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Martrilin-3 (MATN3) Overexpression in Gastric Adenocarcinoma and its Prognostic Significance

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Statistical Analysis C
Data Interpretation D
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Background: The aim of this study was to investigate the expression level of martrilin-3 (MATN3) in patients with gastric adenocarcinoma (GAC) and to investigate the prognostic significance of MATN3.





Material/Methods: Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) data were used to predict the expression and prognostic value of MATN3 mRNA in GAC patients. Seventy-six GAC patients had GAC tissue samples and paired adjacent normal tissue samples collected retrospectively to examine the MATN3 protein expression level by immunohistochemical staining. Furthermore, Kaplan-Meier univariate and Cox multivariate analyses were used to verify the correlation between MATN3 expression and clinicopathological parameters of GAC patients and the prognostic significance of MATN3.

Results: The GEO and TCGA data predicted that MATN3 mRNA levels were significantly higher in GAC tissue compared to normal tissue (all $p < 0.05$). Further survival analyses showed that GAC patients with high mRNA expression of MATN3 had significantly lower disease-free survival (DFS) and overall survival (OS) time than those with low mRNA expression of MATN3 (all $p < 0.05$). Subsequent immunohistochemical staining results confirmed that the MATN3 protein levels in GAC tissues were highly expressed ($p = 0.000$) compared to normal tissues. In addition, GAC patients with high protein expression of MATN3 had remarkably decreased OS compared to patients with low protein expression of MATN3 ($p = 0.000$). Univariate and multivariate survival analyses revealed that MATN3 high expression could be used as an independent predictor of poor prognosis in GAC patients (all $p = 0.000$).

Conclusions: This study confirmed that MATN3 protein was highly expressed in GAC patients, and MATN3 overexpression could be used as an independent predictor of poor prognosis in GAC patients.

MeSH Keywords: **Matrilin Proteins • Prognosis • Stomach Neoplasms**

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Background

Gastric cancer is one of the most common malignant tumors of the digestive tract [1]; gastric adenocarcinoma (GAC) is the main pathologic type. It has the characteristics of easy invasion and metastasis, and a very low early diagnosis rate [2]. Despite the gradual decline in incidence and mortality of gastric cancer in recent years, the prognosis of patients with gastric cancer has not been significantly improved [3]. Thus, to explore biomarkers that can aid in the prognosis of gastric cancer not only would help clinicians predict prognosis and establish individualized treatment plans, but also would help clarify the mechanism of gastric carcinogenesis and development, which has important significance for formulating the treatment strategy of gastric cancer.

MATN3 (*Matrilin-3*), also named *DIPPOA*, *OADIP*, or *EDM5*, is a protein coding gene, which encodes a member of von Willebrand factor A domain containing protein family [4,5]. This family of proteins is thought to be involved in the formation of filamentous networks in the extracellular matrices of various tissues. Previous studies have found that *MATN3* protein is present in the cartilage extracellular matrix and has a role in the development and homeostasis of cartilage and bone [6]. Mutations in this gene result in multiple epiphyseal dysplasia [7,8]. Until now, the expression level of *MATN3* in malignant tumors and their relationship, especially in GAC, have remained unclear.

Therefore, in this study, bioinformatics prediction, selecting both the Gene Expression Omnibus (GEO) and The Cancer Genome

Table 1. Correlation of *MATN3* with clinicopathological parameters of GAC patients.

Clinicopathological parameters	Cases (N)	MATN3 expression level		χ^2	P value
		Low	High		
Age at surgery (years)					
≤60	26	13	13	4.378	0.036
>60	50	13	37		
Gender					
Male	60	19	41	0.819	0.365
Female	16	7	9		
Tumor site					
Antrum	33	16	17	5.280	0.022
Other sites	43	10	33		
Tumor size (cm)					
≤5	38	19	19	8.418	0.004
>5	38	7	31		
Histological grade					
Well/moderate	38	17	21	3.742	0.053
Poor	38	9	29		
T stage					
T1–2	16	9	7	4.374	0.036
T3–4	60	17	43		
N stage					
N0	22	11	11	3.430	0.064
N1–3	54	15	39		
TNM stage					
I–II	34	16	18	4.513	0.034
III–IV	42	10	32		

Atlas (TCGA) data, combined with immunohistochemical validation was used to examine the expression level of MATN3 in GAC and its prognostic value was further investigated by Kaplan-Meier and Cox survival analyses.

