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*Original Article*

# Expression status of cathepsin B may as a prognostic marker for human gastric carcinoma

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**Abstract:** Cathepsin B (CTSB) is reported up-regulated in various tumors. However, data on its expression pattern and clinical relevance in gastric carcinoma are unknown. The aim of this study is to investigate CTSB expression and its prognostic significance in gastric carcinoma. The CTSB expression at mRNA and protein levels was examined by real-time quantitative polymerase chain reaction (RT-PCR) and Western blotting in 10 paired gastric carcinoma and adjacent normal tissues. CTSB protein expression was analyzed in paraffin-embedded gastric carcinoma samples and adjacent non-tumor tissues by immunohistochemistry (IHC). Statistical analyses were also performed to evaluate the clinicopathological significance of CTSB expression. The result shows the expression of CTSB mRNA and protein was higher in gastric carcinoma than in the adjacent normal tissues in 10 paired samples. In paraffin-embedded tissue samples, the expression of CTSB was higher in gastric carcinoma than the adjacent non-cancerous tissues. Compared with adjacent non-cancerous tissues, overexpression of CTSB was detected in 49.06% (52/106) patients. Overexpression of CTSB was significantly associated with age ($P = 0.033$), T Stage ($P = 0.001$), N Stage ($P < 0.001$), TNM Stage ($P < 0.001$), tumor size ($P < 0.001$), and decreased overall survival ($P < 0.001$). In multivariate analysis, expression of CTSB was an independent prognostic factor for OS (overall survival). CTSB is up-regulated in gastric carcinoma and associated with expression of age, T Stage, N Stage, TNM Stage, tumor size and survival. CTSB may serve as a prognostic indicator for patients with gastric carcinoma.

**Keywords:** Gastric carcinoma, cathepsin B, overexpression, prognosis

## Introduction

As one of most common malignant carcinomas, gastric carcinoma (GC) is the fourth popular cancer and the second leading cause of cancer related death worldwide, although exhibiting an decreasing trend of incidence [1, 2]. Despite the progression in diagnosis and treatment over the past few decades, the prognosis of patients with gastric carcinoma remains poor. Due to occult symptoms at early stages, a substantial proportion of gastric cancer patients are already at advanced stages at the time of first diagnosis, causing unfavorable prognosis. Therefore, the early diagnosis and treatment of gastric carcinoma is of critical importance for improving clinical outcomes [3, 4]. CTSB, a lysosomal cysteine protease, has been shown to be an important contributor to the progression and invasion of different types of cancer [5]. In normal cells, CTSB is associated with cell surface caveolae, the specialized membrane microdomains which are involved in endocytosis, proteolysis and signaling pathways [6, 7]. Specifically, CTSB is involved in proteolytic pathways which lead to the degradation of extracellular matrix (ECM) proteins thereby promoting cancer cells' motility and invasion [5, 8]. Increased expression of CTSB has been observed in various tumors including gliomas [9], breast cancer [10, 11], lung tumors [12], melanomas [13], colorectal cancer [14] and prostate cancer [15]. However, few papers demonstrated that CTSB was over-expressed in gastric carcinoma. To the best of our knowledge, correction of CTSB expression in gastric carcinoma with prognosis

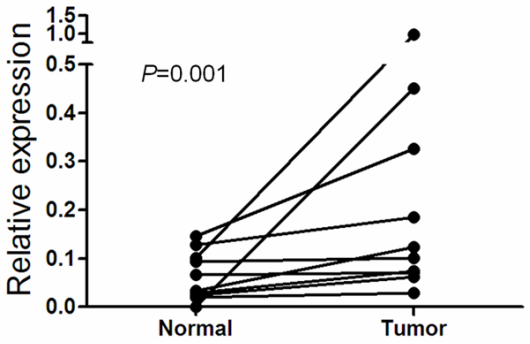


**Figure 1.** Expression levels of *CTSB* mRNA in gastric cancer and adjacent non-cancerous tissues. Expression levels of *CTSB* mRNA in ten paired gastric cancer and adjacent non-cancerous tissues by real-time PCR. Normal, adjacent non-cancerous tissues. Tumor, gastric cancer tissues.

has yet to be determined. In this study, we examined the CTSB expression in gastric carcinoma tissue samples, and revealed its clinicopathological and prognostic significance.

