Original Research

Biglycan expression correlates with aggressiveness and poor prognosis of gastric cancer

Bin Wang[1], Guang-Xin Li[1], Shu-Guang Zhang[1], Quan Wang[2], Yu-Gang Wen[2], Hua-Mei Tang[3], Chong-Zhi Zhou[2], Ai-Yan Xing[4], Jun-Wei Fan[2], Dong-Wang Yan[2], Guo-Qiang Qiu[2], Zhen-Hai Yu[1] and Zhi-Hai Peng[2]

[1]Department of General Surgery, Shandong University Affiliated Qianfoshan Hospital, 16766 Jingshi Road, Jinan 250014; [2]Department of General Surgery; [3]Department of Pathology, Shanghai Jiaotong University Affiliated First People's Hospital, 85 Wujin Road, Shanghai 200080; [4]Department of Pathology, School of Medicine, Shandong University, Jinan 250012, China
Corresponding authors: Dr Zhen-Hai Yu. Email: sdyzh56@163.com or Dr Zhi-Hai Peng. Email: pengpzh@hotmail.com

Abstract

Biglycan, a member of the small leucine-rich proteoglycan family, has been implicated in the development and progression of human cancers. However, the clinical significance of biglycan expression in gastric cancer has not been determined. In the present study, biglycan mRNA and protein concentrations were analyzed using quantitative realtime reverse transcription polymerase chain reaction and Western blot in 69 gastric cancer and adjacent non-tumorous tissues, respectively. Biglycan expression was further assessed using immunohistochemistry in tissue microarrays that contained 264 cases of gastric cancer, and others containing normal or metastasized lymph node tumor tissues. Biglycan was upregulated at the transcriptional and translational levels and there was a correlation between the expression of biglycan mRNA and protein ($P = 0.000$, $\kappa = 0.769$). Over-expression of biglycan was strongly associated with lymph node metastasis, tumor (T) classification, metastasis (M) classification, vascular invasion and Union for International Cancer Control (UICC) stage. Patients with biglycan-positive tumors had a significantly higher disease recurrence rate and poorer survival than patients with biglycan-negative tumors after the radical surgery. Multivariate analysis revealed that biglycan expression is an independent prognostic indicator for survival of patients with gastric cancer. The data from the current study demonstrate that elevated expression of biglycan may play an important role in the development and progression of gastric cancer, and could be further evaluated as a biomarker for predication of a poor clinical outcome.

Keywords: biglycan, gastric cancer, prognosis, tumor aggressiveness

Experimental Biology and Medicine 2011; **236**: 1247–1253. DOI: 10.1258/ebm.2011.011124

Introduction

Gastric cancer remains the fourth most common malignancy diagnosed worldwide and accounts for approximately 800,000 deaths annually – the second leading cause of cancer-related death according to the most recent data on global cancer.[1] The highest incidence of gastric cancer is found in East Asia, with 41% of new cases occurring in China and 11% in Japan.[1] Gastric cancer is frequently asymptomatic or causes only non-specific symptoms in the early stages; by the time symptoms occur, it has often reached an advanced stage. Moreover, despite significant advances in multidisciplinary approaches for treatment including surgery, chemotherapy and radiotherapy, in China, the five-year survival of patients still remains only about 40%, largely as a consequence of late detection and distant metastasis.[2] Therefore, early identification, prevention and prognosis are urgently needed. Use of novel molecular markers that predict tumor metastatic potential and patients' prognosis could also be helpful in designing therapeutic approaches and in prolonging survival.

Extracellular matrix is a complex of proteins that plays an integral role in embryo development, cell migration and proliferation, tissue repair and calcification, as well as cancer development.[3–5] Because of its role in tumorigenesis and cancer metastasis, identification of extracellular matrix molecules may be used to predict tumor metastasis or survival of patients with gastric cancer, since its major constituents include collagens, a large number of non-collagenous glycoproteins and proteoglycans.[6]