Material and Methods

Bioinformatics prediction

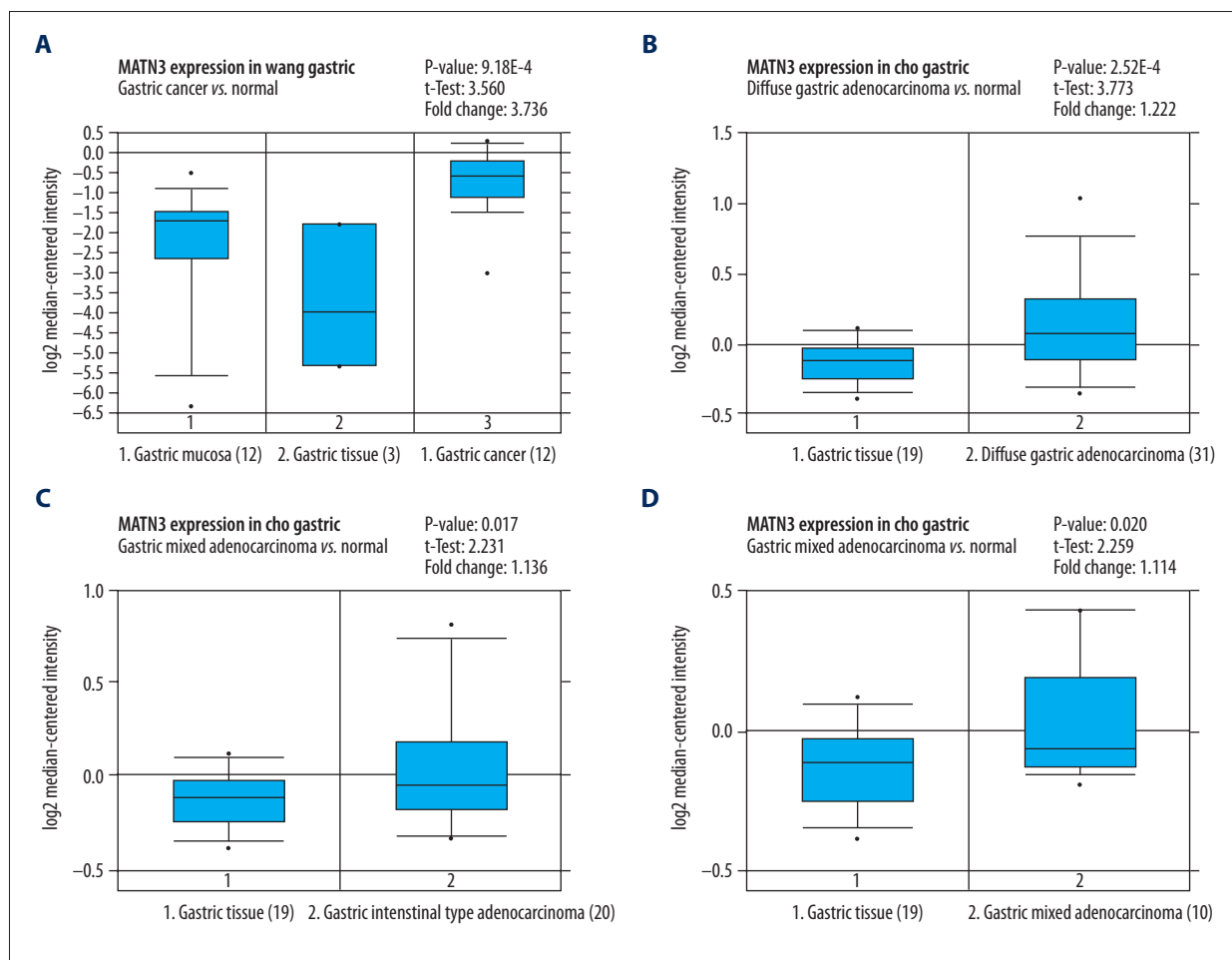
Cancer related public databases Oncomine and TCGA were used for bioinformatics prediction. In the Oncomine database, we entered the gene name “MATN3” and choose the “Differential Analysis” module (gastric cancer versus normal). Gastric cancer related data from the TCGA database was also downloaded to analyze the differential expression level of MATN3 between gastric cancer tissue and normal tissue. Disease-free survival (DFS) and overall survival (OS) curves were drawn based on both TCGA and GEO data (the latter was analyzed in Kaplan-Meier Plotter website, <http://kmplot.com/analysis/index.php?p=service&cancer=gastric>).

