal COL6A2		Cacncer *vs.* Normal COL6A3		Cacncer *vs.* Normal COL8A1		Cacncer *vs.* Normal COL17A1		Cacncer *vs.* Normal COL18A1	
Bladder Cancer	1	3	1	3		2		1		3		2		1		5		3		2		2		1
Brain and CNS Cancer	9	3	13		7		13		12		4		4		4		6				4		3	
Breast Cancer	21		18		15		2				17		22		3		6		8		17		2	
Cervical Cancer							2		1							1		1						
Colorectal Cancer	12		11	3	4	2	13		5		4		14			3	6		6		4		2	
Esophageal Cancer	6		7		9		6		7		7		6		4		6		1		7		2	
Gastric Cancer	11		11		10		10		9		4		11		2		11		7		4		1	
Head and Neck Cancer	8		13		9		14		13		7		12				7		1		7		1	
Kidney Cancer	5	6	6	3	4	5	5		6	1	1	3	3	1	3	1	1	2		1	1	3		2
Leukemia					4	1		1			7	2				1				1	7	2		10
Liver Cancer	4		6		4		7		7		2		1			2	3				2			
Lung Cancer	7		9	1	10		1	1	1	2	6		9					1			6			
Lymphoma	7		15		16	3	10		12		3		7		6		14				3		2	2
Melanoma		1	1	2	1	1		1	1	1	1	2						1			1	2		1
Myeloma						1									1									1
Other Cancer	10		5	1	7		8		8		7		8		2	1	4			1	7		6	1
Ovarian Cancer	2			1			6		5		1	1		1		1		2			1	1	1	
Pancreatic Cancer	5		4		4		3		6		3		2		1		4		1		3		2	
Prostate Cancer				1	1	1			1	3						1		1						1
Sarcoma	10	1	10		9	1	6	2	5	2	9	1	9		9		8	2	1		9	1	1	1
Significant Unique Analyses	117	11	129	15	111	17	105	6	99	12	82	11	107	3	35	16	75	12	25	5	82	11	27	18
Total Unique Analyses	400		442		445		441		450		407		417		393		441		405		407		443	

Figure 3 mRNA levels of collagen isoforms in different cancers (Oncomine). The counts of datasets with statistically significant collagens mRNA down-regulation (blue) or up-regulation (red) (normal tissues versus corresponding different cancers) are shown. Threshold setting: gene rank, top 10%; fold change, 2; P value, 0.01. The figures in the colored box represent the numbers of datasets meeting the threshold.

Prognostic characteristics of COLs in GC patients

Prognostic characteristics of GC patients, including OS, first progression (FP), and post progression survival (PPS), were surveyed in the Kaplan-Meier plotter database. Among these COLs available in the Kaplan-Meier database, most genes showed a positive relationship between high expression and significantly worse OS in GC patients (*Figure 6A*), except COL3A1, COL5A2 and COL17A1. The data showed FP reduction with low COL17A1 (*Figure 6B*) and high levels of the other collagen genes. The significant, inverse relationship was shown between PPS and collagen genes, except for COL5A2 and COL17A1 (*Figure 6C*). High COL1A1, COL1A2, COL4A1, COL4A2, COL5A1, COL6A2, COL6A3, COL8A1 and COL18A1 mRNA expression levels led to reduced OS, FP and PPS in GC patients. Furthermore, increased COL17A1 mRNA levels significantly correlated only with increased FP, but was not correlated with OS or PPS. In Lauren's classification, GC is divided into three categories: diffuse, intestinal and mixed. Therefore, the Kaplan-Meier plotter online tool can be used to determine the prognostic value of COL gene isoforms in different GC subtypes. The data showed that high expression levels of COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A1, COL6A2, COL6A3, and COL18A1 led to reduced OS, FP and PPS in intestinal and diffuse-type GC patients. Additionally, in the mixed-type GC patients, most of the COLs were with no-significance because the number of the cases were too small for statistical analysis (*Table S4*). The different transcript levels of COL17A1 had no effect on the three subtypes, except the OS in intestinal type, which corresponded to the result where the COL17A1 mRNA expression level showed no difference between the normal and tumor tissues. The complex relationship of these GC subtype survival time (OS, FP, PPS) with the COLmRNA expression was shown in the supplementary materials (*Figures S1-S3*).

mRNA expression of COL3A1 and COL5A1 in different GC cells

Except for COL6A2 and COL17A1, COLs were highly expressed according to the TCGA-STAD databases. We

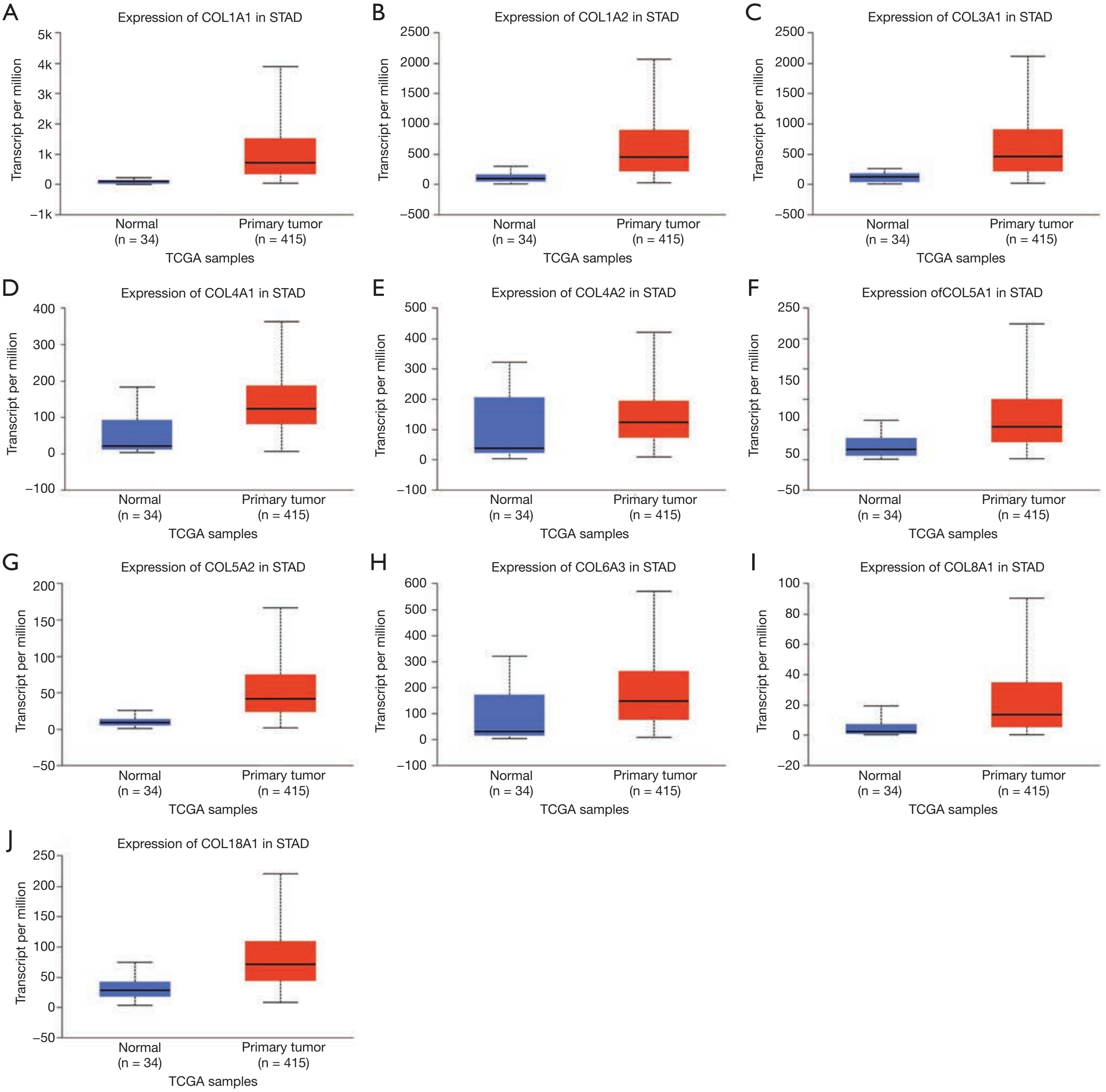


Figure 4 Uaclan database showed that mRNA expression of collagen family genes differed between primary tumor and corresponding normal tissues in gastric cancer patients using (A-J). The blue box represents normal tissue; red box represents tumor tissue. Only P<0.05 was shown.