ISSN: 1535-3702

Copyright © 2011 by the Society for Experimental Biology and Medicine

Biglycan, a member of the family of small leucine-rich proteoglycans, is composed of glycosaminoglycan side chains covalently bound to a central core protein.[7] It resides at the cell surface or in the pericellular space of various tissues. A previous study using biglycan knockout mice demonstrated a major physiological role for biglycan in bone formation.[8] More importantly, aberrant expression of biglycan has been linked to a number of pathological conditions such as osteoporosis, glomerulonephritis, pancreatic cancer and mesothelioma.[9–11] Recently, cDNA microarray analyses and a review of the serial analysis of gene expression databases have shown significantly higher expression levels of biglycan in tumor tissues from the ovary, colon and liver compared with adjacent normal tissues.[12–14] In addition, Weber *et al.*[15] found that biglycan is over-expressed in pancreatic cancer tissues. Our recent microarray analysis revealed an elevated biglycan expression in gastric cancer tissues compared with their normal counterparts.[16] Therefore, in this study we aimed to detect and compare biglycan expression in gastric cancer tissues and adjacent normal mucosae, and note any differences in associations between these respective tissues. We also sought to determine whether there is a correlation between the expression of biglycan and clinicopathological parameters of gastric cancer patients and their survival.

Materials and methods

Tissue specimens and patient information

Between 2004 and 2009, 264 sets of gastric tumor and adjacent non-tumorous tissues (at least 5 cm away from the tumor edge) were recruited from patients who underwent curative surgery at Shanghai Jiaotong University Affiliated First People's Hospital. These patients consisted of 157 men and 107 women, and the age of the patients ranged from 27 to 89 y, with a median age of 66 y. A portion of the tissue specimens from each case was immediately frozen in liquid nitrogen after surgical resection. None of the patients received any preoperative chemo- or radiotherapy. A research protocol to use tissue specimens and clinicopathological data was approved by the Institutional Review Board for the Use of Human Subjects at Shanghai Jiao Tong University School of Medicine. Each patient signed a written informed consent form before surgery. The clinicopathological data were collected and tumor diagnosis was made according to the classification of malignant tumors by the World Health Organization and the Union for International Cancer Control (UICC)'s tumor-node-metastasis (TNM) staging system.[17] All tumor tissues were diagnosed histopathologically by at least two well-trained pathologists. Of these 264 cases, 95 (36.0%) were in stage I, 48 (18.2%) in stage II, 89 (33.7%) in stage III and 32 (12.1%) in stage IV. One hundred forty-eight cases had tumors metastasized to lymph nodes.

Follow-up of patients

After surgery and discharge from the hospital, the patients were followed up regularly. The patients were subjected to a tumor screening test every two or three months, which included computed tomography scans of the chest, abdomen and pelvis, serum carcinoembryonic antigen concentration, and other hematological tests. A diagnostic gastroscopy was also performed on these patients annually. Overall survival was defined as the interval between the dates of surgery and demise. Disease-free survival was defined as the interval between the dates of surgery and recurrence; if recurrence was not diagnosed, patients were accounted either dead or alive based on the date of death or last follow-up, respectively. The last patient follow-up was on 15 September 2009 and the mean follow-up time was 45 months (range: 9–69 months).

Paraffin block collections for tissue microarray construction

After reviewing hematoxylin and eosin-stained sections for optimal tumor contents, we collected paraffin blocks from the Department of Pathology for the 264 matched pairs of primary gastric cancer and adjacent gastric tissues, and 104 metastatic lymph node tissues. These paraffin blocks were used by Shanghai Biochip (Shanghai, China) to construct tissue microarrays. For tissue microarray construction, we selected 2/3 punches from tissue cylinders (diameter: 2 mm) from two different representative areas of each paraffin block (i.e. the center of the tumor tissue and the gastric tissue adjacent to the tumor). In addition, different controls and metastatic lymph node tissues were also selected to ensure reproducibility and homogeneous staining of the tissue microarray sections.

Immunohistochemistry

Immunohistochemistry was used to detect biglycan expression in these tissue microarray sections. Briefly, the sections were given a heat pretreatment of 60°C for one hour, then dewaxed in xylene, re-hydrated in an ethanol series (100–50%) and treated in 0.01 mol/L citrate buffer (pH 6.0) for antigen retrieval. After the endogenous peroxidase was inhibited by 3% H_2O_2 in methanol, the sections were incubated with a mouse anti-biglycan monoclonal antibody (Abcam, Cambridge, MA, USA) at a dilution of 1:300 at 4°C overnight. The next day, sections were thoroughly washed with phosphate-buffered saline, the corresponding secondary antibody was then applied and kept at room temperature for 30 min, followed by an incubation with avidin–biotin complex for 30 min. The color was subsequently developed with 3,3′-diaminobenzidine solution and then a counterstain with hematoxylin. Negative controls were achieved by substituting isotype-matched IgG2a (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for the first antibody.