GAC tissue samples and corresponding clinicopathological data

Seventy-six cases of tumor tissue samples and paired adjacent normal tissues were retrospectively collected from GAC patients who had received radical surgery from December 2009 to June 2010. Patient-related clinical information, including age, gender, tumor size, histologic grade, and TNM stage are summarized in Table 1. This study was approved by the Ethics Committee of Anhui Provincial Hospital and all patients signed the informed consent.

Immunohistochemical staining and interpretation of the results

Immunohistochemistry was performed with the MATN3 antibody (ab106388, Abcam, UK) at a dilution of 1: 100 based on the commercial protocol. The results were judged and evaluated by two pathologists using a double-blind method. Based on a previous confirmation method [9], an immunoreactive score (IRS) for each case was calculated. The IRS ranged from 0–12, which was generated by staining intensity (SI) x number of stained cells (PP).



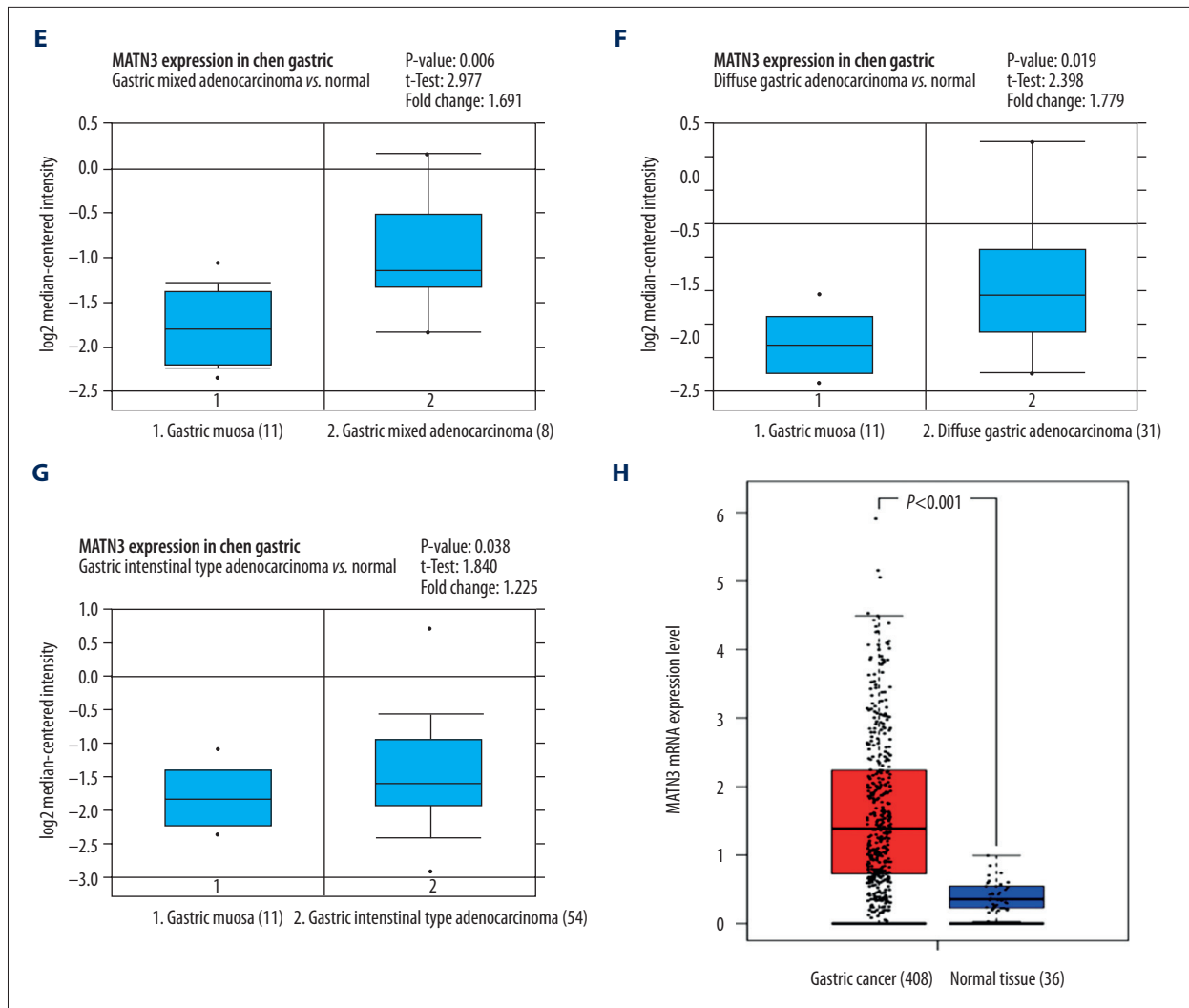


Figure 1. MATN3 mRNA overexpression in gastric adenocarcinoma predicted based on the GEO and TCGA data. MATN3 mRNA levels in (A) Wang Gastric (GEO: GSE19826), (B–D) Cho Gastric (GEO: GSE13861), (E–G) Chen Gastric (Oncomine Reporter: IMAGE119728), and (H) TCGA database grouped by gastric cancer versus gastric muosa or gastric tissue.

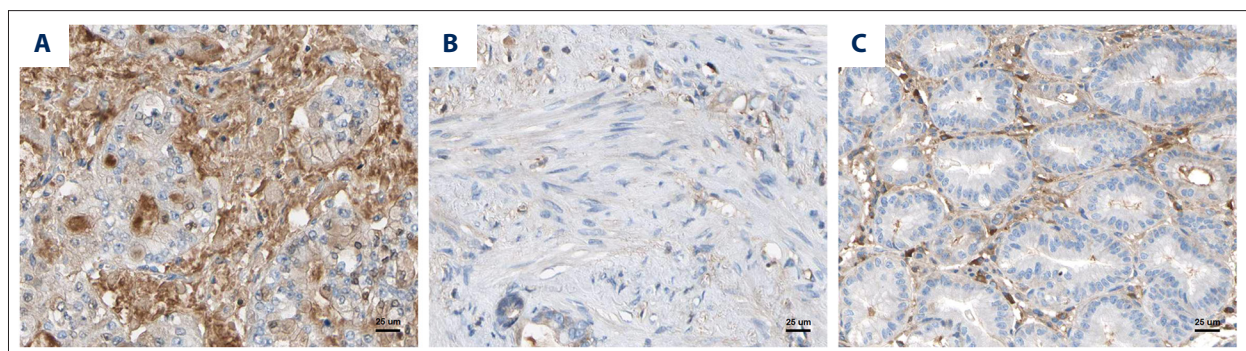


Figure 2. Representative immunohistochemical images of MATN3 in 76 cases of paired GAC and adjacent normal tissues. (A) High expression of MATN3 in GAC tissue; (B) low expression of MATN3 in GAC tissue; (C) low expression of MATN3 in paired adjacent normal tissue. Bar=25 um.

Table 2. MATN3 overexpression in 76 cases of GAC compared to the paired adjacent normal tissues.

Samples	MATN3 expression level (Cases, %)		χ^2	P
	Low	High		
GAC tissues	26 (34.2)	50 (65.8)	17.802	0.000
Adjacent normal tissues	52 (68.4)	24 (31.6)		