chose the *COL3A1* and *COL5A1* genes, which lacked experimental verification in GC but have already been shown to play roles in other cancers (15,16), for RT-qPCR experiments to validate our analysis results. As shown in *Figure* 7, the COL3A1 level was 1.310×10^6 folds higher in HGC27, 185 folds higher in SGC7901, 96 folds higher in

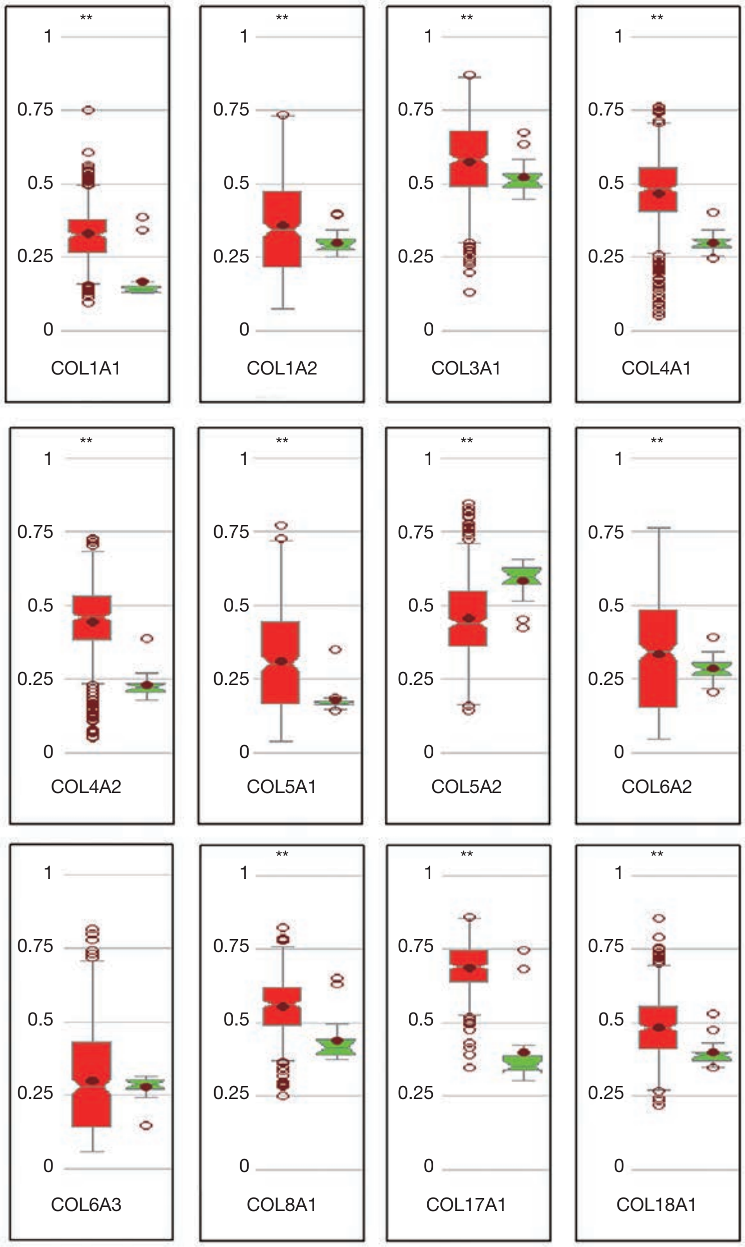
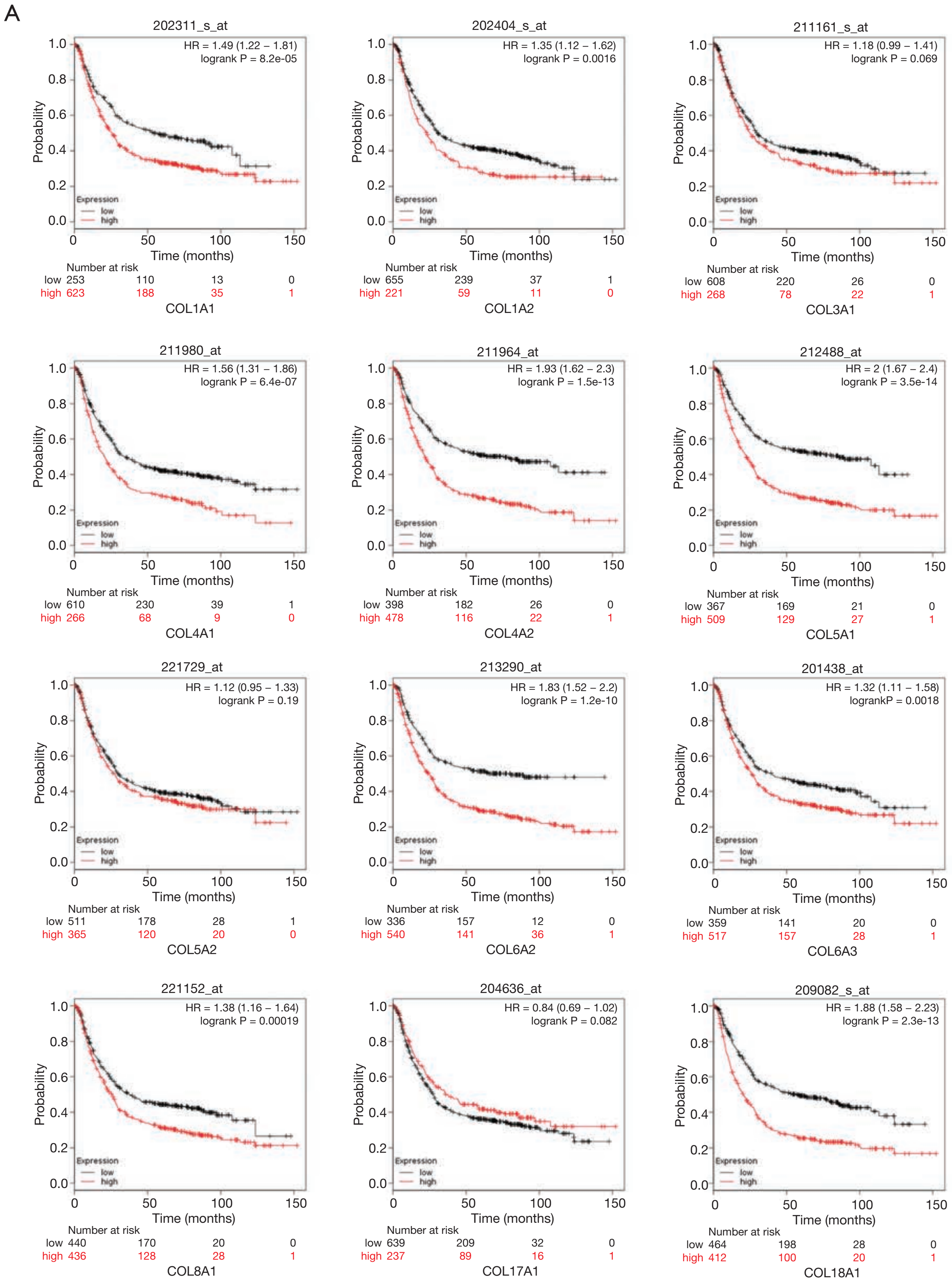