Review and scoring of the stained sections

The biglycan-stained sections were reviewed and scored by two pathologists who were blinded to clinical data related to the patients. Biglycan staining data were expressed as the percentage of stained cells over the total number of tumor cells in the tissue, and assigned to one of four categories,

based on the percentage of positively stained cells: 0, 0%; 1, up to 10%; 2, 10–50%; and 3, >50%. The intensity of immunostaining was graded on a semiquantitative scale (0–3): 0, absent; 1, weak; 2, positive; and 3, strong positive. These two scores were then multiplied and produced a final total score for each tissue core. A final score ranging between 0 and 2 was considered negative for biglycan protein expression. Scores 3 and 4 were considered weakly positive, and scores 5 and 6 were strongly positive.

RNA isolation and quantitative realtime reverse transcription polymerase chain reaction

Total RNA from frozen tissues was isolated using Trizol reagent (Life Technologies, Rockville, MD, USA) according to the manufacturer's instructions. Reverse transcription was performed on 2 μg of total RNA from each sample to convert RNA to cDNA for quantitative realtime polymerase chain reaction (PCR) analysis of biglycan mRNA. SYBR Green reagent (Bio-Rad, Hercules, CA, USA) was used for quantitative realtime reverse transcription polymerase chain reaction (qRT-PCR) in a Mastercycler ep realplex Real-Time Quantitative Thermal Block (Eppendorf AG, Hamburg, Germany). The PCR primers were designed and chemically synthesized according to the human biglycan and β-actin cDNA sequences reported in GenBank. Primers for biglycan were 5′ AAGGTGCCCAA GGGAGTGTTC 3′ (forward) and 5′ TGGTCTAGGTGG AGTTCATTCAGG 3′ (reverse), and for β-actin 5′ CTCCTT AATGTCACGCACGAT 3′ (forward) and 5′ CATGTA CGTTGCTATCCAGGC 3′ (reverse). The PCR reactions were carried out at 95°C for two minutes to activate the enzyme, then 40 cycles of 95°C for 20 s, 55°C for 15 s, and 72°C for 20 s, and a final extension at 72°C for 10 min. The specificity of the PCR products was confirmed by examining the dissociation reaction plot subsequent to qRT-PCR. β-Actin served as the loading control. PCR reactions of each sample were conducted in triplicate. The data were analyzed with the comparative threshold cycle method described previously.[18]

Protein extraction and Western blot

Total proteins were extracted from frozen tissues using a Whole Cell Extraction Kit (Chemicon, Billerica, MA, USA) and protein concentrations were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts (50 μg) of protein were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and then electrotransferred to polyvinylidene fluoride membranes. The membranes were subsequently incubated in 5% skim milk at room temperature for two hours, and then incubated at 4°C overnight with a mouse antibiglycan monoclonal antibody (#ab54855 from Abcam) at a dilution of 1:1000, or monoclonal rabbit anti-β-actin antibody (Cell Signaling Technology, Beverly, MA, USA) at 1:1000, followed by horseradish peroxidase-conjugated secondary antibody incubations. Positive protein bands were visualized using an electrochemiluminescence (ECL)-plus kit (GE Healthcare, Piscataway, NJ, USA) and exposed to X-ray film.

Statistical analysis

Chi-square (χ^2) and kappa (κ) tests were used to analyze the association between biglycan expression and clinicopathological data. Survival curves were plotted by using the Kaplan–Meier method and the log-rank test. Survival data were further evaluated using univariate and multivariate Cox regression analyses. All statistical analyses were performed using SPSS version 13.0 software (SPSS Inc, Chicago, IL, USA) for Microsoft Windows. A probability (P) value <0.05 was considered to be statistically significant.

Results

Expression of biglycan mRNA and protein in gastric cancer tissue specimens

We first quantitatively determined the expression of biglycan mRNA levels in these gastric cancer tissue samples using qRT-PCR. Our data showed that 47 of 69 (68.1%) analyzed samples expressed a higher level (at least a two-fold change) of biglycan mRNA in gastric cancer tissue specimens than in non-cancerous tissue specimens.

To investigate whether the difference in biglycan expression between tumor and non-tumorous samples also occurs at the protein concentration, we performed a Western blot analysis of biglycan protein expression in these 69 paired samples. As expected, changes in 62 paired tissues (62/69, 89.9%) observed by Western blot analysis were in accordance with the findings in the qRT-PCR study, including upregulated in 43 tumor tissues and downregulated in 19 non-neoplastic tissues. The expression of biglycan protein in tumor samples was significantly higher than that in non-tumorous tissues (1.69 ± 0.73 versus 1.16 ± 0.89, $P = 0.003$). Representative blots are shown in Figure 1. The Kappa test showed that there was a correlation between the expression of biglycan mRNA and protein (Table 1, $P = 0.000$, $\kappa = 0.769$).

