

Global Journal of Medicine

2024; 5(2): 38-53.

ISSN Online: 2766-8894; ISSN Print: 2766-8908

Website: <http://naturescholars.com>Email: Glo_J_Med@126.com

Publisher: Scholars Publishing, LLC

**Research Article****Biglycan Overexpression Predicts Poor Prognosis of Gastric Cancer****Xin-zhou Huang^{1,3,#}, Hui Chen^{1,2,#}, Wen-ming Song^{9,#}, Ying-ying Wang^{4,5}, Mei-yuan Zhou⁸, Hui-chuan Zhao⁸, Na Li⁶, Bing Zheng^{1,2}, Quan Gong^{1,2,*}, Ke-bin Liu^{3,*}, Hong-wu Xin^{4,7,*}**

¹Department of Immunology, School of Medicine, Yangtze University, Jingzhou, Hubei Province 434023, PR China; ²Clinical Molecular Immunology Center, School of Medicine, Yangtze University, Jingzhou, Hubei Province 434023, PR China; ³Department of Orthopedics, Affiliated the First Hospital Yangtze University, Jingzhou, Hubei Province 434023, PR China; ⁴Laboratory of Oncology, Center for Molecular Medicine, School of Basic Medicine, Faculty of Medicine, Yangtze University, Jingzhou, Hubei Province 434023, PR China; ⁵Gynaecology Research Unit, Hannover Medical School, Hannover, D30625, Germany; ⁶Department of Oncology, Affiliated the First Hospital Yangtze University, Jingzhou, Hubei Province 434023, PR China; ⁷Department of Biochemistry and Molecular Biology, School of Basic Medicine, Faculty of Medicine, Yangtze University, Jingzhou, Hubei Province 434023, PR China; ⁸Department of Pathology, Affiliated the First Hospital Yangtze University, Hubei 434000, China; ⁹Department of Neurology, Lianjiang people's Hospital, Lianjiang, Guangdong Province 524400, PR China.

#, These authors contributed equally to this work.

*, Correspondence

Quan Gong, Ph.D., Department of Immunology, School of Medicine, Yangtze University, Jingzhou, Hubei Province 434023, PR China. Email: gongquan1998@163.com.Ke-bin Liu, M.M., Department of Orthopedics, First Affiliated Hospital of Yangtze University, Jingzhou, Hubei Province 434023, PR China. Email: liukb@sina.com.Hong-wu Xin, Ph.D., Laboratory of Oncology, Center for Molecular Medicine, School of Basic Medicine, Faculty of Medicine, Yangtze University, Jingzhou, Hubei Province 434023, PR China. Email: hongwu_xin@126.com.**Received:** July 23, 2023; **Accepted:** May 1, 2024; **Published online:** September 5, 2024.**Cite this paper:** Xin-zhou Huang, Hui Chen, Wen-ming Song, Ying-ying Wang, Mei-yuan Zhou, Hui-chuan Zhao, Na Li, Bing Zheng, Quan Gong, Ke-bin Liu, Hong-wu Xin. (2024) Biglycan Overexpression Predicts Poor Prognosis of Gastric Cancer. *Global Journal of Medicine*, 5(2):38-53. <http://naturescholars.com/gjm.050203>.<https://doi.org/10.46633/gjm.050203>.**Copyright**© 2024 by Scholars Publishing, LLC.**Abstract**

Biglycan (BGN) encodes an extracellular matrix (ECM) proteoglycan. However, the potential diagnostic and prognostic value of BGN in gastric cancer (GC) have not yet been reported. In this analysis, BGN expression in GC was evaluated across the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), and Oncomine databases, and verified using immunohistochemistry (IHC). In this analysis, the expression of BGN in gastric cancer was evaluated by bioinformatics analysis and validated by immunohistochemistry (IHC). The relationship between BGN expression and clinicopathological parameters was assessed by chi-square test and logistic regression. We analyzed the prognostic value of BGN. Then, Gene set enrichment analysis (GSEA) was used to screen the signaling pathways involved in high BGN expression datasets in GC. Finally, CIBERSORT was used to evaluate the infiltration of immune cells in GC tissues, and the correlation between BGN and infiltrating immune cells was analyzed. Then the signaling pathways involved in the BGN highly expressed data set in GC were screened. Finally, the infiltration of