## Materials and methods

### *Patients and tissue specimens*

This study was conducted on a total of 106 paraffin-embedded specimens of gastric carcinoma from the Third Affiliated Hospital of Sun Yat-sen University between January 2001 and December 2004 for immunohistochemical (IHC) assay. The median age of these patients was 54 years (range 29-72 years), the median tumor size was 6.0 cm (range 0.8-15.0 cm). Among these 106 patients, 32 of adjacent non-cancerous tissue were collected as control. All these patients were pathologically diagnosed as gastric adenocarcinoma. None of the patients received any type of neoadjuvant therapy, and all of them underwent a curative surgery. Clinical information of these samples is summarized in **Table 1**. The surgical treatment time of the patients was defined as the initial event of survival analysis, and the time of patient death was defined as the end time; the interval was defined as the survival time of the patients.

In addition, ten paired gastric carcinoma and adjacent normal tissues (the adjacent normal tissue was defined as at least 5-cm distance from the tumor edge) were collected from the Third Affiliated Hospital of Sun Yat-sen University between June 2013 and February 2015 for real-time PCR and Western blot analysis. Tissues were collected immediately after operation.

Clinicopathological classification and staging were determined according to the AJCC (American Joint Committee on Cancer Seventh Edition) criteria. Patient consent to the use of these clinical specimens for research purposes was gained prior and the protocol was approved by the Institutional Research Ethics Committee.

### *Real-time PCR (RT-PCR) analysis*

Total RNA samples were extracted from primary gastric tumor materials using Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Extracted RNA was pretreated with RNase-free DNase. 2 μg RNA of each sample was used for cDNA synthesis. For the PCR amplification of *CTSB* cDNA, an initial amplification step using CTSB-specific primers were performed with a denaturation at 95°C for 10 min, and then followed by 28 denaturation cycles at 95°C for 60 s, then primer annealing at 58°C for 30 s, and then primer extension phase at 72°C for 30 s. On the completion of these cycling steps, a final extension at 72°C for 5 min was carried out before the reaction mixture was stored at 4°C. Then Real-time PCR was performed to determine the fold increase of CTSB mRNA in each of the gastric tumors and paired normal gastric tissue from the same patient. The primer sequences were as follows: CTSB sense 5'-GCAGGCCGGGCACAAC-3', antisense 5'-GGAGGCCCAGAGCTGCCACAT-3'. GAPDH (sense 5'-TGTTGCCATCAATGACCCC-3', antisense 5'-CTCCACGACGTACTCAGC-3') was used as an internal control. The primers were designed by Primer Express v 2.0 software (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control, and all experiments were performed in triplicate.

### *Western blotting analysis*

Cells at 70% to 80% confluence were washed twice by ice-cold phosphate-buffered saline (PBS) and then lysed on ice by radio immunoprecipitation assay buffer (RIPA; Cell Signaling Technology, MA) which contained complete protease inhibitor cocktail (Roche Applied Sciences, Germany). Fresh tissue samples were

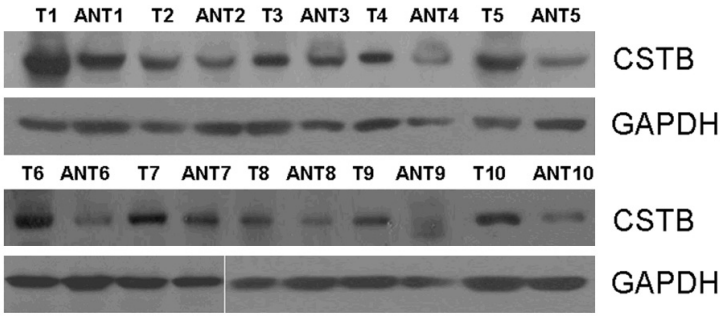


**Figure 2.** CTSB protein expression in 10 paired gastric cancer and adjacent non-cancerous tissues in patients with gastric cancer. Expression levels of CTSB protein in 10 paired gastric cancer and adjacent non-cancerous tissues by Western blotting. T, gastric cancer tissues, ANT, adjacent non-cancerous tissues.

ground into powder in liquid nitrogen and lysed by SDS-PAGE sample buffer. 20 μg protein samples were separated on 10.5% SDS polyacrylamide gels and then transferred to PVDF membranes (Immobilon P, Millipore, MA). Membranes were then blocked with 5% fat-free milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 1 h at room temperature. PVDF membranes were incubated with anti-CTSB antibody (1:1000, proteintech, USA) overnight at 4°C, and then with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, SC-2004). CTSB expression was detected by ECL Western blotting detection reagent (Amersham) according to the manufacturer's instructions. GAPDH (1:1000, Proteintceh, USA) was used as loading control.