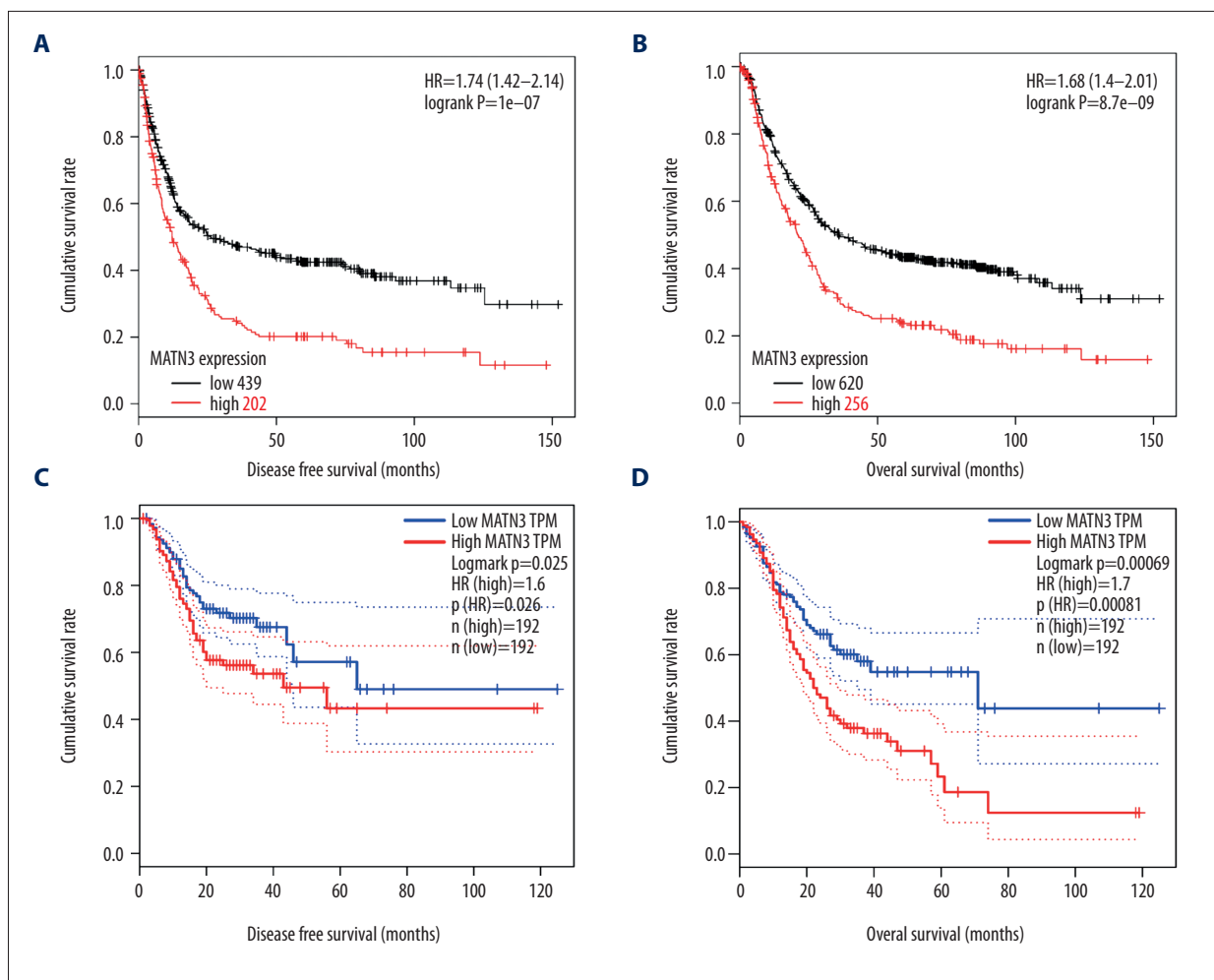


Figure 3. Kaplan-Meier analysis of disease-free survival (DFS) and overall survival (OS) curves of GAC patients based on MATN3 mRNA expression (high versus low). **(A)** Relationship between MATN3 mRNA expression level and DFS of GAC patients based on GEO data; **(B)** relationship between MATN3 mRNA expression level and OS of GAC patients based on GEO data; **(C)** relationship between MATN3 mRNA expression level and DFS of GAC patients based on TCGA data; **(D)** relationship between MATN3 mRNA expression level and OS of GAC patients based on TCGA data.

If IRS >4 was founds, MATN3 high expression was defined and if IRS ≤4 score was found, MATN3 low expression was defined.

Statistical analysis

SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) was performed to analyze the data. Quantitative data were expressed as mean

± standard deviation (SD). The association between MATN3 expression and clinicopathological parameters was analyzed by chi-square test. Survival analysis was done by the Kaplan-Meier method and the log-rank test. Cox regression model was established for the multivariate survival analysis to determine prognostic factors that were significant in the univariate analysis. A p value less than 0.05 was considered to be statistically significant.