Expression of biglycan protein in gastric cancer tissues is associated with the clinicopathological parameters of patients

Using immunohistochemistry, we assessed biglycan expression in all 264 cases of gastric cancer tissue specimens, which included 104 cases of lymph node metastasis of gastric cancer cells. The immunostaining of biglycan was mostly found in the cytoplasm of epithelial cells (Figure 2). We found that biglycan was not expressed in 93 (35.2%) cases, weakly expressed in 70 (26.5%) cases and

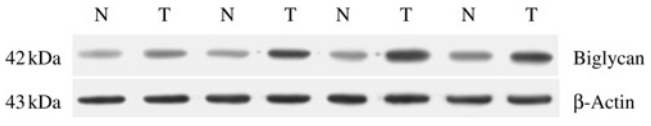

Figure 1 Western blot analysis of biglycan expression. Gastric cancer and the corresponding normal tissues were subjected to protein extraction and Western blot analysis of biglycan expression. T, primary gastric cancer tissue; N, adjacent non-neoplastic tissues paired from the same patient

Table 1 Association of biglycan mRNA with protein in gastric cancer tissues

	Biglycan mRNA		*P* value	κ
	High	Low		
Biglycan protein				
High	43	3	0.000	0.769
Low	4	19		

strongly expressed in 101 (38.3%) cases in gastric cancer tissues. In contrast, in adjacent non-neoplastic tissues, biglycan expression was negative in 247 (93.6%) cases, weak in 13 (4.9%) cases and strong in 4 (1.5%) cases (Table 2). In addition, biglycan was upregulated in 60 cases of tumor tissues with lymph node metastases; only six cases of the lymph node metastases downregulated biglycan expression. These data clearly indicate that biglycan is over-expressed in gastric cancer and lymph node metastases.

We then evaluated the association between biglycan expression and the clinicopathological data of the patients. As summarized in Table 3, we found that upregulated biglycan protein expression was associated with lymph node metastasis ($P = 0.004$), tumor (T) classification ($P = 0.002$), metastasis (M) classification ($P = 0.041$), vascular invasion ($P = 0.001$) and UICC tumor stage ($P = 0.002$). However, biglycan protein expression in tumor tissues was not significantly associated with other clinicopathological parameters, such as gender ($P = 0.768$), age ($P = 0.332$), tumor size ($P = 0.376$), tumor location ($P = 0.178$) or grade of tumor differentiation ($P = 0.102$; Table 3).

Table 2 Expression of biglycan protein in normal and cancerous gastric tissue specimens

Tissue types	*n*	Negative (%)	Weak (%)	Strong (%)	*P* value
Tumor	264	93 (35.2)	70 (26.5)	101 (38.3)	$<0.001^{*}$
Adjacent non-neoplastic tissues	264	247 (93.6)	13 (4.9)	4 (1.5)	$<0.001^{\dagger}$
Lymph node metastases (LNM)	104	12 (11.5)	20 (19.2)	72 (69.2)	$<0.001^{\ddagger}$

*Between LNM and primary tumor tissues
†Between primary tumor and adjacent non-neoplastic tissues
‡Between LNM and adjacent non-neoplastic tissues

Association of biglycan expression with prognosis of patients with gastric cancer

After surgery, patients were followed-up for overall and disease-free survival. The mean follow-up period was 45 months (range: 9–69 months). Using these data, we determined if there was an association between the overall survival and disease-free survival with biglycan expression. We found that both overall and disease-free survival of patients were significantly better in those who had negative biglycan protein expression than for those who had biglycan expression. Overall, patients with biglycan-positive gastric cancer had an approximately 11-times higher risk than patients with biglycan-negative gastric cancer of tumor recurrence and metastasis (hazard ratio [HR]: 10.83; 95% confidence interval [CI]: 4.23–32.97; $P < 0.001$). Based on a