immune cells in GC tissues was evaluated. The results showed that the mRNA levels of BGN were significantly up-regulated in GC compared with normal tissues (all $p < 0.001$). The Kaplan-Meier plotter online database suggested that patients with high BGN expression had a poor prognosis ($P=1.3e-10$). In addition, using gene sets analysis, we found that pathways of bladder cancer, Wnt-signaling, TGF-beta signaling, and ECM-receptor interaction were differentially activated in high-expression BGN tissues. In addition, we found that multiple signaling pathways are activated to varying degrees in the highly expressed BGN tissues. Furthermore, CIBERSORT analysis for the proportion of tumor initiating cells (TICs) revealed that macrophages M2 was positively correlated with BGN expression. We provided the first evidence that BGN overexpression predicts poor prognosis of GC and BGN is a potential biomarker for GC prognosis. The finding may have significant implication for the diagnosis, prognosis and treatment of GC.

Key words: Biglycan; Gastric cancer; TCGA; GSEA; CIBERSORT; Macrophages M2.

Introduction

Gastric cancer (GC) is a high mortality disease and is often found more frequently in men all over the world (1). Although the overall incidence of GC is declining, when the GC tissue infiltrates into submucosa, enters into muscle layer or has passed through muscle layer to serosa, the overall survival rate of advanced GC is less than one year (2). Even after various treatments, the 5 year survival rate was only 36%-47% 15-25% (3). Poor survival may be closely related to late detection. At present, the diagnosis of GC mainly depends on gastroscopy and nuclear magnetic resonance imaging. Moreover, researchers have recently proposed oncolytic virus-mediated fluorescence imaging combined with endoscopic imaging, which may improve the accurate imaging and treatment of gastrointestinal tumors (4). However, even these imaging modalities are ineffective in some patients with GC (5, 6). Therefore, it has clinical significance to discover effective biomarkers or target molecules for the diagnosis and treatment of GC.

Biglycan (BGN) encodes an extracellular matrix (ECM) proteoglycan that binds effectively to TGF- β (7) and has carcinogenic effect (8). BGN not only participates in the long-distance migration of cells, but also has the effect of adhesion (9). In previous reports, the expression level of BGN has been studied as a potential biomarker for

esophageal and lung cancer (10, 11). It has been shown that BGN can inhibit the proliferation of pancreatic cancer (12). BGN has the function of regulating the size of collagen fibers and may be deposited in blood vessels when combined with collagen (13). A large number of studies have pointed out that BGN plays an important and positive role in promoting angiogenesis (14). There is strong evidence for the involvement of BGN in neovascularization of tumors. In addition, BGN regulates osteocyte differentiation through BMP signaling pathway (15). However, the potential diagnostic and prognostic value of BGN in GC have not yet been determined.

Here, we provided the first evidence that BGN overexpression predicts poor prognosis of GC and BGN is a potential biomarker for GC prognosis. The higher expression of BGN in GC tissues compared to normal tissues was further validated using immunohistochemistry. We identified the correlation between BGN and clinical features of GC. Furthermore, BGN regulation signaling pathways in GC were explored and enriched by Gene Set Enrichment Analysis (GSEA). The prognostic role of BGN in GC and its relationship with immune infiltration were also studied. The finding may have significant implication for the diagnosis, prognosis and treatment of GC.

Materials and methods

Microarray data Oncomine analysis

The Oncomine database (<http://www.oncomine.org>) is an expression database for most cancer gene chips (16, 17). The database is a whole-genome expression analysis aimed at formulating new therapeutic goals. We investigated the transcriptional levels of BGN in GC and corresponding normal tissues by using Oncomine.

GEO microarray series (GSE29272, GSE29998, GSE54129, GSE66229) containing GC and non-tumor samples were obtained from (<http://www.ncbi.nlm.nih.gov/geo/>). In addition, the gene expression data of GC were also downloaded from the TCGA database (<https://cancergenome.nih.gov/>). The data were standardized, and \log_2 transformed. Gene expression of BGN was determined by GC and non-tumor samples. Meanwhile, relevant clinical information was obtained from the TCGA website. All analysis operating procedures were run by Perl and R software. All analysis operating procedures were run by Perl and R software (18).

Immunohistochemistry and evaluation of immunostaining intensity

The study consisted of 30 samples from 15 patients diagnosed with GC. All participants signed informed consent forms. Samples were collected and approved by the Ethics Committee of the Jingzhou First People's Hospital, Yangtze University. The paraffin section of GC tissue was dewaxed, and the endogenous peroxidase was blocked by 3% H₂O₂, the surface antigen was repaired by microwave oven, and goat serum was blocked. The appropriate amount of primary antibody BGN (1 : 300, ab209234; Abcam) was added, overnight at 4 °C. After rewarming, the second antibody was added and incubated in 37 °C incubator for 60 min, then 3, 3-diaminobenzidine tetrahydrochloride (DAB) chromogenic solution was added. Then add hematoxylin, dehydrate step by step, seal the film and observe under microscope. ImagePro Plus 6.0 was used to analyze and count

the results. The relative expression of BGN was measured by the integrated optical density (IOD).