*Immunohistochemical analysis*

Immunohistochemical (IHC) staining was performed to study altered protein expression in 106 human gastric cancer tissues, and 32 paired adjacent non-cancerous tissue. Briefly, 4-μm-thick paraffin sections of the tissue were deparaffinized with xylene and rehydrated. Antigenic retrieval was performed by submerging the slides into EDTA antigenic retrieval buffer and microwaving. In order to quench endogenous peroxidase activity, the slides were treated with 3% hydrogen peroxide in methanol, and then incubated with 1% bovine serum albumin to block nonspecific binding. After that, sections were incubated with anti-CTSB mouse monoclonal antibody (1:100, SANTA) at 4°C over-night. Normal goat serum was used as a negative control. The tissue sections were incubated with a biotinylated anti-rabbit secondary anti-body (Abcam) after 3 times washing, and followed by a further incubation with streptavidin-horseradish peroxidase complex (Abcam). Slides were immersed in 3-amino-9-ethyl car-bazole and then counterstained with 10% Mayer's hematoxylin, finally dehydrated and mounted in Crystal Mount.

As for evaluation of immunostaining, the degree of immunostaining was viewed and scored separately by two pathologists, who were blind to the histopathological characteristics and patient information of the samples. Scores given by the two independent pathologists were averaged for further comparative evaluation of CTSB expression. The intensity of CTSB staining was graded according to the following criteria: 0, no staining; 1, weak staining = light yellow; 2, moderate staining = yellow brown; 3, strong staining = brown. The percentage of stained tumor cells was scored as follows: 0, no positive tumor cells; 1, 1-25% positive tumor cells; 2, 26-50% positive tumor cells; 3, 51-75% positive tumor cells; 4, > 75% positive tumor cells.

The staining score was calculated as the product of the proportion of positive tumor cells and the staining intensity score. The expression level of CTSB was defined as follows: "-" (score 0, negative), "+" (score 1-4, weakly positive), "++" (score 5-8, positive), "+++" (score 9-12, strongly positive). Cut-off values for CTSB were chosen on the basis of the heterogeneity using log-rank test with respect to overall survival (OS). The optimal cut-off value was estimated as follows: a staining index score of $\geq 4$ was used to define tumors with high CTSB expression and < 4 indicated low CTSB expression.

*Statistical analysis*

The duration from the date of each patient's randomization to the date of death for any cause or the censoring of the patient at the last follow-up date was defined as OS. All the statistical analyses were conducted using the SPSS 20.0 statistical software packages. The difference of CTSB expression between gastric cancer tissues and adjacent non-cancerous tissues were analyzed by chi-square test. Survival

**Table 1.** Correlation of CTSB expression with clinicopathologic features

| Characteristics | Total (n = 106) | CTSB | | *P* value |
|---|---|---|---|---|
| | | Negative (n = 54) | Positive (n = 52) | |
| Gender | | | | 0.727 |
| Male | 67 (63.2%) | 35 (52.2%) | 32 (8.7%) | |
| Female | 39 (36.8%) | 19 (48.7%) | 20 (51.3%) | |
| Age (years) | | | | 0.033 |
| ≥ 60 | 46 (43.4%) | 18 (39.1%) | 28 (60.9%) | |
| < 60 | 60 (56.6%) | 36 (60.0%) | 24 (40.0%) | |
| T stage | | | | 0.001 |
| 1 | 10 (9.4%) | 10 (100%) | 0 (0%) | |
| 2 | 10 (9.4%) | 7 (70%) | 3 (30%) | |
| 3 | 84 (79.2%) | 36 (42.9%) | 48 (57.1%) | |
| 4a | 2 (1.9%) | 1 (50%) | 1 (50%) | |
| N stage | | | | 0 |
| 0 | 21 (19.8%) | 21 (100%) | 0 (0%) | |
| 1 | 38 (35.8%) | 20 (52.6%) | 18 (47.4%) | |
| 3 | 47 (44.3%) | 13 (27.7%) | 34 (72.3%) | |
| M stage | | | | 0.734 |
| 0 | 99 (93.4%) | 50 (50.5%) | 49 (49.5%) | |
| 1 | 7 (6.6%) | 4 (57.1%) | 3 (42.9%) | |
| TNM stage | | | | 0 |
| I | 13 (12.3%) | 13 (100%) | 0 (0%) | |
| II | 18 (17.0%) | 13 (72.2%) | 5 (27.8%) | |
| II | 68 (64.2%) | 26 (38.2%) | 42 (61.8%) | |
| IV | 7 (6.6%) | 2 (28.6%) | 5 (71.4%) | |
| Tumor size (cm) | | | | 0 |
| ≥ 5 | 74 (69.8%) | 29 (39.2%) | 45 (60.8%) | |
| < 5 | 32 (30.2%) | 25 (78.1%) | 7 (21.9%) | |
| Grade | | | | 0.174 |
| 1 | 4 (3.8%) | 4 (100%) | 0 (0%) | |
| 2 | 25 (23.6%) | 12 (48.0%) | 13 (52.0%) | |
| 3 | 76 (71.7%) | 37 (48.7%) | 39 (51.3%) | |
| 4 | 1 (9%) | 1 (100%) | 0 (0%) | |
| Infiltration | | | | 0.675 |
| 0 | 101 (95.3%) | 52 (51.5%) | 49 (48.5%) | |
| 1 | 5 (4.7%) | 2 (40.0%) | 3 (60.0%) | |