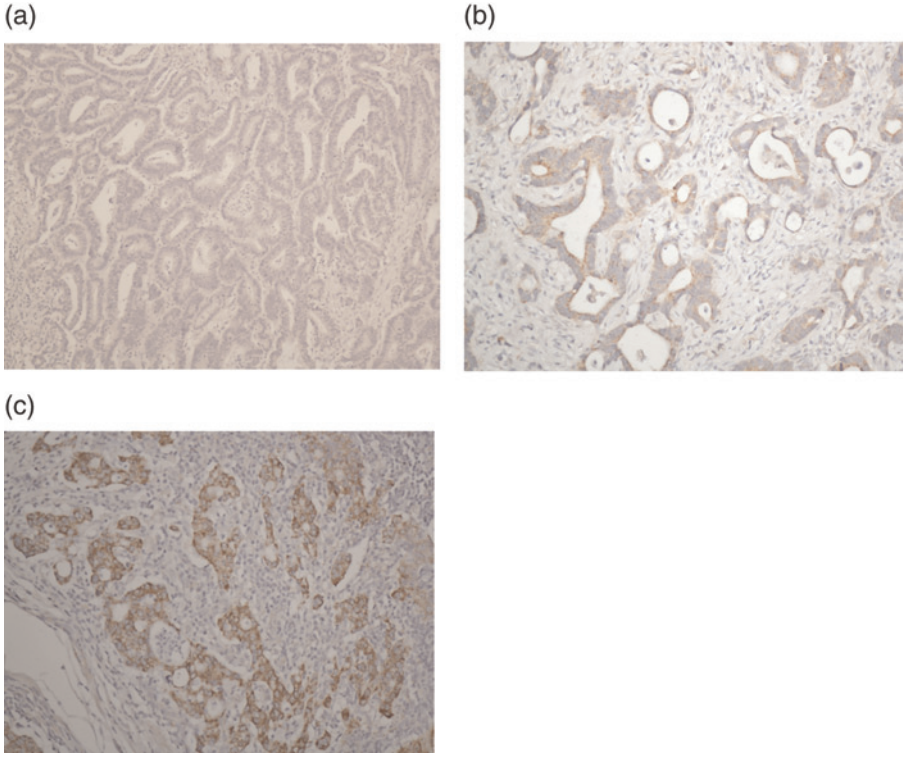

Figure 2 Immunohistochemical analysis of biglycan expression in gastric cancer and the corresponding normal tissues. Tissue microarray sections were immunohistochemically stained with antibiglycan antibody and scored as negative (a), weakly positive (b) and strongly positive (c). (A color version of this figure is available in the online journal)

Table 3 Association of biglycan expression with clinicopathological parameters from gastric cancer patients

Clinicopathological variables	*N*	Biglycan expression		*P* value
		Negative (*n* = 93)	Positive (*n* = 171)	
Age (years)				
<65	121	45	76	0.768
≥65	143	48	75	
Gender				
Male	157	59	98	0.332
Female	107	34	73	
Tumor location				
Gastric fundus	11	5	6	0.156
Gastric corpus	123	36	87	
Pylorus	130	52	78	
Tumor size (cm)				
≥3	187	69	118	0.376
<3	77	24	53	
T classification				
T1	76	37	39	0.002
T2	42	17	25	
T3	118	36	82	
T4	28	3	25	
Lymph node metastases				
Absent	116	52	64	0.004
Present	148	41	107	
M classification				
M0	254	93	161	0.041
M1	10	0	10	
UICC stage				
I	95	46	49	0.002
II	48	17	31	
III	89	25	64	
IV	32	5	27	
Vessel invasion				
Absent	186	77	109	0.001
Present	78	16	62	
Differentiation				
Well	47	11	36	0.102
Moderate	42	13	29	
Poor	175	69	106	

UICC, Union for International Cancer Control; T classification, tumor classification; M classification, metastasis classification
Note: Positive biglycan expression included all positive cases, such as weak and strong

univariate analysis, factors such as age, UICC stage, tumor classification, lymph node metastasis, distant metastasis, vascular invasion, differentiation and biglycan expression showed a significantly higher HR for a poor prognosis. However, biglycan expression does not associate with the disease-free survival (HR: 1.63, CI: 0.65–3.96) of the patients. Additionally, multivariate analysis revealed that biglycan expression was recognized as an independent prognostic factor of patient outcome (Table 4). Taken together, the data from our current study suggest that biglycan might represent a novel and potentially useful independent biomarker for determining the prognosis of patients with gastric cancer.

Discussion

To the best of our knowledge, the current study is the first report demonstrating over-expression of biglycan mRNA and protein in gastric cancer tissues. Moreover, biglycan over-expression is shown to be strongly associated with lymph node metastasis, tumor classification, metastasis, vascular invasion and UICC stage. In addition, according to the statistical analysis, patients with higher biglycan expression had a shorter overall and disease-free survival, whereas patients with lower biglycan expression had better survival. Considering these factors in conjunction, the current study demonstrates that over-expression of biglycan is associated with tumor aggressiveness and that biglycan expression could be further evaluated as an independent prognostic factor for gastric cancer patients.