Gene Set Enrichment Analysis

GSEA is a method of enrichment analysis and calculation based on existing gene sets (19). GSEA could determine which group of predefined genes was closer to the high BGN group and the low BGN group to find significant differential expression. Genome replacement tests were set up by 1000 times. The BGN gene expression was used as phenotype label. $p < 0.05$ and $q\text{-value} < 0.25$ were set as the cut-off criteria.

TICs Profile

CIBERSORT calculation method was used to estimate the TICs abundance spectrum of all GC tumor samples. After mass filtration, the correlation of 22 kinds of infiltrating immune cells was visualized. Spearman correlation analysis was conducted between BGN gene and TICs, and the results were displayed.

Statistical analysis

The significance between characteristic clinical information in GC and genes, such as BGN, were determined by Kruskal-Wallis test, Wilcoxon test and logistic regression method. The expression of BGN in normal tissues and GC tissues was shown by scatter plot and paired difference analysis plot. The Kaplan-Meier plotter database (www.kmplot.com) can be used to analyze the survival trends of 54675 genes, including 1440 GC samples. Kaplan-Meier plotter online database was used to analyze the overall survival rate of BGN in GC patients. The clinical information data of GC were analyzed by univariate Cox, and potential positive variables were selected. The correlation between BGN expression and survival along with other clinical factors was compared by multivariate Cox analysis ($p < 0.05$).

Results

The mRNA expression of BGN in GC

The expression of BGN in various tumor types and corresponding normal tissues was analyzed by

Oncomine database (Figure 1A). The result shows that there are 421 unique analyses for BGN, respectively. In GC, BGN was significantly increased in 73 datasets and 5 datasets showed a reduced level. We performed a meta-analysis of BGN expression in 6 analyses with a threshold set as $p\text{-value} \leq 1E-4$, fold change ≥ 2 and top 10% gene rank in the Oncomine database (Figure 1B, $p < 1.24E-6$). This analysis showed that high expression of BGN in GC tissues, and its gene rank was higher, with statistical significance. The details of the GEO series were summarized in Table 1.

As illustrated in Figure 2, the expression of BGN in GC samples was all significantly higher than non-tumor samples in GSE29272, GSE29998, GSE54129 and GSE66229 (Figure 2A-D, all $p < 0.05$).

Interestingly, the expression level of BGN was also found to be significantly higher in GC than in normal tissues in TCGA datasets (Figure 2E, $p < 1.159e-16$). The paired difference analysis also showed that BGN expression was higher in GC than that in normal tissues (Figure 2F, $p < 1.527e-9$).

Immunohistochemical analysis of BGN in GC

We have collected 15 clinical GC cases and determined the BGNs' protein expression in GC tissues and normal tissues using immunohistochemistry (IHC). The positive expression of BGN was shown as brown granules in cytoplasm (Figure 3A). We found that BGN are highly expressed in the GC tissues than that in the normal tissues (Figure 3B, $p < 0.001$).

GC patient characteristics

A total of 443 GC samples were downloaded from the TCGA online website, which included clinical information and gene expression datasets (Table 2). The average age of GC patients was 67 years old, 47.63% of the patients were younger than 67 years old, 52.37% of the patients were older than or equal to 67 years old. The gender analysis showed 158 females (35.67%) and 285 males (64.33%).

Among the patients, the histological types of GC could be classified into G1 (2.71%, $n = 12$), G2 (35.89%, $n = 159$), G3 (59.37%, $n = 263$) and GX (2.03%, $n = 9$). These patients were at all stages I-IV. Stage I disease was discovered in 63 patients (14.22%), stage II in 132 (29.80%), stage III in 201 (45.37%) and stage IV in 47 (10.61%). According to the TNM classification, samples from our study included 5.19% ($n = 23$) at T1, 20.77% ($n = 92$) at T2, 44.70% ($n = 198$) at T3, 26.86% ($n = 119$) at T4 and 2.26% ($n = 10$) at TX; M0 in 391 patients (88.26%), M1 in 30 (6.77%), MX in 22 (4.97%); N0 in 133 patients (30.02%), N1 in 119 (26.86%), N2 in 85 (19.19%), N3 in 88 (19.86%), NX in 18 (4.06%). One hundred and seventy-three of 443 patients (39.05%) had died.