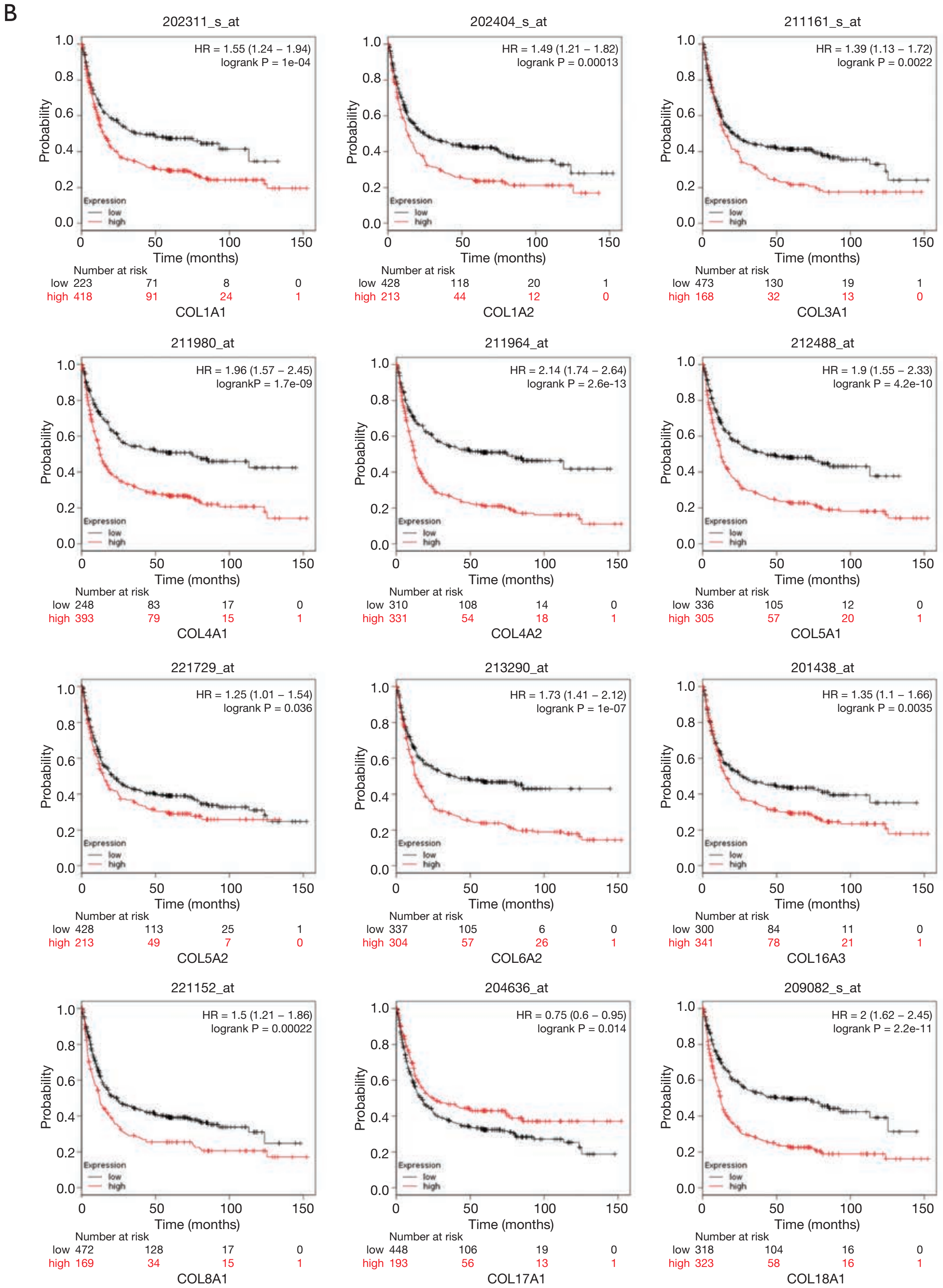
Figure 5 The methylation of collagen isoforms in gastric cancer and normal tissues (MethHC). Box plots in red color represent cancer samples and those in green color represent normal samples. **, indicates P<0.005. GC, gastric cancer.

MKN45 and six folds higher in AGS human GC cell lines, compared with GES-1 normal human gastric mucosal epithelial cell line, and these results were consistent with the analyses from the TCGA-STAD databases. Interestingly, COL5A1 was highly expressed in HGC27, MKN45 and SGC7901, at about 3-7 folds, which were also consistent with the above results. However, in the AGS cell lines, COL5A1 was 400 folds lower than in GES-1. These results require additional in-depth exploration.

Discussion

In recent years, significant efforts have been made in order to understand better the early diagnosis, targeted therapy


A
202311_s_at
HR = 1.49 (1.22 – 1.81)
logrank P = 8.2e-05
Probability
Time (months)
Expression
low
high
Number at risk
low 253 110 13 0
high 623 188 35 1
COL1A1
202404_s_at
HR = 1.35 (1.12 – 1.62)
logrank P = 0.0016
low 655 239 37 1
high 221 59 11 0
COL1A2
211161_s_at
HR = 1.18 (0.99 – 1.41)
logrank P = 0.069
low 608 220 26 0
high 268 78 22 1
COL3A1
211980_at
HR = 1.56 (1.31 – 1.86)
logrank P = 6.4e-07
low 610 230 39 1
high 266 68 9 0
COL4A1
211964_at
HR = 1.93 (1.62 – 2.3)
logrank P = 1.5e-13
low 398 182 26 0
high 478 116 22 1
COL4A2
212488_at
HR = 2 (1.67 – 2.4)
logrank P = 3.5e-14
low 367 169 21 0
high 509 129 27 1
COL5A1
221729_at
HR = 1.12 (0.95 – 1.33)
logrank P = 0.19
low 511 178 28 1
high 365 120 20 0
COL5A2
213290_at
HR = 1.83 (1.52 – 2.2)
logrank P = 1.2e-10
low 336 157 12 0
high 540 141 36 1
COL6A2
201438_at
HR = 1.32 (1.11 – 1.58)
logrankP = 0.0018
low 359 141 20 0
high 517 157 28 1
COL6A3
221152_at
HR = 1.38 (1.16 – 1.64)
logrank P = 0.00019
low 440 170 20 0
high 436 128 28 1
COL8A1
204636_at
HR = 0.84 (0.69 – 1.02)
logrank P = 0.082
low 639 209 32 0
high 237 89 16 1
COL17A1
209082_s_at
HR = 1.88 (1.58 – 2.23)
logrank P = 2.3e-13
low 464 198 28 0
high 412 100 20 1
COL18A1
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150


B
202311_s_at
HR = 1.55 (1.24 – 1.94)
logrank P = 1e-04
Probability
1.0
0.8
0.6
0.4
0.2
0.0
Expression
low
high
0
50
100
150
Time (months)
Number at risk
low 223 71 8 0
high 418 91 24 1
COL1A1
202404_s_at
HR = 1.49 (1.21 – 1.82)
logrank P = 0.00013
low 428 118 20 1
high 213 44 12 0
COL1A2
211161_s_at
HR = 1.39 (1.13 – 1.72)
logrank P = 0.0022
low 473 130 19 1
high 168 32 13 0
COL3A1
211980_at
HR = 1.96 (1.57 – 2.45)
logrankP = 1.7e-09
low 248 83 17 0
high 393 79 15 1
COL4A1
211964_at
HR = 2.14 (1.74 – 2.64)
logrank P = 2.6e-13
low 310 108 14 0
high 331 54 18 1
COL4A2
212488_at
HR = 1.9 (1.55 – 2.33)
logrank P = 4.2e-10
low 336 105 12 0
high 305 57 20 1
COL5A1
221729_at
HR = 1.25 (1.01 – 1.54)
logrank P = 0.036
low 428 113 25 1
high 213 49 7 0
COL5A2
213290_at
HR = 1.73 (1.41 – 2.12)
logrank P = 1e-07
low 337 105 6 0
high 304 57 26 1
COL6A2
201438_at
HR = 1.35 (1.1 – 1.66)
logrank P = 0.0035
low 300 84 11 0
high 341 78 21 1
COL16A3
221152_at
HR = 1.5 (1.21 – 1.86)
logrank P = 0.00022
low 472 128 17 0
high 169 34 15 1
COL8A1
204636_at
HR = 0.75 (0.6 – 0.95)
logrank P = 0.014
low 448 106 19 0
high 193 56 13 1
COL17A1
209082_s_at
HR = 2 (1.62 – 2.45)
logrank P = 2.2e-11
low 318 104 16 0
high 323 58 16 1
COL18A1