Indeed, recent microarray-based studies have documented over-expression of biglycan in different human cancers, which may serve as a potential marker for diagnosis of these cancers.[12–14,19] For instance, using proteomic and microarray analyses, Mikula *et al.*[14] identified an upregulation of biglycan in colon adenocarcinoma samples at both protein and mRNA levels compared with normal colon mucosa, suggesting that dysregulation of biglycan may be involved in colorectal cancer development. However, to date, biglycan expression in gastric cancer has not been determined.

We first analyzed biglycan mRNA and protein concentrations in 69 paired sets of gastric cancer and adjacent nontumorous tissues. We found that biglycan expression was significantly higher in neoplastic compared with adjacent non-neoplastic tissues at both mRNA and protein concentrations, which was consistent with previous findings in pancreatic cancer tissues.[15] Additionally, our data showed that biglycan positively correlates with lymph node metastasis, T and M classification, vascular invasion, and UICC stage in gastric cancer. Previous studies have shown that biglycan, a transforming growth factor β (TGF-β)-binding protein, is capable of increasing the probability of an interaction between TGF-β and its specific surface receptors and thereby contributing to the early progression of pancreatic cancers.[10,15] We therefore speculated that future study may focus on TGF-β and assess whether the latter is likely to be one of the underlying mechanisms for the function of biglycan in gastric cancers. More importantly, our data also demonstrate that biglycan expression is associated with poor patient survival. The univariate and multivariate analyses in the current study revealed that biglycan expression might serve as an independent prognostic factor for gastric cancer patients. Collectively, these findings suggest that upregulated biglycan in gastric cancer tissues may contribute to gastric cancer development or progression.

The finding of over-expression of biglycan in gastric cancer tissues could lead researchers to the development of a novel antigastric cancer strategy. Nonetheless, further studies are needed to elucidate the molecular mechanisms by which biglycan participates in the development and progression of gastric cancer. To this end, biglycan has been shown to be involved in regulation of extracellular matrix assembly, cell adhesion and migration, and growth factor activation.[20–22] Previous *in vitro* and *in vivo* experiments revealed that TGF-β can promote cancer metastasis through its effects on altered tumor microenvironment,

Table 4 Univariate and multivariate analyses of various prognostic parameters in patients with gastric cancer

Variables	Overall survival				Disease-free survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years)								
< 65								
≥ 65	1.88 (1.10–3.20)	0.020			1.86 (1.09–3.17)	0.022		
Gender								
Male								
Female	1.00 (0.60–1.67)	0.992			1.01 (0.61–1.67)	0.982		
Tumor location								
Gastric fundus								
Gastric corpus	2.02 (0.70–5.81)	0.192			2.01 (0.70–5.78)	0.195		
Pylorus	1.40 (0.83–2.36)	0.203			1.42 (0.84–2.38)	0.189		
Tumor size								
≥ 3 cm								
< 3 cm	1.66 (0.91–3.01)	0.097			1.66 (0.91–3.01)	0.097		
T classification								
T1								
T2	2.78 (0.79–9.89)	0.113	2.03 (0.34–7.60)	0.078	2.87 (0.81–10.18)	0.103	1.98 (0.30–6.94)	0.060
T3	7.60 (2.71–21.30)	<0.001	5.87 (1.32–17.58)	<0.001	7.54 (2.69–21.15)	<0.001	4.65 (1.07–15.74)	0.002
T4	12.06 (3.93–37.04)	<0.001	10.63 (1.70–29.55)	<0.001	12.20 (3.97–37.47)	<0.001	11.02 (1.83–31.35)	<0.001
N classification								
N0								
N1	4.02 (1.80–9.00)	0.001	2.95 (1.25–6.79)	0.056	4.05 (1.81–9.05)	0.001	2.58 (1.09–5.97)	0.063
N2	9.65 (4.24–21.96)	<0.001	5.54 (2.56–13.25)	0.003	9.49 (4.17–21.59)	<0.001	4.64 (2.53–11.95)	0.002
N3	16.91 (6.67–42.90)	<0.001	11.23 (3.04–24.73)	<0.001	18.15 (7.15–46.09)	<0.001	13.53 (4.80–31.87)	<0.001
M classification								
M0								
M1	3.08 (1.23–7.71)	0.016	1.67 (0.53–4.05)	0.045	3.41 (1.56–8.52)	0.009	2.10 (0.78–6.25)	0.023
UICC stage								
I								
II	6.80 (1.87–24.70)	0.004	4.32 (1.24–17.38)	0.113	6.87 (1.89–24.96)	0.003	5.16 (1.43–20.48)	0.235
III	13.46 (4.10–44.18)	<0.001	8.05 (2.96–30.65)	<0.001	13.36 (4.07–43.83)	<0.001	7.37 (2.59–25.82)	<0.001
IV	32.07 (9.43–109.00)	<0.001	14.63 (5.79–60.42)	<0.001	34.58 (10.17–117.6)	<0.001	16.20 (6.67–71.05)	<0.001
Vessel invasion								
Absent								
Present	2.56 (1.54–4.21)	<0.001			2.55 (1.54–4.21)	<0.001		
Differentiation								
Well								
Moderate	5.44 (1.16–25.63)	0.032	3.27 (0.54–14.86)	0.726	5.50 (1.17–25.91)	0.031		
Poor	8.51 (2.07–34.96)	0.003	2.89 (1.08–6.17)	0.033	8.58 (2.09–35.27)	0.003		
Biglycan expression								
Negative								
Positive	3.71 (1.82–9.96)	0.001	2.05 (1.49–4.89)	0.019	2.87 (1.22–4.97)	<0.001	1.63 (0.65–3.96)	0.047