BGN overexpression in GC

As shown in Fig. 4A-E BGN expression was closely related to clinical stage ($p = 0.002$), histological grade ($p = 0.004$), and T classification ($p = 6.605e-6$). As an independent variable, BGN has a certain correlation with other clinical features (Table 3). Relevance was judged by logistic regressive univariate analysis. Increased BGN expression in GC was statistically significantly correlated with higher stages (OR = 3.40 for I vs. II, and OR = 3.18 for I vs. III) and higher T classes (OR = 12 for T1 vs. T2, OR = 21.27 for T1 vs. T3, and OR = 24.86 for T1 vs. T4). The results suggest that BGN overexpression in GC was more likely to develop into advanced cancer and worse prognosis.

Survival outcomes, univariate and multivariate Cox analyses

By using Kaplan Meier plotter analysis, we found that BGN overexpression in GC had a worse overall survival rate ($p = 1.3e-10$, Figure 5). The median OS of the low expression of BGN group was 89.43 months, while the median OS of the high expression of BGN group was 23.6 months.

Because survival was significantly correlated with BGN expression in GC patients. According to Cox proportional hazard regression model, 375 patients with GC were analyzed by univariate and multivariate analysis to evaluate the effect of BGN

expression and other clinicopathological factors on survival rate.

We showed that high expression of BGN was associated with poor survival (hazard ratio [HR] = 1.20, 95% confidence interval [CI]: 1.04-1.37, $p = 0.009$). Higher age, stages, TNM stages were also found to be associated with lower survival (Table 4). In multivariate analysis, BGN, along with age, gender and N/M classification, was shown to be an independent factor in the overall survival rate of GC (hazard ratio [HR] = 1.16, 95% confidence interval [CI]: 1.00-1.34, $p = 0.047$, Figure 6A).

GSEA identifies a BGN-related signaling pathway

To find signaling pathways associated with BGN in GC, we explored the function of BGN and its related signal transduction pathway through GSEA. The result showed significant differences ($FDR < 0.25$, $NOM\ p < 0.05$) in the enrichment of MSigDB Collection (c2.cp.kegg.v6.2.symbols.gmt). We chose the most significant five up-regulated signaling pathways (Table 5). Gene sets related to bladder cancer, Wnt-signaling pathway, TGF- β signaling pathway, ECM-receptor interaction, and pathways in cancer were differentially enriched in BGN overexpression phenotype (Figure 6B).

Immune Cell Infiltration Results

To further validate the correlation of BGN expression with the immune microenvironment, the proportion of tumor-infiltrating immune subsets was analyzed using CIBERSORT algorithm, and 21 different kinds of immune cell profiles in GC samples were constructed (Figure 7A-B).

Correlation Analysis between BGN and Infiltrating Immune Cells

The violin plot of the immune cell infiltration difference showed that, plasma cells decreased in the high expression of BGN group, while macrophages M2 showed an increase in the high BGN expression group (Figure 8A). Correlation analysis showed that BGN was positively correlated with macrophages M2 ($r = 0.26$, $p = 0.0031$), and negatively correlated with activated dendritic cells ($r = -0.2$, $p = 0.023$) and Plasma cells (Figure 8B, $r = -0.3$, $p = 0.00057$). The results from the difference and correlation analyses showed that a total of two kinds of TICs (M2 macrophage and plasma cells) were correlated with the expression of BGN (Figure 8C).

Table 1. Table 1 Details of GEO series included in this analysis.

GEOseries	Contributor(s)	Year	Tumor	Non-Tumor	Platform
GSE29272	Wang G et al.,	2011	134	134	[HG-U133A] Affymetrix Human Genome U133A Array
GSE29998	Holbrook JD	2011	49	50	Illumina HumanHT-12 V3.0 expression beadchip
GSE54129	Liu B et al.	2014	21	111	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0
GSE66229	Oh SC et al.,	2015	100	300	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0

Table 2 Clinical characteristics of the gastric cancer patients.