curves were plotted by Kaplan-Meier method and compared using the log-rank test. The relationship between CTSB expression and other clinicopathological characteristics was analyzed by chi-square test and Fisher's exact test. Bivariate correlations between the clinicopathological characteristics were calculated by Spearman's rank correlation coefficients. Clinicopathological characteristics used to predict prognosis in clinical practice were evaluated by univariate and multivariate Cox regression analyses. The chosen type of Cox model for univariate analysis was enter method, and for multivariate analysis was forward method. A *p*-value of less than 0.05 was considered as statistically significant.

## Results

### *CTSB is overexpressed in gastric cancer tissues*

To determine whether CTSB is high-expressed in human gastric cancer samples, we performed RT-PCR and Western blotting analyses on 10 gastric tumor samples and adjacent non-cancerous tissues. As illustrated in **Figure 1**, *CTSB* mRNA was expressed at higher levels in all of the 10 gastric cancer tissues than in adjacent non-cancerous tissues, with the differential expression level ranging from 2.3 to 52.5 fold. Consistent with these data, CTSB protein was also found to be upregulated in fresh gastric cancer tissues compared with adjacent non-cancerous tissues (**Figure 2**). For immunostaining result, overexpression of CTSB was observed in 49.06% (52/106) gastric cancer patients. CTSB protein staining was weak or no staining in the adjacent non-tumor tissues, only 3.13% (2/32) in the adjacent non-tumor tissues. The difference between gastric cancer group and the adjacent non-tumor group was statistically significant ($X^2 = 21.921$, $P < 0.001$).

### *CTSB overexpression is associated with gastric cancer clinical features*

For better understanding of the potential roles of CTSB in gastric cancer development and progression, we investigated the relationship of CTSB expression and other clinicopathological features in 106 paraffin-embedded archived gastric cancer tissues, including 10 stage I tumors, 10 stage II tumors, 84 stage III tumors, and 2 stage IVa tumors. Among 106 samples, high CTSB protein expression was detected in 52 samples (49.06%) and weak or no staining was observed in 54 tumor samples (50.94%, **Table 1**). As shown in **Figure 3**, CTSB was highly expressed in gastric cancer tissues. In con-