HR, hazard radio; CI, confidence interval; UICC; Union for International Cancer Control; T classification, tumor classification; M classification, metastasis classification

enhanced invasive properties and inhibition of immune cell function.[23,24] Because of pericellular localization and function as a TGF-β binding protein, biglycan is believed to increase the probability of an interaction of TGF-β with its specific surface receptors. The data from the current study showed that patients with biglycan-positive gastric cancer had an approximately 11 times higher risk than patients with biglycan-negative gastric cancer of tumor recurrence and metastasis. This suggests that biglycan may play a role in cancer metastasis and that activation of TGF-β is likely to represent one underlying mechanism for biglycan-mediated metastasis. Future study will need to explore these associations and their functions.

In summary, the data from the current study have shown that biglycan is over-expressed in gastric cancer and associated with lymph node metastasis, T and M classification, vascular invasion, and UICC stage, as well as poor prognosis of gastric cancer patients. Furthermore, biglycan concentrations appear to be an independent predictor of survival for patients with gastric cancer.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. BW and G-XL contributed equally to this work.

ACKNOWLEDGEMENTS

This study was supported in part by grants from the National High Technology Research and Development Program (863 program) of China (2007AA022003), the Fundamental Key Science Foundation of Science and Technology Commission of Shanghai Municipality (No. 09JC1412400 and 10411967300), the Public Scientific Research Platform of Hospitals of Grade A at the Tertiary

Level of Shanghai (No. SHDC12007704) and the Natural Science Foundation of the Shanghai Municipality (# 07ZR14064). We would like to thank Dr Peng Gao of the Department of Pathology, School of Medicine, Shandong University, Jinan, People's Republic of China for his excellent technical assistance.