Characteristic	n (%)
Age	
<67 years	211 (47.63)
≥ 67 years	232 (52.37)
Gender	
Female	158 (35.67)
Male	285 (64.33)
Histological grade	
G1	12 (2.71)
G2	159 (35.89)
G3	263 (59.37)
GX	9 (2.03)

Stage	
I	63 (14.22)
II	132 (29.80)
III	201 (45.37)
IV	47 (10.61)
T classification	
T1	23 (5.19)
T2	92 (20.77)
T3	198 (44.70)
T4	119 (26.86)
TX	10 (2.26)
M classification	
M0	391 (88.26)
M1	30 (6.77)
MX	22 (4.97)
N classification	
N0	133 (30.02)
N1	119 (26.86)
N2	85 (19.19)
N3	88 (19.86)
NX	18 (4.06)
Status	
Alive	270 (60.95)
Dead	173 (39.05)

Table 3. Biglycan expression associated with clinical characteristics (logistic regression).

clinical characteristics	Total(N)	Odds ratio in BGN expression	p-Value
Age(continuous)	375	0.782 (0.520-1.174)	0.236
Grade			
G1 vs. G2	147	1.069 (0.292-4.341)	0.921
G1 vs. G3	229	1.887 (0.524-7.554)	0.336
Stage			
I vs. II	164	3.399 (1.692-7.138)	0.001
I vs. III	203	3.184 (1.629-6.523)	0.001
I vs. IV	91	3.441 (1.441-8.515)	0.066
T classification			
T1 vs. T2	99	12 (2.297-221.218)	0.018
T1 vs. T3	187	21.272 (4.242-387.099)	0.003
T1 vs. T4	119	24.857 (4.839-455.953)	0.002
M classification (m0 vs. m1)	355	0.976 (0.786-1.211)	0.826
N classification			
N0 vs. N1	208	0.914 (0.628-1.326)	0.635
N0 vs. N2	186	1.164 (0.675-2.012)	0.585
N0 vs. N3	185	0.958 (0.532-1.723)	0.885

Notes: Categorical dependent variable, greater or less than the median expression level.

Table 4. Univariate analysis and multivariate analysis of the correlation of biglycan expression with overall survival among gastric cancer patients.

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	p	HR	95% CI	p
Age	1.02	1.00-1.04	0.021	1.03	1.01-1.05	0.001
Gender	1.46	1.00-2.12	0.050	1.48	1.01-2.16	0.042
grade	1.40	0.99-1.96	0.055	1.35	0.95-1.93	0.091
Stage	1.45	1.17-1.78	0.000	1.01	0.68-1.51	0.948
T classification	1.31	1.06-1.62	0.014	1.20	0.90-1.60	0.204
N classification	1.33	1.14-1.55	0.000	2.18	1.03-4.59	0.041
M classification	1.96	1.08-3.56	0.026	1.26	1.01-1.58	0.043
BGN	1.20	1.04-1.37	0.009	1.16	1.00-1.34	0.047

Table 5 Gene sets enriched in the high and low biglycan expression phenotype.

Gene set name	NES	NOM <i>p</i> -value	FDR <i>q</i> -value
KEGG_BLADDER_CANCER	1.832	0.006	0.021
KEGG_ECM_RECEPTOR_INTERACTION	2.473	0.000	0.000
KEGG_PATHWAYS_IN_CANCER	2.074	0.000	0.003
KEGG_TGF_BETA_SIGNALING_PATHWAY	2.125	0.002	0.003
KEGG_WNT_SIGNALING_PATHWAY	1.880	0.004	0.014

Abbreviation: NES: normalized enrichment score; NOM: nominal; FDR: false discovery rate. Gene sets with NOM *p*-value < 0.05 and FDR *q*-value < 0.25 were considered as significantly enriched.