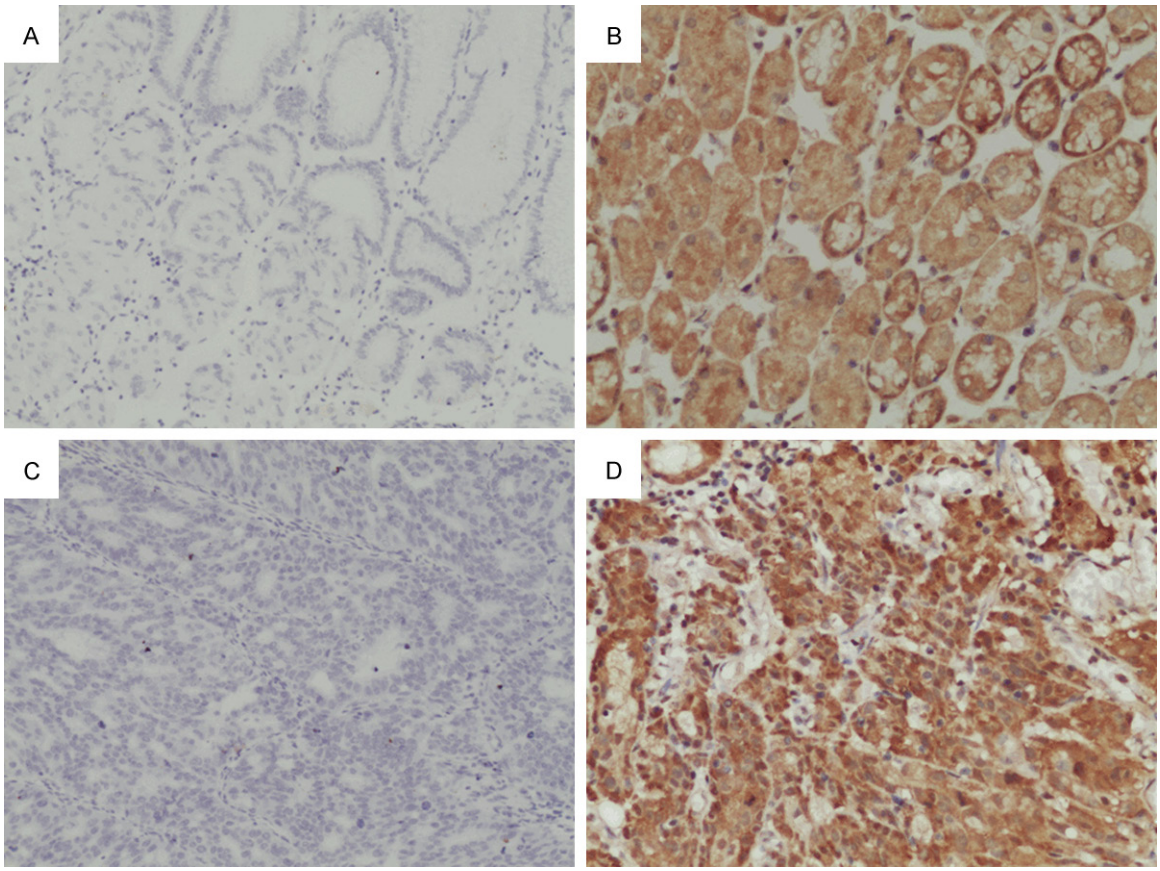


**Figure 3.** Expression analysis of CTSB protein by immunohistochemistry. CTSB expression was mainly localized in the cytoplasm of gastric tumor cells. CTSB is weak or not expressed in normal gastric epithelial cells. A. Negative Staining of CTSB in normal gastric tissues, B. Expression of CTSB in normal gastric tissues, C. Negative Staining of CTSB in gastric cancer tissues, D. Expression of CTSB in gastric cancer tissues.

trast, no signals or only weak signals were detected in adjacent non-cancerous tissues. The subcellular location of CTSB was mainly at the cytoplasm.

We further analyzed the correlation between CTSB expression and the clinicopathological characteristics of patients. As was summarized in **Table 1**, there were no significant correlations between the expression of CTSB protein and patient gender, M Stage, Grade, and infiltration in patients with gastric cancer. However, the CTSB expression was markedly associated with age ($P = 0.033$), T Stage ($P = 0.001$), N Stage ($P < 0.001$), TNM Stage ($P < 0.001$), and tumor size ($P < 0.001$).

*Association between CTSB expression and patient survival*

Survival analysis showed a clear negative correlation between CTSB protein expression level and the OS of patients with gastric cancer ($P < 0.001$, **Figure 4A**). In addition, Cox regression revealed that CTSB expression, T Stage, and N Stage were independent prognostic factors for OS (**Table 2**).

Furthermore, we analyzed the prognostic value of CTSB in selective patient subgroups stratified by age, T Stage, N Stage, TNM Stage, and tumor size, respectively. The expression of CTSB was strongly associated with OS duration of the patients both under 60 years old (**Figure 4B**, log-rank test, $P < 0.001$) and over 60 years old (**Figure 4C**, log-rank test, $P < 0.001$). For patients with late-stage tumors (Stage III-IVa), the expression of CTSB was strongly associated with OS duration (**Figure 4E**, log-rank test, $P < 0.001$), but not for patients with early-stage tumors (Stage I-II, log-rank test, $P = 0.088$, **Figure 4D**). Similarly, when it was evaluated according to T Stage, the impact on outcome associated with the expression of CTSB contin-