Table 3. Kaplan-Meier survival analysis of MATN3 and other clinicopathological parameters in GAC patients.

clinicopathological parameters	Mean survival time (months)	95% CI	P value
MATN3 expression			
Low	71.538	65.240–77.837	0.000
High	25.100	18.695–31.505	
Age at surgery (years)			
≤60	50.231	38.460–62.001	0.045
>60	36.180	28.069–44.291	
Gender			
Male	39.417	31.930–46.903	0.372
Female	46.875	30.740–63.010	
Tumor site			
Antrum	49.333	38.923–59.744	0.029
Other sites	34.581	25.959–43.204	
Tumor size (cm)			
≤5	56.211	46.874–65.547	0.000
>5	25.763	18.435–33.092	
Histological grade			
Well/moderate	50.579	41.075–60.083	0.009
Poor	31.395	22.517–40.273	
T stage			
T1–2	64.438	53.201–75.674	0.003
T3–4	34.733	27.355–42.111	
N stage			
N0	56.500	44.389–68.611	0.004
N1–3	34.667	26.998–42.335	
TNM stage			
I–II	57.029	47.549–66.510	0.000
III–IV	28.000	20.235–35.765	

Results

Overexpression of MATN3 mRNA and protein levels in GAC

First, GEO and TCGA data were used to predict the MATN3 mRNA expression levels in GAC and normal tissues. Compared to normal tissue, the expression level of MATN3 mRNA was dramatically higher in GAC tissue (all *p* values <0.05, Figure 1A–1H). To confirm the study predictive findings, immunohistochemical assays were used to examine 76 cases of GAC and paired adjacent tissues (Figure 2A–2C). The results showed that MATN3 protein was mainly positively located in cytoplasm and extracellular matrix of GAC cells (Figure 2A). The expression level

of MATN3 protein in GAC tissue was significantly higher than that in the paired normal tissue ($\chi^2=17.802$, *p*=0.000; Table 2).

Correlation between MATN3 protein differential expression and clinicopathological factors of GAC patients

The correlation between MATN3 protein differential expression and clinicopathological parameters of GAC patients was investigated, including age, gender, tumor site, tumor size, histological grade, T stage, N stage, and TNM stage. As shown in Table 1, MATN3 protein differential expression levels were significantly associated with age, tumor site, tumor size, T stage, and TNM stage of GAC patients, respectively (all *p* values <0.05).

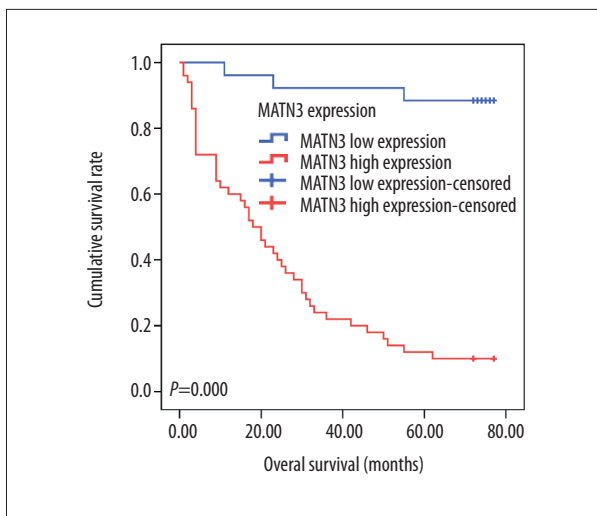


Figure 4. Kaplan-Meier analysis of overall survival (OS) curve of GAC patients based on MATN3 protein expression (low versus high) using our immunohistochemical data.

Prognostic value of MATN3 expression in GAC patients

Finally, we also explored the relationship between the differential expression of MATN3 gene and the prognosis of GAC patients. The results based on the GEO and TCGA data showed that both of DFS and OS of GAC patients with high expression of MATN3 mRNA was significantly lower than those with high expression of MATN3 mRNA (all *p* values <0.05, Figure 3A–3D). To verify these findings, we subsequently conducted a statistical analysis on the results of immunohistochemistry and found that the OS of GAC patients with high expression of MATN3 protein was significantly lower than that of patients with low expression (*p*=0.000, Figure 4). Besides, as shown in Table 3, Kaplan-Meier univariate survival analysis revealed that MATN3 expression (low versus high), age at surgery (≤60 versus >60 years), tumor site (antrum versus other sites), tumor size (≤5 versus >5 cm), histological grade (well/moderate versus poor), T stage (T1–2 versus T3–4), N stage (N0 versus N1–3), and TNM

stage (stage I–II versus III–IV) were the significant factors influencing the survival time of GAC patients. Then, these eight significant single factors were substituted into the Cox multivariate survival analysis and the findings confirmed that MATN3 high expression level was the only independent factor to predict the poor prognosis of GAC patients (*p*=0.000, Table 4).