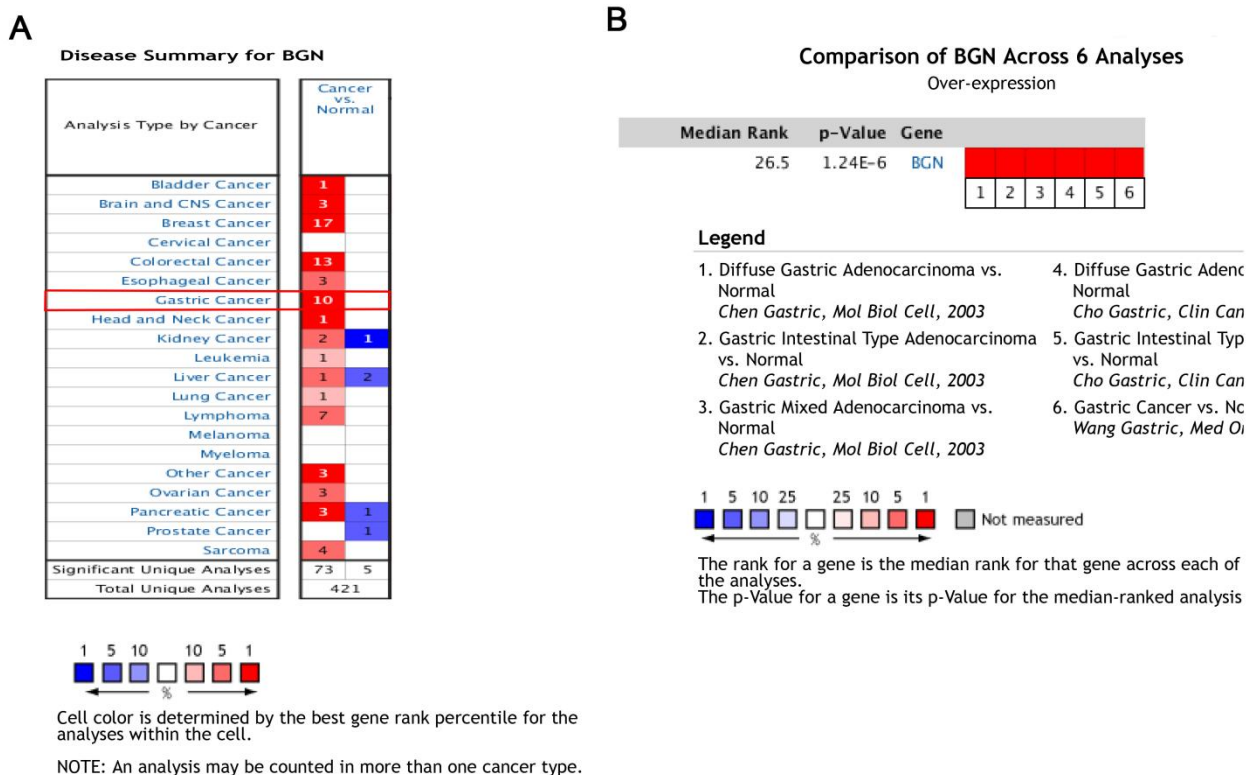


Figure 1. OncoPrint analysis of the mRNA expression levels of biglycan in different cancers. (A) The differences in expression levels of the biglycan genes between tumor and normal tissues are summarized. The red box indicates the high expression of biglycan in gastric cancer tissues. Red cells represent overexpression of the biglycan gene in tumor tissues compared to normal tissues, whereas blue cells indicate downregulation of the biglycan gene. (B) Meta-analysis results show that the expression of biglycan in gastric cancer tissues is significantly higher than that of normal tissues (*p* < 0.05).