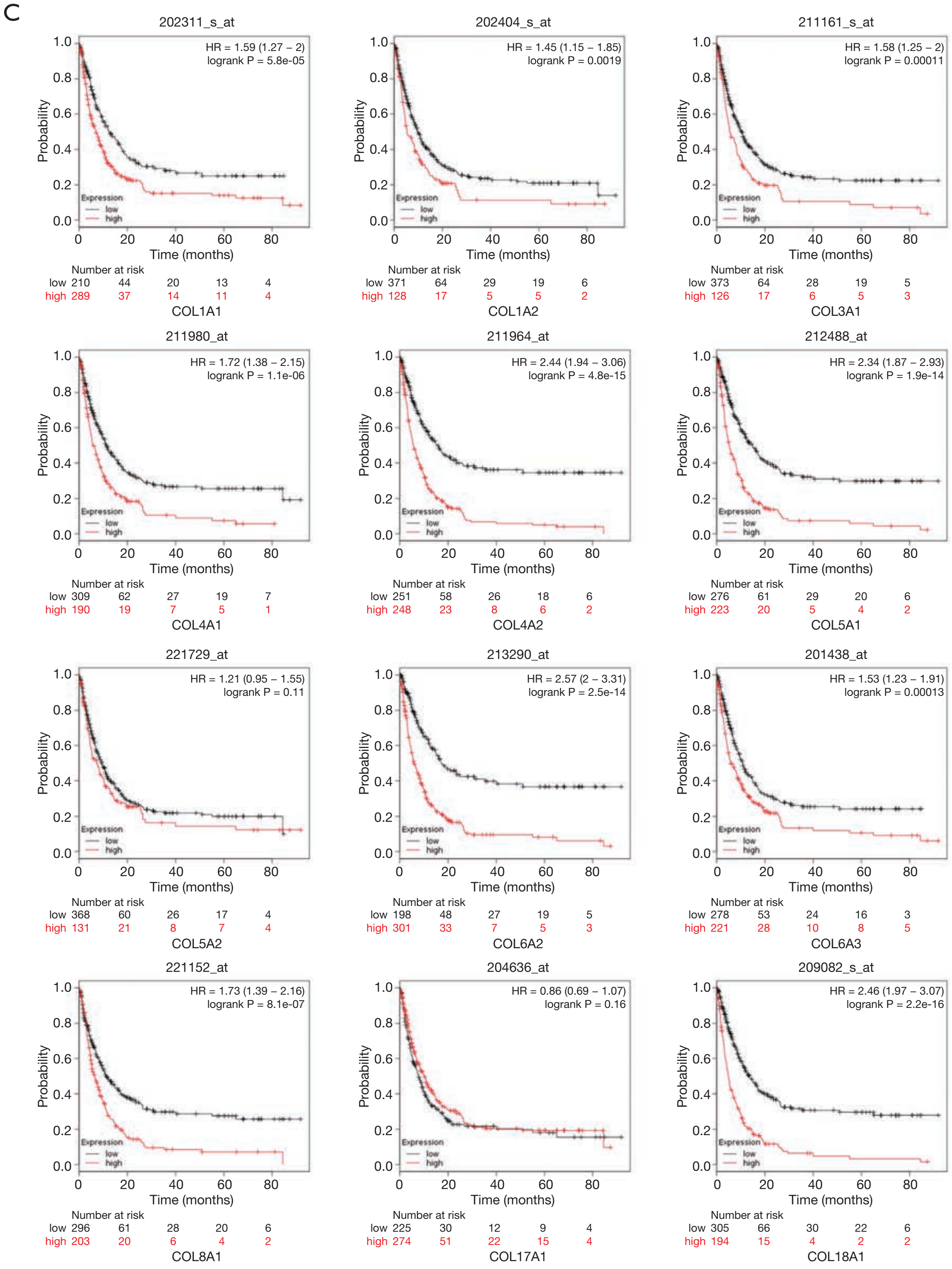


Figure 6 Different mRNA levels of collagen genes prognostic values in gastric cancer patients (Kaplan-Meier plotter). Kaplan-Meier plots show the relationship between OS (A), FP (B) and PPS (C) and the expression of collagens in gastric cancer patients, with hazard ratio (HR) and statistical significance.

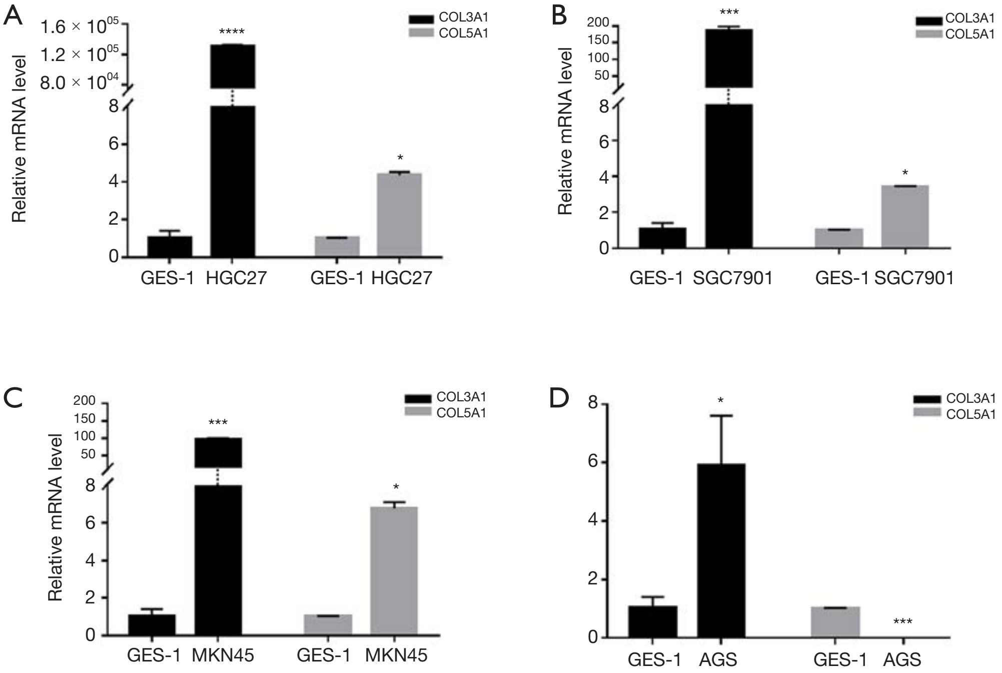


Figure 7 The expression of COL3A1 and COL5A1 mRNA in different gastric cancer cells. *, indicates folds change from 2 to 10; ***, indicates folds change from 100 to 500; ****, indicates folds higher than 1,000.

and prognosis of GC (17,18). However, the OS of patients with GC remains unimproved, particularly in developing countries (19,20). Our study aimed to identify nuclear genes with similar functions that are highly expressed in GC, compared to normal controls, and to reveal their underlying mechanisms. In the present study, we downloaded the gene expression series of GSE79973, GSE26899, GSE54129 and GSE29272 from the GEO database and found 132 upregulated and 275 downregulated overlap DEGs between GC and normal controls. GO term analysis showed that upregulated DEGs were related primarily with ECM. As reported in previous studies, several ECM-related genes had impacts on the development of GC (21-23). Increased deposition of matrix proteins favors tumor progression by interfering with cell polarity, cell-cell adhesion and, ultimately, amplifying growth factor signaling. As the most significant ECM component (24), collagen determines the functional properties of the matrix and changes in the deposition or degradation of collagen can lead to a decline of ECM homeostasis. It has been reported that increased collagen cross-linking and deposition leads to tumor progression via increased integrin signaling (25). PPI network analysis showed that the IGFBP, SERPIN, and COL gene families were enriched in several submodules. Previous studies have shown that IGFBPs play a protective role in the process of GC development (26-28). However, in our meta-analysis, we found that IGFBP3, IGFBP4, and IGFBP7 were upregulated in GC patients, which was opposed to normal tissues. This might be a self-protection mechanism in GC patients, and additional experiments and analyses are required to investigate this unusual situation. Wang *et al.* (29), Ju *et al.* (30), and Yang *et al.* (31) found that SERPINs can be used as a novel prognostic factor in GC. Additionally, Tian *et al.* found that SERPINH1 was overexpressed in GC patients and took part in the regulation of EMT (32), which supported the results of our analysis.