Discussion

MATN3, as a member of the matrilin protein family, is a non-collagenous extracellular matrix. It has attracted extensive attention in recent years, especially in bone and cartilage related fields [10]. According to previous reports [6,11,12], it is mainly distributed in cartilage around the whole body, such as articular cartilage, costal cartilage, and tracheal cartilage. In primary human chondrocytes, MATN3 can induce the expression of MMP1, MMP3, MMP13, proinflammatory cytokines (TNFα, IL-1β, IL-6, and IL-8), iNOS, and COX2, indicating that MATN3 can regulate extracellular matrix degradation [12]. The mutation of MATN3 gene may lead to some diseases [13,14] such as multiple epiphyseal dysplasia (MED), epiphyseal dysplasia (BHMED), and epiphyseal dysplasia of the vertebral body (SEMD). So far, although many studies about MATN3 have been limited to epiphyseal disease, few studies have been reported on other conditions such as malignant tumors. Therefore, in the present study, the expression of MATN3 gene in GAC and its prognostic significance were explored for the first time.

We initially performed bioinformatics to predict the high expression of MATN3 gene in GAC tissues by using the GEO and TCGA public data. Then, immunohistochemistry was used to testify that MATN3 protein expression level was significantly higher in the GAC tissue group compared to the normal control group and the difference was statistically significant. These results were consistent with those of bioinformatics predictions. These findings also suggested that the MATN3 gene might play a role in promoting the occurrence and development of GAC acting as an oncogene.

Table 4. Cox multivariate analysis of MATN3 and other clinicopathological parameters in GAC patients.

Covariates	HR	95% CI for HR	P value
MATN3 expression level (low vs. high)	17.800	5.061–62.603	0.000
Age at surgery (≤60 vs. >60 years)	1.915	0.917–3.997	0.084
Tumor site (antrum vs. other sites)	1.040	0.543–1.993	0.906
Tumor size (≤5 vs. >5 cm)	1.726	0.761–3.916	0.192
Histological grade (well/moderate vs. poor)	1.367	0.683–2.734	0.377
T stage (T1–2 vs. T3–4)	1.718	0.518–5.698	0.377
N stage (N0 vs. N1–3)	1.131	0.417–3.070	0.808
TNM stage (I–II vs. III–IV)	2.187	0.794–6.027	0.130

Subsequently, the clinical prognostic significance of MATN3 gene expression levels in GAC patients was examined. First, MATN3 protein differential expression levels were significantly associated with age, tumor site, tumor size, T stage, and TNM stage of GAC patients, respectively. Second, Kaplan-Meier univariate survival analysis showed that GAC patients with MATN3 mRNA and protein high levels had remarkably shorter OS time than those with MATN3 low levels. Then, Cox multivariate analysis determined that MATN3 high expression level was an independent factor to predict unfavorable prognosis of GAC patients. These confirmatory results, especially MATN3 overexpression in GAC and its prognostic significance in OS of GAC patients, were consistent with those deduced by GEO and TCGA data. Together, all these findings suggested that high expression of MATN3 could indicate a poor prognosis for GAC patients, and *MATN3* might be a pivotal target gene involved in the process of GAC cell growth and metastasis.

Nevertheless, there were still some limitations to our study. First, due to the lack of large numbers of tumor samples, the study data results need to be validated in the future. Second, DFS data was not accessible in our department, so we could not verify the predictive results deduced by GEO and TCGA data. Third, only immunohistochemical staining, a semi-quantitative method, was used to validate the results of the bioinformatics

prediction. In future studies, western blot or real-time PCR will need to be added to confirm the predictive results further. Lastly, the detailed underlying molecular mechanisms were not unraveled. Thus, this will need to be more deeply examined in future studies.

Conclusions

This study revealed that the MATN3 gene was overexpressed in GAC, and overexpression of MATN3 can be used as an independent predictor to indicate the poor prognosis of GAC patients. In the future, MATN3 is expected to be a new potential therapeutic target for the treatment of GAC patients.

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Conflicts of interest

None.

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