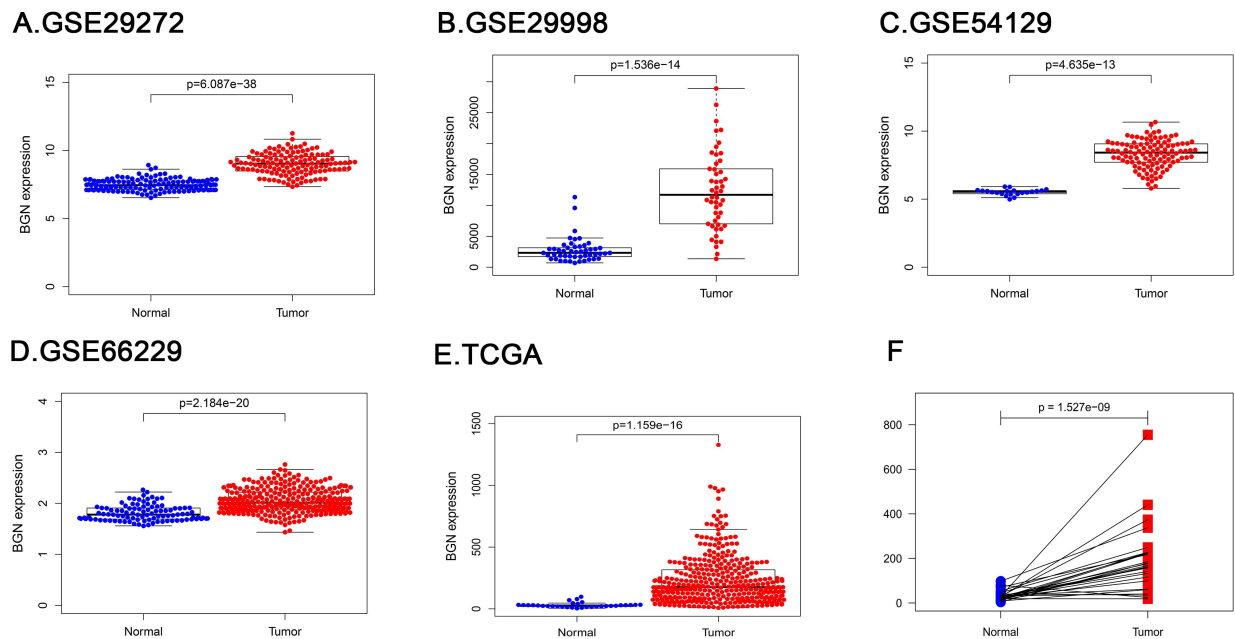


Figure 2. The mRNA expression levels of biglycan between tumor and normal samples in gastric cancer patients on the GEO database series including GSE29272 (A), GSE29998 (B), GSE54129 (C), GSE66229 (D), TCGA database (E, F). The blue points represent normal, red points represent gastric cancer, and "/" means that biglycan is upregulated between gastric cancer tissues and adjacent tissues in the same sample. $p < 0.05$ was considered statistically significant. GEO: Gene Expression Omnibus, The Cancer Genome Atlas (TCGA).

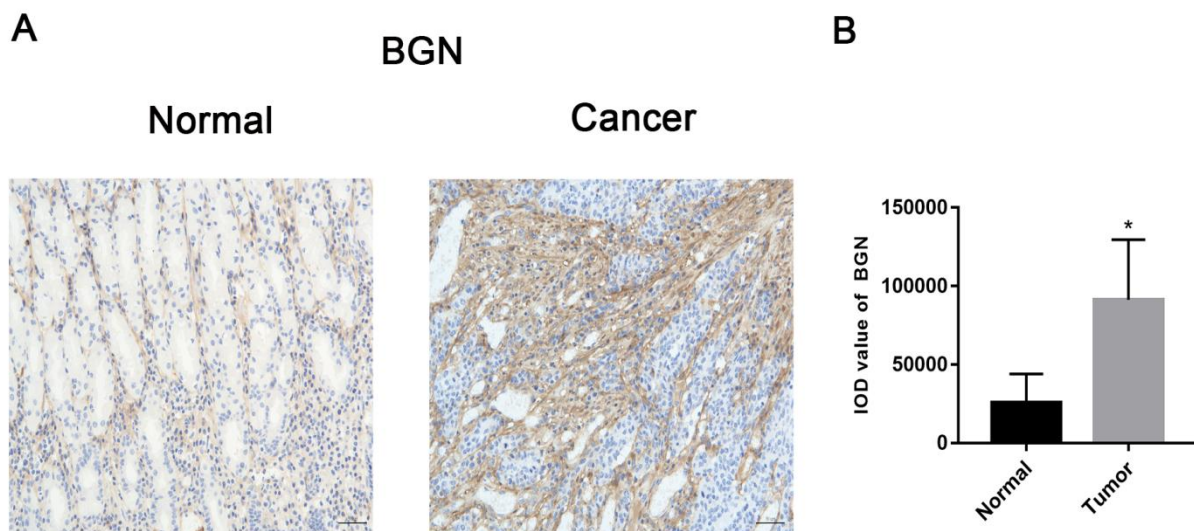


Figure 3. (A) Representative adjacent normal gastric tissues and gastric cancer tissues staining. (B) The protein expression of biglycan in gastric cancer tissues and their adjacent normal gastric tissues were compared using immunohistochemistry staining ($p < 0.001$).