REFERENCES

1 Global Cancer Facts and Figures in 2007. See www.cancer.org (last checked 26 August 2011)
2 Zhao ZS, Wang YY, Chu YQ, Ye ZY, Tao HQ. SPARC is associated with gastric cancer progression and poor survival of patients. *Clin Cancer Res* 2010;**16**:260–8
3 Sommer B, Bickel M, Hofstetter W, Wetterwald A. Expression of matrix proteins during the development of mineralized tissues. *Bone* 1996;**19**:371–80
4 Zhao M, Lu Y, Takata T, Ogawa I, Miyauchi M, Mock D, Nikai H. Immunohistochemical and histochemical characterization of the mucosubstances of odontogenic myxoma: histogenesis and differential diagnosis. *Pathol Res Pract* 1999;**195**:391–7
5 Martinez EF, Araujo VC. *In vitro* immunoexpression of extracellular matrix proteins in dental pulpal and gingival human fibroblasts. *Int Endod J* 2004;**37**:749–55
6 Hall RC, Embery G. The use of immunohistochemistry in understanding the structure and function of the extracellular matrix of dental tissues. *Adv Dent Res* 1997;**11**:478–86
7 Bidanset DJ, LeBaron R, Rosenberg L, Murphy-Ullrich JE, Hook M. Regulation of cell substrate adhesion: effects of small galactosaminoglycan-containing proteoglycans. *J Cell Biol* 1992;**118**:1523–31
8 Young MF, Bi Y, Ameye L, Chen XD. Biglycan knockout mice: new models for musculoskeletal diseases. *Glycoconj J* 2002;**19**:257–62
9 Schaefer L, Hausser H, Altenburger M, Ugorcakova J, August C, Fisher LW, Schaefer RM, Kresse H. Decorin, biglycan and their endocytosis receptor in rat renal cortex. *Kidney Int* 1998;**54**:1529–41
10 Chen WB, Lenschow W, Tiede K, Fischer JW, Kalthoff H, Ungefroren H. Smad4/DPC4-dependent regulation of biglycan gene expression by transforming growth factor-beta in pancreatic tumor cells. *J Biol Chem* 2002;**277**:36118–28
11 Gulyas M, Hjerpe A. Proteoglycans and WT1 as markers for distinguishing adenocarcinoma, epithelioid mesothelioma, and benign mesothelium. *J Pathol* 2003;**199**:479–87
12 Nishino R, Honda M, Yamashita T, Takatori H, Minato H, Zen Y, Sasaki M, Takamura H, Horimoto K, Ohta T, Nakanuma Y, Kaneko S. Identification of novel candidate tumour marker genes for intrahepatic cholangiocarcinoma. *J Hepatol* 2008;**49**:207–16
13 Pan S, Cheng L, White JT, Lu W, Utleg AG, Yan X, Urban ND, Drescher CW, Hood L, Lin B. Quantitative proteomics analysis integrated with microarray data reveals that extracellular matrix proteins, catenins, and p53 binding protein 1 are important for chemotherapy response in ovarian cancers. *OMICS* 2009;**13**:345–54
14 Mikula M, Rubel T, Karczmarski J, Goryca K, Dadlez M, Ostrowski J. Integrating proteomic and transcriptomic high-throughput surveys for search of new biomarkers of colon tumors. *Funct Integr Genomics* 2010;**11**:215–24
15 Weber CK, Sommer G, Michl P, Fensterer H, Weimer M, Gansauge F, Leder G, Adler G, Gress TM. Biglycan is overexpressed in pancreatic cancer and induces G1-arrest in pancreatic cancer cell lines. *Gastroenterology* 2001;**121**:657–67
16 Wang Q, Wen YG, Li DP, Xia J, Zhou CZ, Yan DW, Tang HM, Peng ZH. Upregulated INHBA expression is associated with poor survival in gastric cancer. *Med Oncol* 2010 [Epub ahead of print] DOI: 10.1007/s12032-010-9766-y
17 Sobin LH, Fleming ID. TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. *Cancer* 1997;**80**:1803–4
18 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;**25**:402–8
19 Galamb O, Sipos F, Spisák S, Galamb B, Krenács T, Valcz G, Tulassay Z, Molnár B. Potential biomarkers of colorectal adenoma-dysplasia-carcinoma progression: mRNA expression profiling and *in situ* protein detection on TMAs reveal 15 sequentially upregulated and 2 downregulated genes. *Cell Oncol* 2009;**31**:19–29
20 Nelimarkka L, Kainulainen V, Schönherr E, Moisander S, Jortikka M, Lammi M, Elenius K, Jalkanen M, Järveläinen H. Expression of small extracellular chondroitin/dermatan sulfate proteoglycans is differentially regulated in human endothelial cells. *J Biol Chem* 1997;**272**:12730–7
21 Kinsella MG, Tsoi CK, Järveläinen HT, Wight TN. Selective expression and processing of biglycan during migration of bovine aortic endothelial cells. The role of endogenous basic fibroblast growth factor. *J Biol Chem* 1997;**272**:318–25
22 Ruoslahti E, Yamaguchi Y. Proteoglycans as modulators of growth factor activities. *Cell* 1991;**64**:867–9
23 Perera M, Tsang CS, Distel RJ, Lacy JN, Ohno-Machado L, Ricchiuti V, Samaranayake LP, Smejkal GB, Smith MG, Trachtenberg AJ, Kuo WP. TGF-beta1 interactome: metastasis and beyond. *Cancer Genom Proteom* 2010;**7**:217–29
24 Ikushima H, Miyazono K. TGFbeta signalling: a complex web in cancer progression. *Nat Rev Cancer* 2010;**10**:415–24

(Received April 8, 2011, Accepted July 6, 2011)