Among the identified DEGs, 12 collagen genes were found. Most of these collagen genes with high mRNA and DNA methylation levels ExceptingCOL6A2, COL8A1, COL17A1 and COL5A2, these collagen genes were found to have high mRNA and DNA methylation levels. DNA methylation causes gene silencing. Our results showed, however, high DNA methylation in the promoter region (except for COL5A2), similar to the mRNA levels, in the GC cells. The results showed that methylation in the promotor region did not influence mRNA expression levels COL genes and suggested that methylation may exist in another region or some other mechanism may have affected mRNA levels. Kaplan-Meier analysis revealed that most of the COLs showed a positive relationship between high expression and significantly worse prognoses in GC patients, which supported the idea that COLs could be prognostic markers in GC patients. Previously, only a few isoforms of COLs involved in GC were reported. Previous studies have demonstrated that upregulated expression of COL1A1 (33), COL1A2 and COL6A3 (34) enhanced the invasive properties of GC cells. COL4A3 was confirmed as a prognostic factor in GC (13). The role of other COLs in GC has not been published (14). Accordingly, additional experimental verification is required to confirm our results and evaluate their meaning. It has been shown that COL3A1 and COL5A1 can be a diagnostic marker in breast cancer and plays a role in non-small cell lung cancer (15,16,35). Therefore, we chose these two COLs for RT-qPCR experiments. After our repeated experiments, data showed that COL3A1 was highly expressed in the four cell lines, and that COL5A1 was highly expressed, in except AGS cells. The differing expression levels between GC cell lines suggested to determine the differences between the cell lines. We found that between these four GC cell lines, HGC27 had the highest degree of malignancy, while AGS was the lowest (36-39).These results are consistent with the expression levels of COL3A1 and COL5A1 in each of the cell lines, which provided a basis for COL3A1 and COL5A1 as markers for the progression and prognosis of GC.

Conclusions

Additional experimentation is required in order to determine whether the COL gene family can be utilized as markers of GC progression and prognosis. Our analysis provides a feasible basis for the idea that COLs may be used as progression and prognosis markers of GC.

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Footnote

Reporting Checklist: The authors have completed the MDAR checklist. Available at http://dx.doi.org/10.21037/tcr-20-1726

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-1726). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 Features of module and five submodules of protein-protein interaction (PPI) networks

Characteristics	Nodes	Edges	Average node degree	Average local clustering coefficient	PPI enrichment P value	Key genes	Functional enrichment		
							BP	MF	CC
Module	365	371	2.03	0.36	<1.0e-16	*QSOX1, FN1, TIMP1, C3, MSLN*	Extracellular structure organization	Platelet-derived growth factor binding	Endoplasmic reticulum lumen
Submodules									
1	13	78	12	1	<1.0e-16	*IGFBP7, IGFBP3, QSOX1, VCAN, TIMP1*	Post-translational protein modification	Insulin-like growth factor binding	Endoplasmic reticulum lumen
2	13	78	12	1	<1.0e-16	*COL1A1, COL1A2, SERPINH1, COL17A1, COL4A2*	Extracellular matrix organization	Platelet-derived growth factor binding	Collagen trimer
3	6	15	5	1	<1.0e-16	*MGAM, PLAU, SIRPA, FCER1G, CYSTM1*	Neutrophil degranulation	-	Tertiary granule membrane
4	5	10	4	1	2.03E-13	*SERPINE1, SERPING1, THBS1, ISLR, SPARC*	Platelet degranulation	Extracellular matrix binding	Platelet alpha granule lumen
5	7	15	4.29	0.886	<1.0e-16	*METTL7A, CHI3L1, LTF, TCN1, TNFAIP6*	Neutrophil degranulation	Carbohydrate derivative binding	Tertiary granule lumen

Nodes, the gene numbers in the modules. Edges, the interaction numbers in the modules. PPI enrichment P value indicate that the nodes are not random and that the observed number of edges is significant.

Table S2 The mRNA levels of collagen isoforms in normal and different types of gastric cancer tissues (ONCOMINE)