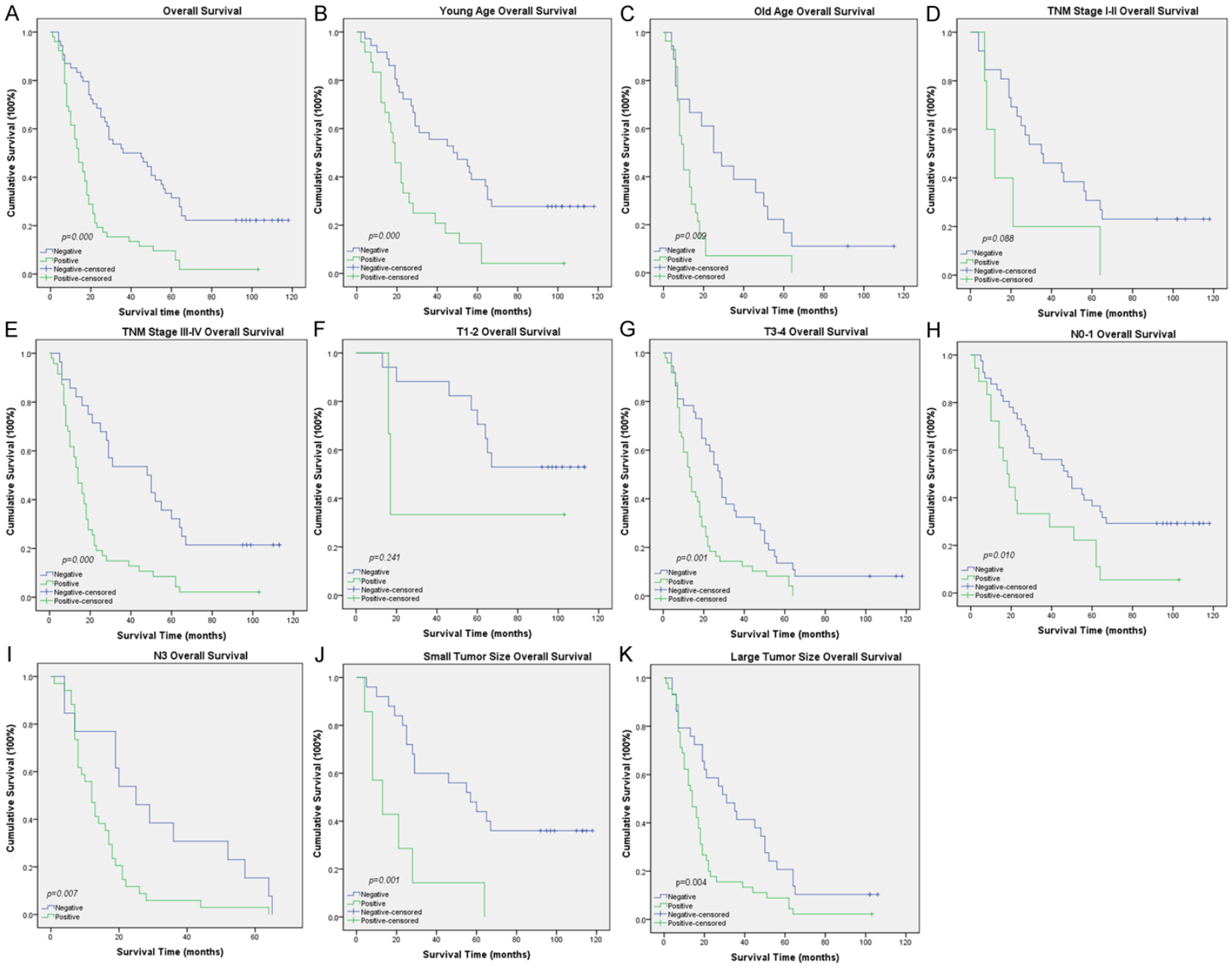
A
Overall Survival
p=0.000
Negative
Positive
Negative-censored
Positive-censored
Cumulative Survival (100%)
Survival time (months)
B
Young Age Overall Survival
p=0.000
Survival Time (months)
C
Old Age Overall Survival
p=0.009
Survival Time (months)
D
TNM Stage I-II Overall Survival
p=0.088
Survival Time (months)
E
TNM Stage III-IV Overall Survival
p=0.000
Survival Time (months)
F
T1-2 Overall Survival
p=0.241
Survival Time (months)
G
T3-4 Overall Survival
p=0.001
Survival Time (months)
H
N0-1 Overall Survival
p=0.010
Survival Time (months)
I
N3 Overall Survival
p=0.007
Survival Time (months)
J
Small Tumor Size Overall Survival
p=0.001
Survival Time (months)
K
Large Tumor Size Overall Survival
p=0.004
Survival Time (months)