Collagen family	Types of Gastric Cancer *vs.* normal	Fold change	*t*-test	P value	Reporter
COL1A1	Gastric Cancer *vs.* Normal	3.201	8.724	1.81E-15	Cui Gastric Statistics;3762198
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	5.483	12.628	3.47E-21	Chen Gastric Statistics; IMAGE:153646
	Diffuse Gastric Adenocarcinoma *vs.* Normal	9.047	8.649	1.65E-07	Chen Gastric Statistics IMAGE:418193
	Gastric Mixed Adenocarcinoma *vs.* Normal	11.917	7.514	1.90E-05	Chen Gastric Statistics; IMAGE:153647
	Diffuse Gastric Adenocarcinoma *vs.* Normal	5.607	7.715	4.58E-10	Cho Gastric Statistics ILMN_1701308
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	4.081	5.196	4.71E-06	Cho Gastric Statistics ILMN_1701308
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.652	3.295	0.002	Cho Gastric Statistics ILMN_1701308
	Gastric Cancer *vs.* Normal	5.808	6.795	2.99E-06	Wang Gastric Statistics;202310_s_at
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	6.017	8.766	5.20E-11	DErrico Gastric Statistics 202311_s_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	5.538	5.334	8.77E-04	DErrico Gastric Statistics 202311_s_at
	Gastric Mixed Adenocarcinoma *vs.* Normal	5.471	8.023	6.74E-04	DErrico Gastric Statistics 202310_s_at
COL1A2	Gastric Cancer *vs.* Normal	7.491	7.308	2.81E-07	Wang Gastric Statistics; 202404_s_AT
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	4.548	15.552	6.07E-25	Chen Gastric Statistics;IMAGE839991
	Diffuse Gastric Adenocarcinoma *vs.* Normal	5.193	11.394	2.23E-10	Chen Gastric Statistics;IMAGE839991
	Gastric Mixed Adenocarcinoma *vs.* Normal	6.984	8.952	4.51E-05	Chen Gastric Statistics;IMAGE839991
	Diffuse Gastric Adenocarcinoma *vs.* Normal	5.876	9.253	1.89E-12	Cho Gastric Statistics;ILMN_2104356
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	4.062	5.226	9.69E-06	Cho Gastric Statistics;ILMN_2104356
	Gastric Mixed Adenocarcinoma *vs.* Normal	3.404	4.337	4.87E-04	Cho Gastric Statistics;ILMN_2104356
	Gastric Cancer *vs.* Normal	2.277	7.245	9.49E-12	Cui Gastric Statistics;3013054
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	7.433	10.405	5.42E-14	DErrico Gastric Statistics;202404_s_at
	Gastric Mixed Adenocarcinoma *vs.* Normal	3.453	9.816	2.37E-08	DErrico Gastric Statistics;202403_s_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	6.424	5.785	4.63E-04	DErrico Gastric Statistics;202404_s_at
COL3A1	Gastric Cancer *vs.* Normal	2.333	7.397	4.15E-12	Cui Gastric Statistics;2519577
	Diffuse Gastric Adenocarcinoma *vs.* Normal	4.458	10.994	2.57E-11	Chen Gastric Statistics IMAGE:122159(1)
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	3.466	12.075	5.06E-19	Chen Gastric Statistics IMAGE:122159(2)
	Gastric Mixed Adenocarcinoma *vs.* Normal	5.675	8.902	2.65E-06	Chen Gastric Statistics IMAGE:122159(2)
	Gastric Cancer *vs.* Normal	2.766	6.322	2.41E-06	Wang Gastric Statistics;215076_s_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.656	5.201	2.04E-06	Cho Gastric Statistics;ILMN_1773079
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.225	3.13	0.002	Cho Gastric Statistics;ILMN_1773079
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.864	7.241	2.31E-05	DErrico Gastric Statistics;215076_s_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.7	4.516	9.72E-04	DErrico Gastric Statistics;201852_s_at
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.425	5.784	2.29E-07	DErrico Gastric Statistics;215076_s_at
COL4A1	Diffuse Gastric Adenocarcinoma *vs.* Normal	5.045	14.254	4.54E-13	Chen Gastric Statistics;IMAGE:145292
	Gastric Mixed Adenocarcinoma *vs.* Normal	6.23	10.438	6.43E-07	Chen Gastric Statistics;IMAGE:145292
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	4.104	15.779	6.04E-18	Chen Gastric Statistics;IMAGE:145292
	Gastric Cancer *vs.* Normal	2.276	5.853	5.67E-06	Wang Gastric Statistics;211980_at
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	3.207	10.716	7.08E-14	DErrico Gastric Statistics;211980_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	3.705	3.88	0.002	DErrico Gastric Statistics;211981_at
	Gastric Mixed Adenocarcinoma *vs.* Normal	7.956	6.055	0.001	DErrico Gastric Statistics;211981_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.884	6.164	3.10E-07	Cho Gastric Statistics;ILMN_1653028
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.25	3.838	2.35E-04	Cho Gastric Statistics;ILMN_1653028
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.676	3.972	4.37E-04	Cho Gastric Statistics;ILMN_1653028
COL4A2	Diffuse Gastric Adenocarcinoma *vs.* Normal	3.14	10.501	1.63E-09	Chen Gastric Statistics;IMAGE:769959
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.133	10.669	2.38E-17	Chen Gastric Statistics;IMAGE:769959
	Gastric Mixed Adenocarcinoma *vs.* Normal	3.82	9.131	4.23E-06	Chen Gastric Statistics;IMAGE:769959
	Gastric Cancer *vs.* Normal	2.483	5.93	1.85E-06	Wang Gastric Statistics;211964_at
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.632	9.368	3.54E-12	DErrico Gastric Statistics;211964_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	3.047	3.886	0.002	DErrico Gastric Statistics;211966_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.425	5.644	4.41E-07	Cho Gastric Statistics ILMN_1724994
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.515	4.902	1.57E-05	Cho Gastric Statistics ILMN_1724994
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.618	3.618	0.002	Cho Gastric Statistics ILMN_1724994
COL5A1	Gastric Cancer *vs.* Normal	3.946	7.332	5.77E-07	Wang Gastric Statistics;212488_at
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.981	6.907	1.45E-08	DErrico Gastric Statistics;203325_s_at
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.714	6.924	7.43E-04	DErrico Gastric Statistics;212488_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.625	3.883	0.005	DErrico Gastric Statistics;212488_at
COL5A2	Gastric Cancer *vs.* Normal	2.294	8.708	2.52E-15	Cui Gastric Statistics;2591643
	Gastric Cancer *vs.* Normal	3.287	5.94	2.89E-06	Wang Gastric Statistics;221730_at
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	3.534	12.611	2.05E-17	Chen Gastric Statistics;IMAGE429203
	Diffuse Gastric Adenocarcinoma *vs.* Normal	3.589	7.761	4.05E-07	Chen Gastric Statistics;IMAGE429203
	Gastric Mixed Adenocarcinoma *vs.* Normal	4.988	7.028	4.00E-05	Chen Gastric Statistics;IMAGE429203
	Diffuse Gastric Adenocarcinoma *vs.* Normal	3.393	5.78	3.98E-07	Cho Gastric Statistics;ILMN_1729117
	Gastric Adenocarcinoma *vs.* Normal	3.03	3.59	0.007	Cho Gastric Statistics;ILMN_1729117
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.54	3.819	2.53E-04	Cho Gastric Statistics;ILMN_1729117
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.166	3.229	0.002	Cho Gastric Statistics;ILMN_1729117
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	3.77	7.502	8.64E-09	DErrico Gastric Statistics;221730_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.885	4.174	0.003	DErrico Gastric Statistics;221730_at
COL6A2	Gastric Cancer *vs.* Normal	2.819	6.496	5.69E-07	Wang Gastric Statistics;209156_s_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.668	4.98	7.94E-04	DErrico Gastric Statistics;209156_s_at
COL6A3	Gastric Cancer *vs.* Normal	5.087	7.295	6.06E-08	Wang Gastric Statistics;201438_at
	Gastric Mixed Adenocarcinoma *vs.* Normal	5.37	16.537	1.25E-13	DErrico Gastric Statistics;201438_at
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	3.92	8.91	9.71E-12	DErrico Gastric Statistics;201438_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	4.619	6.311	2.79E-04	DErrico Gastric Statistics;201438_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	3.409	8.89	9.13E-12	Cho Gastric Statistics;ILMN_1706643
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.819	5.326	8.78E-06	Cho Gastric Statistics;ILMN_1706643
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.583	3.174	0.003	Cho Gastric Statistics;ILMN_2307861
	Gastric Mixed Adenocarcinoma *vs.* Normal	4.552	10.63	1.09E-07	Chen Gastric Statistics;IMAGE:138991
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	3.131	11.751	4.40E-19	Chen Gastric Statistics;IMAGE:138991
	Diffuse Gastric Adenocarcinoma *vs.* Normal	4.647	9.783	5.85E-09	Chen Gastric Statistics;IMAGE:138991
	Gastric Cancer *vs.* Normal	2.021	5.82	1.60E-08	Cui Gastric Statistics;2605321
COL8A1	Diffuse Gastric Adenocarcinoma *vs.* Normal	5.2	9.378	3.63E-12	Cho Gastric Statistics;ILMN_2402392
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	4.754	7.45	4.92E-08	Cho Gastric Statistics;ILMN_1685433
	Gastric Adenocarcinoma *vs.* Normal	6.156	5.203	0.005	Cho Gastric Statistics;ILMN_1685433
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.759	4.424	4.73E-04	Cho Gastric Statistics；ILMN_2402392
	Gastric Cancer *vs.* Normal	2.735	6.252	1.81E-09	Cui Gastric Statistics;2633390
	Gastric Cancer *vs.* Normal	5.094	4.553	7.70E-05	Wang Gastric Statistics;214589_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.055	3.461	0.001	DErrico Gastric Statistics;221152_at
COL17A1	Gastric Mixed Adenocarcinoma *vs.* Normal	-3.494	-6.925	9.86E-08	Chen Gastric Statistics;IMAGE:252259
	Diffuse Gastric Adenocarcinoma *vs.* Normal	-2.167	-4.414	7.09E-05	Chen Gastric Statistics;IMAGE:501981
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	-2.447	-5.491	1.16E-06	Chen Gastric Statistics;IMAGE:252259
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	-2.469	-2.936	0.002	DErrico Gastric Statistics;204636_at
COL18A1	Gastric Cancer *vs.* Normal	3.074	7.036	1.14E-07	Wang Gastric Statistics;209081_s_at
	Gastric Mixed Adenocarcinoma *vs.* Normal	2.233	7.572	3.30E-05	Chen Gastric Statistics;IMAGE:301061
	Gastric Intestinal Type Adenocarcinoma *vs.* Normal	2.111	8.088	6.14E-11	DErrico Gastric Statistics;209081_s_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.155	4.947	0.001	DErrico Gastric Statistics;209082_s_at
	Diffuse Gastric Adenocarcinoma *vs.* Normal	2.118	4.757	1.02E-05	Cho Gastric Statistics;ILMN_1806733

Notes: P value was analyzed using the *t*-test. Reporters of the datasets meeting the threshold was shown.

Table S3 The relationship between DNA methylation and mRNA expression in the collagen gene members of gastric cancer patients (MethHC)

Gene name	*COL1A1*	*COL1A2*	*COL3A1*	*COL4A1*	*COL4A2*	*COL5A1*	*COL5A2*	*COL6A2*	*COL6A3*	*COL8A1*	*COL17A1*	*COL18A1*
R value	−0.0634	−0.162	0.0552	−0.114	−0.24	−0.157	0.0779	−0.128	0.0166	−0.259	−0.262	−0.00749
P value	0	0	3.33E-16	0	0	0	0	0.341	0	3.33E-16	0.259	0

Table S4 The prognostic values of collagen isoforms in different subtypes of gastric cancer patients (Kaplan-Meier plotter)

Collagen family	Lauren classification	OS				PPS				FP			
		Cases	HR	95% CI	P value	Cases	HR	95% CI	P value	Cases	HR	95% CI	P value
COL1A1	Intestinal	320	2.08	1.43–3.02	8.70E-05	192	2.27	1.39–3.71	0.0007	263	1.97	1.33–2.92	0.00052
	Diffuse	241	1.52	1.05–2.21	0.026	176	1.86	1.26–2.74	0.0016	231	1.56	1.07–2.29	0.0202
	Mixed	32	0.62	0.2–1.97	0.417	16	–	–	–	28	0.32	0.06–1.74	0.1643
COL1A2	Intestinal	320	1.79	1.31–2.46	0.00026	192	1.6	1.04–2.47	0.032	263	1.98	1.38–2.82	0.00014
	Diffuse	241	1.71	1.21–2.42	0.002	176	2.13	1.44–3.16	0.00011	231	1.64	1.16–2.32	0.005
	Mixed	32	0.4	0.11–1.47	0.154	16	–	–	–	28	0.63	0.21–1.86	0.3955
COL3A1	Intestinal	320	1.49	1.09–2.05	0.0123	192	2.02	1.34–3.06	0.0006	263	1.93	1.35–2.77	0.00028
	Diffuse	241	1.42	1.01–1.99	0.045	176	2.02	1.38–2.96	0.00022	231	1.47	1.04-2.08	0.029
	Mixed	32	2.82	1–7.99	0.042	16	–	–	–	28	1.53	0.57–4.15	0.3988
COL4A1	Intestinal	320	1.63	1.15–2.32	0.0056	192	1.76	1.15–2.69	0.0082	263	2	1.36–2.95	0.00033
	Diffuse	241	2.08	1.4–3.1	0.00022	176	1.99	1.26–3.12	0.0024	231	2.17	1.5–3.16	2.90E-05
	Mixed	32	1.97	0.55–7.09	0.2922	16	–	–	–	28	2.21	0.79–6.17	0.1222
COL4A2	Intestinal	320	2.58	1.81–3.67	5.60E-08	192	2.89	1.91–4.37	1.60E-07	263	2.38	1.64–3.46	2.50E-06
	Diffuse	241	2.45	1.72–3.49	3.40E-07	176	2.63	1.77–3.89	5.60E-07	231	2.7	1.83–3.98	2.10E-07
	Mixed	32	2.2	0.49–9.93	0.29	16	–	–	–	28	2.66	0.6–11.81	0.1811
COL5A1	Intestinal	320	2.55	1.85–3.5	2.50E-09	192	2.79	1.85–4.22	3.80E-07	263	2.25	1.58–3.2	3.30E-06
	Diffuse	241	1.86	1.31–2.64	0.00043	176	2.46	1.67–3.67	2.60E-06	231	1.82	1.27–2.58	0.00083
	Mixed	32	3.83	1.35–10.87	0.0067	16	–	–	–	28	2.33	0.86–6.35	0.0892
COL5A2	Intestinal	320	1.49	1.07–2.08	0.0167	192	1.34	0.89–2.02	0.1596	263	1.51	1.04–2.18	0.029
	Diffuse	241	1.26	0.89–1.79	0.1984	176	1.54	1.03–2.32	0.036	231	1.31	0.93–1.85	0.118
	Mixed	32	2.45	0.84–7.12	0.0886	16	–	–	–	28	4.83	1.46–15.94	0.0048
COL6A2	Intestinal	320	2.78	1.98–3.91	8.10E-10	192	3.54	2.33–5.39	3.50E-10	263	2.17	1.52–3.09	1.40E-05
	Diffuse	241	2.13	1.48–3.06	3.10E-05	176	2.51	1.7–3.72	2.00E-06	231	2.08	1.43–3.01	8.00E-05
	Mixed	32	3.58	1.26–10.22	0.0111	16	–	–	–	28	0.52	0.16–1.65	0.2597
COL6A3	Intestinal	320	2.26	1.65–3.1	1.90E-07	192	2.51	1.67–3.79	5.40E-06	263	1.95	1.37–2.79	0.0002
	Diffuse	241	1.43	1–2.03	0.048	176	1.92	1.29–2.86	0.0011	231	1.56	1.06–2.31	0.024
	Mixed	32	1.77	0.62–5.03	0.279	16	–	–	–	28	1.71	0.61–4.73	0.3001
COL8A1	Intestinal	320	1.94	1.38–2.72	8.80E-05	192	2.19	1.43–3.35	0.0002	263	1.57	0.99–2.48	0.054
	Diffuse	241	1.31	0.93–1.86	0.13	176	2.21	1.5–3.25	3.90E-05	231	1.31	0.93–1.86	0.1209
	Mixed	32	0.39	0.13–1.25	0.1012	16	–	–	–	28	0.4	0.14–1.1	0.0674
COL17A1	Intestinal	320	0.69	0.5–0.94	0.0181	192	1.27	0.83–1.95	0.2725	263	0.71	0.5–1	0.051
	Diffuse	241	0.72	0.47–1.09	0.1189	176	1.28	0.83–1.98	0.26	231	0.69	0.46–1.05	0.0815
	Mixed	32	2.84	0.94–8.53	0.053	16	–	–	–	28	0.63	0.21–1.87	0.4016
COL18A1	Intestinal	320	2.76	2.01–3.8	7.90E-11	192	3.14	2.08–4.76	1.20E-08	263	2.3	1.61–3.3	2.90E-06
	Diffuse	241	1.76	1.22–2.53	0.002	176	1.97	1.34–2.89	0.00042	231	1.75	1.21–2.54	0.0025
	Mixed	32	2.59	0.81–8.31	0.0967	16	–	–	–	28	2/36	0.84–6.6	0.0935

Notes: P value was analyzed using the survival analysis test. OS, overall survival; PPS, post-progression survival; FP, first progression; HR, hazard ratio.

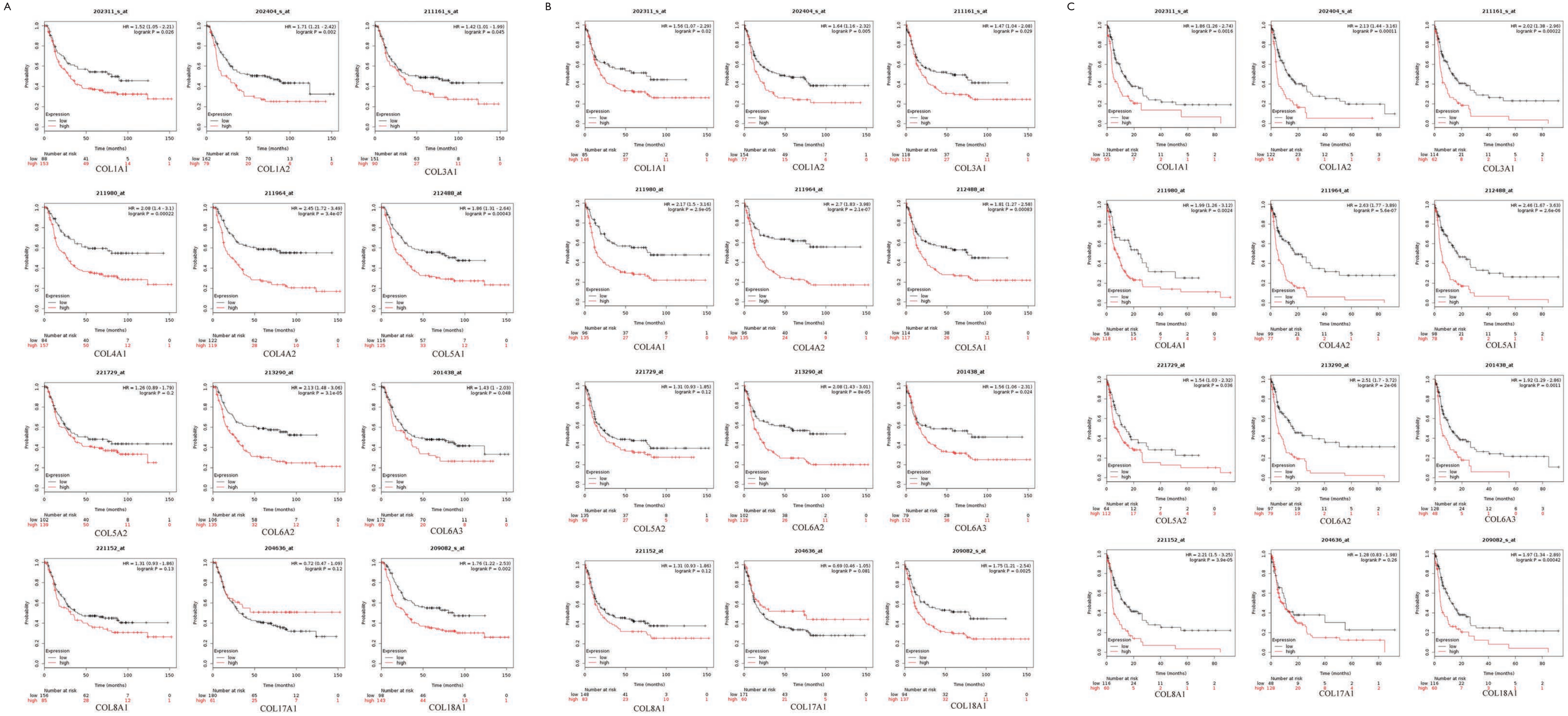

Figure S1 Different mRNA level of collagens' prognostic values in diffuse subtype gastric cancer patients (Kaplan-Meier plotter). Notes: Kaplan-Meier plots show the relationship between OS (A), FP (B) and PPS (C) and the expression of collagens in gastric cancer patients, respectively, with hazard ratio (HR) and statistical significance.