**Figure 4.** Kaplan-Meier curves with univariate analysis (log-rank). A. OS rates for cases with high CTSB expression versus those with low CTSB expression levels in all patients. B. OS rate for young age cases (< 60) with high CTSB expression versus those with low CTSB expression levels. C. OS rate for old age cases (≥ 60) with high CTSB expression versus those with low CTSB expression levels. D. OS rate for early TNM stage cases (Stage I-II) with high CTSB expression versus those with low CTSB expression levels. E. OS rate for late stage cases (stage III-IVa) with high CTSB expression versus those with low CTSB expression levels. F. OS rate for cases with high CTSB expression versus cases with low CTSB expression in patients with T1-2 grade gastric tumors. G. OS rate for cases with high CTSB expression versus cases with low CTSB expression in patients with T3-4 grade gastric tumors. H. OS rate for cases with high CTSB expression versus cases with low CTSB expression in patients with N0-1 grade gastric tumors. I. OS rate for cases with high CTSB expression versus cases with low CTSB expression in patients with N3 grade gastric tumors. J. OS rate for cases with high CTSB expression versus cases with low CTSB expression in patients with small tumor size (under 5 cm). K. OS rate for cases with high CTSB expression versus cases with low CTSB expression in patients with large tumor size (over 5 cm).

**Table 2.** Cox-regression analysis of various prognostic parameters in patients for all patients

| Factor | Univariate | | Multivariate | |
|---|---|---|---|---|
| | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| N Stage | | | | |
| 0 | Reference | 0 | Reference | 0.005 |
| 1 | 4.022 (1.955-8.274) | 0 | 1.133 (0.521-2.464) | |
| 3 | 7.015 (3.421-14.386) | 0 | 2.314 (1.049-5.105) | |
| Age | | | | |
| ≥ 60 | Reference | | | |
| < 60 | 0.481 (0.319-0.727) | 0.001 | — | — |
| Tumor size (cm) | | | | |
| < 5 | Reference | 0.001 | — | — |
| ≥ 5 | 0.439 (0.272-0.709) | | | |
| CB expression | | | | |
| Negative | Reference | 0 | 0.574 (0.361-0.913) | 0.019 |
| Positive | 0.354 (0.231-0.542) | | | |
| T stage | | | | |
| 1 | Reference | 0.001 | Reference | 0.002 |
| 2 | 17.539 (2.207-139.398) | 0.007 | 10.828 (1.264-92.735) | |
| 3 | 36.233 (4.970-264.173) | 0 | 22.422 (2.807-179.093) | |
| 4 | 16.855 (1.516-188.064) | 0.022 | 5.269 (0.425-65.333) | |

ued to be more favorable only in T3-4 subgroups (**Figure 4G**, log-rank test, $P = 0.001$), but not in the T1-2 subgroup (**Figure 4F**, log-rank test, $P = 0.241$).

However, when it was evaluated according to Tumor size and N Stage, the expression of CTSB was strongly associated with OS duration of the patients in both N0-1 subgroups (**Figure 4H**, log-rank test, $P = 0.01$) and N1-3 subgroup (**Figure 4I**, log-rank test, $P = 0.007$). It was also strongly associated with OS duration of the patients in both small tumor size (under 5 cm) (**Figure 4J**, log-rank test, $P = 0.001$) and large tumor size (over 5 cm) (**Figure 4K**, log-rank test, $P = 0.004$).

## Discussion

Despite the incidence of gastric carcinoma has declined in the recent decades, it remains the fourth most common malignant carcinoma and the second highest cause of cancer-associated mortality worldwide with an estimated 951,600 new cases and 723,100 deaths occurring in 2012 [1]. While the incidence of gastric carcinoma has decreased substantially in most Northern and Western Europe and in North America, it is still prevalent in Central and South America, Eastern Europe, East Asia and Russia [16]. Although there have been important clinical progression in the diagnosis and treatment of gastric carcinoma over the past few decades,

it is commonly diagnosed in patients with late stage disease, which cause high treatment cost and abrogate successful curative surgery for many patients [17, 18]. The 5-year overall survival rate (OS) of gastric carcinoma is closely correlated with tumor stage. Patients at stage I had a 5-year survival rate of over 90%, whilst patients at stage IV had a less than 5% survival rate after 5 years [19, 20]. Classical serum tumor markers such as CEA and CA19-9 had certain implication for gastric carcinoma diagnosis but the lack of specificity and sensitivity impaired their function [21]. In the recent years, there are several novel tissue-based prognostic and therapeutic biomarkers for gastric carcinoma, including vascular endothelial growth factor receptor 2 (VEGFR-2) [22], excision repair cross-complementation group 1 (ERCC1) [23], human epidermal growth factor receptor-2 (HER2) [24, 25], B-cell lymphoma-2 (Bcl-2) and Ki-67 [26]. However, most of these biomarkers are not routinely used in clinical practice because they could not predict clinical outcome or therapeutic efficiency accurately and efficiently. Novel tumor markers are thus required for improving detection, diagnosis, and prognosis of gastric carcinoma.