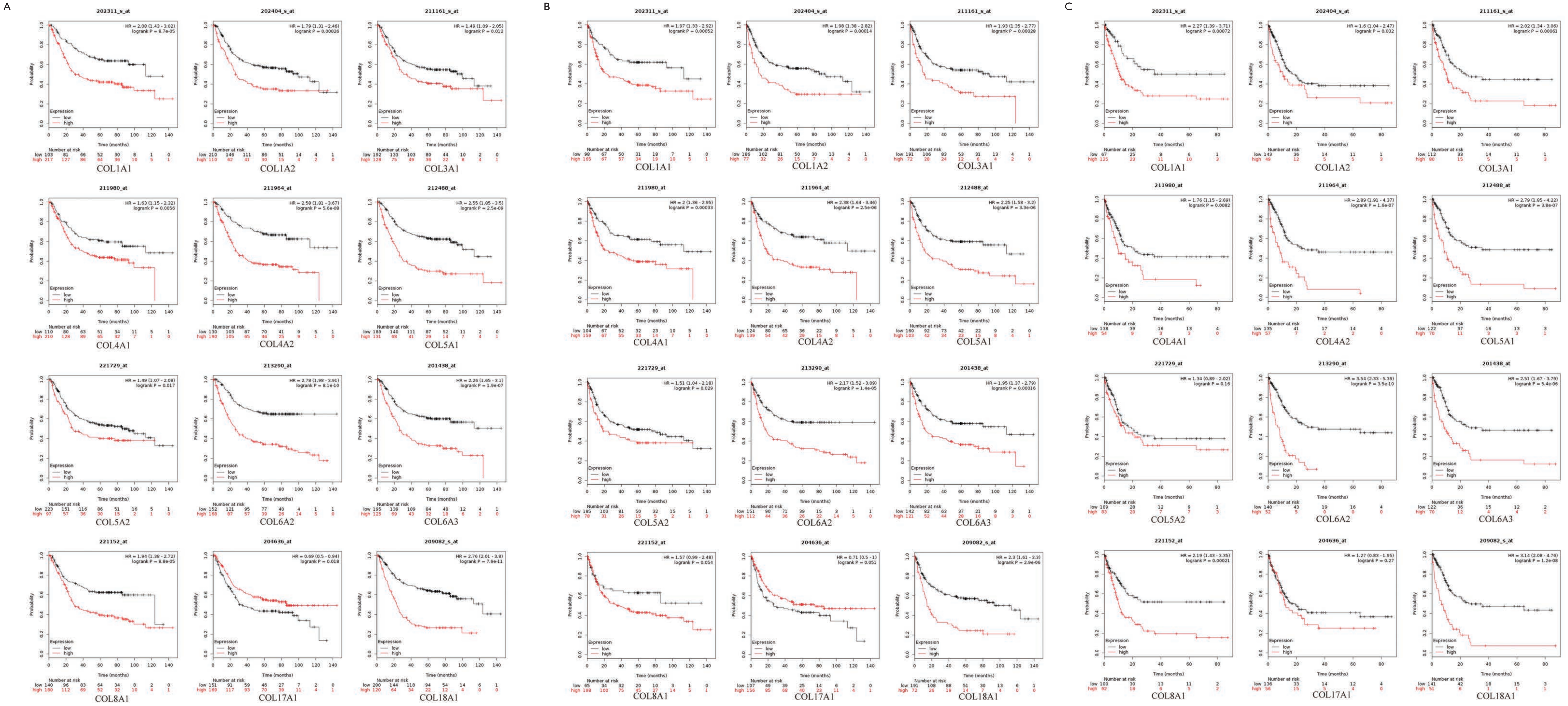

Figure S2 Different mRNA level of collagens' prognostic values in intestinal subtype gastric cancer patients (Kaplan-Meier plotter). Notes: Kaplan-Meier plots show the relationship between OS (A), FP (B) and PPS (C) and the expression of collagens in gastric cancer patients, respectively, with hazard ratio (HR) and statistical significance.

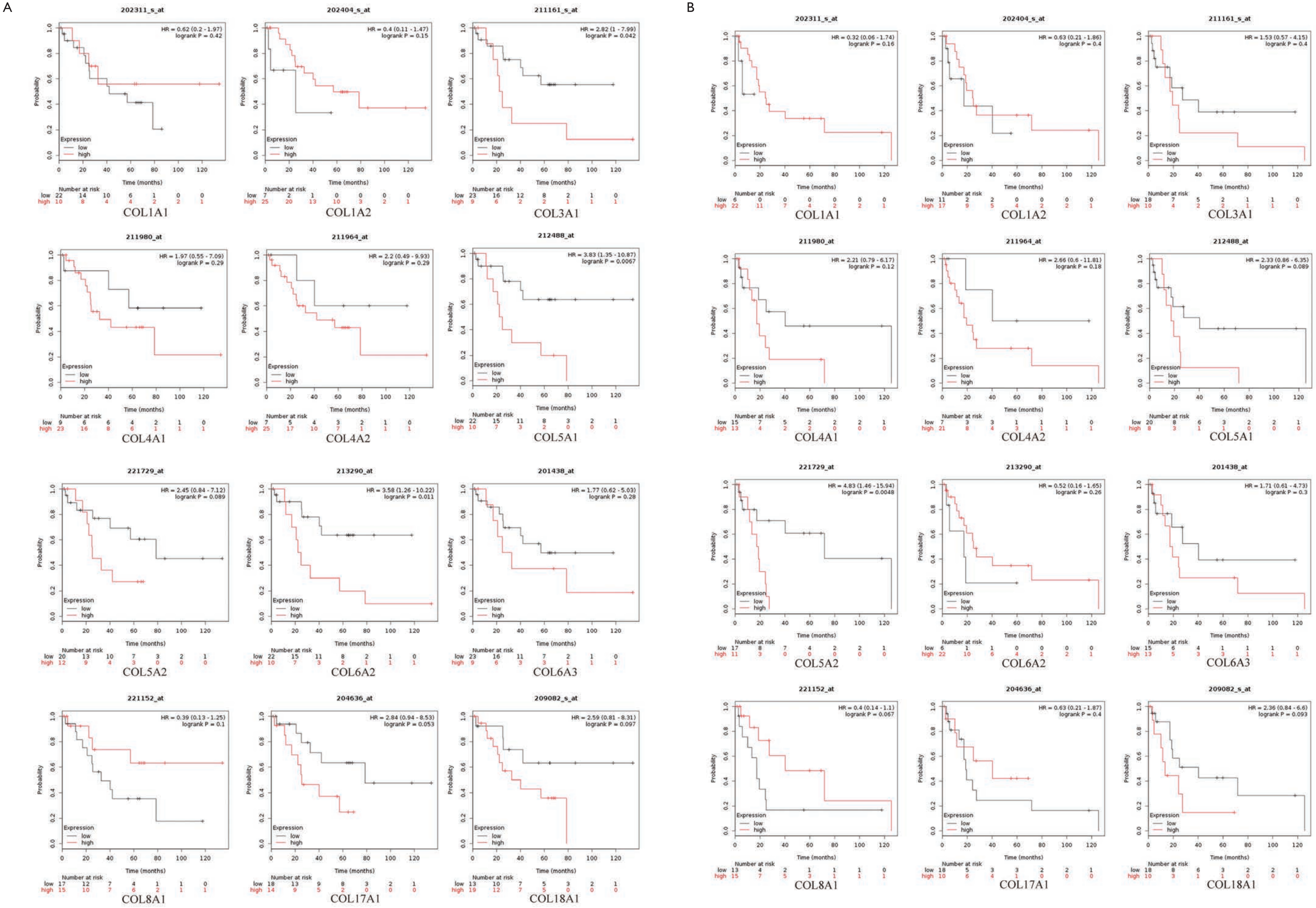

Figure S3 Different mRNA level of collagens' prognostic values in mixed subtype gastric cancer patients (Kaplan-Meier plotter). Notes: Kaplan-Meier plots show the relationship between OS (A), FP (B) and PPS (C) and the expression of collagens in gastric cancer patients, respectively, with hazard ratio (HR) and statistical significance.