Cathepsins are lysosomal proteases which belong to the papain family. More than a dozen cathepsins have been identified in different organisms. Of all these cathepsins, studies have shown that CTSB is very important as it is involved in diverse pathologies and oncogenic processes.

CTSB has been mapped to chromosome 8p22 [27-29]. Its gene consists of 13 exons, including exons 2a and b. The portion of CTSB is approximately 1 kb in size, composed of a heavy chain of 25-26 kDa and a light chain of 5 kDa. The overall gene spanning is at least 27 kb [30]. It has been observed that the CTSB promoter contains a GC-rich region which includes many SP1 sites, similar to a housekeeping gene [31]. And these SP1 sites are known to increase in cancer cells [32]. Numerous studies have shown that the increased expression of CTSB is correlated with invasive and metastatic cancers including gliomas [9], breast cancer [10, 11], lung tumors [12], melanomas [13], colorectal cancer [14], and prostate cancer [15]. In cancer cells, CTSB is shuttled to the plasma membrane where it can activate the serine protease pro-urokinase-type plasminogen activator (pro-uPA) to uPA [33, 34], and lead to the activation of latent transforming growth factor [35]. CTSB also enhances the activity of the matrix metalloproteinases (MMPs, a family of proteolytic enzymes that are major participants in extracellular matrix degradation and cancer cell motility and invasion [36], directly by the activation of the proenzymes [37], and indirectly by destroying their inhibitors (TIMPs) [19], thereby promoting ECM degradation and angiogenesis [38]. It is also known to interact with cystatins [39, 40] and annexin II tetramer (p11), which is also called S100A10 [41]. The interactions place CTSB at a crucial position for the proteolytic activation of ECM components, which can enable ECM degradation. Taken together, activity of CTSB has key roles in tumorigenesis, angiogenesis, invasion and metastasis [42, 43]. Therefore, targeting CTSB would have significant clinical relevance in the diagnosis and treatment of various cancers.

In this report, we present new evidence that the up-regulation of CTSB is associated with poor prognosis in gastric carcinoma patients, especially for those with late-stage diseases. Our results clearly showed that gastric carcinoma lesions displayed higher CTSB expression at the mRNA and protein level as compared with adjacent non-cancerous tissues. So we consider CTSB is an important molecular marker of gastric carcinoma and can help precise diagnoses. However, at present the precise roles of CTSB in human cancers are still obscure. To understand the precise signaling pathways of CTSB in gastric carcinoma still requires further studies.

We further analyzed the relationship between the expression of CTSB and clinical characteristics of patients affected by gastric carcinoma. There was a significant correlation between CTSB expression and age, T Stage, N Stage, TNM Stage, and tumor size, which strongly suggested that the overexpression of CTSB would be useful as an independent biomarker for the identification of subsets of gastric carcinoma patients with more aggressive disease. Meanwhile, there were no significant correlations between the expression of CTSB and patient gender, M Stage, Grade, and infiltration in patients with gastric cancer. Moreover, patients in the high CTSB expression group had a 1.92% cumulative 10-year survival rate, which was significantly lower than that of patients with low CTSB expression levels (22.22%). Multivariate analysis revealed that CTSB expression might

be an independent prognostic indicator for OS in gastric carcinoma patients (**Table 2**). This finding indicates the possibility of using high expression levels of CTSB as a predictor for prognosis and survival. Interestingly, sub-group analysis revealed that CTSB overexpression patients with a significantly poor prognosis among patients whose tumors demonstrated the features of late TNM Stage and late T Stage, respectively.

In conclusion, to our knowledge, this is the first report addressing CTSB expression and its clinicopathological and prognostic significance in gastric carcinoma. Our findings suggest that CTSB is up-regulated in gastric carcinoma and associated with age, T Stage, N Stage, TNM Stage, and tumor size. Multivariate analysis revealed that CTSB might be an independent biomarker for the prediction of gastric carcinoma prognosis and survival. Therefore, testing the CTSB protein level may be helpful for stratifying patients for a novel therapeutic strategy and establishing a rational treatment selection criterion for gastric carcinoma patients. Further investigation is also needed to investigate the molecular mechanism of CTSB involvement in the development and progression of gastric carcinoma.

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## Disclosure of conflict of interest

None.

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