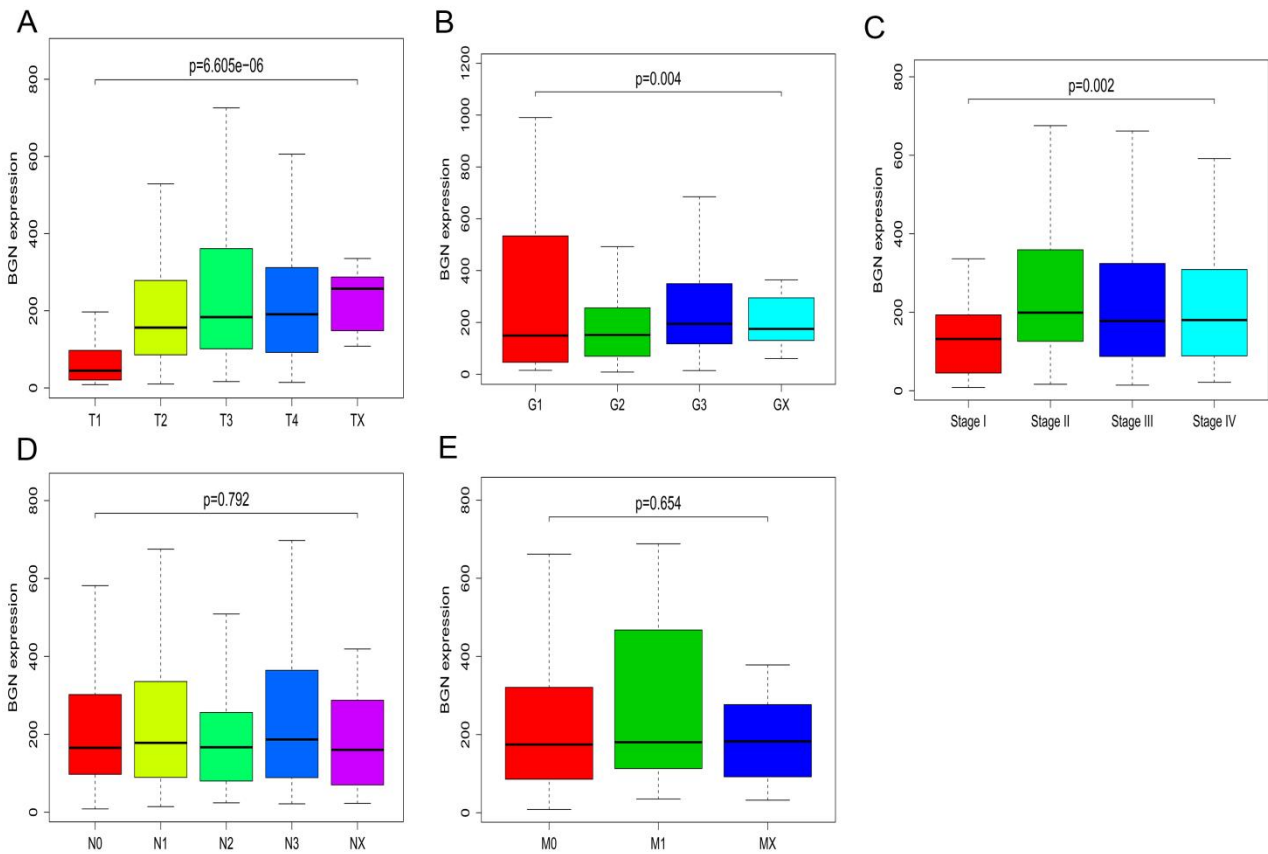


Figure 4. Biglycan expression in gastric cancer. (A) T classification; (B) histologic grade; (C) clinical stage; (D) N classification; (E) M classification. TX, GX, NX and MX means patient information were incomplete.

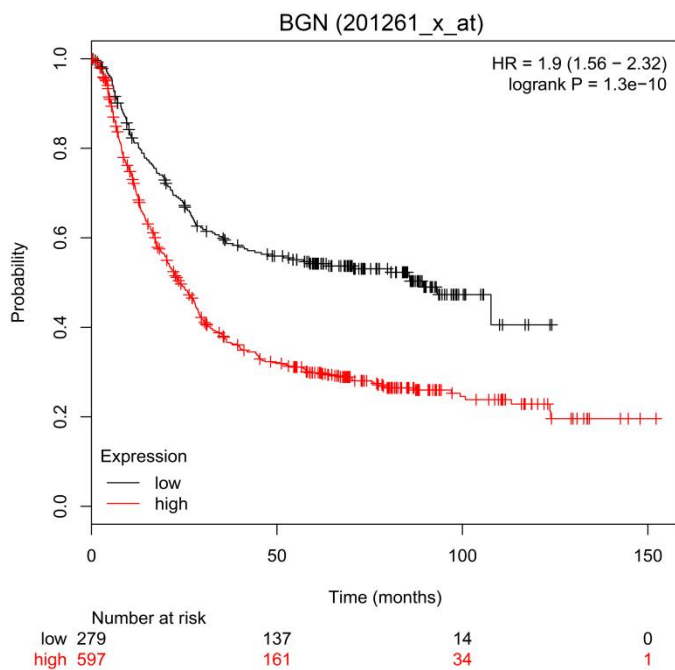


Figure 5. The survival prognostic value of biglycan expression on overall survival in gastric cancer patients. Red lines represent high expression of genes in gastric cancer, and black lines represent low expression of genes in gastric cancer.

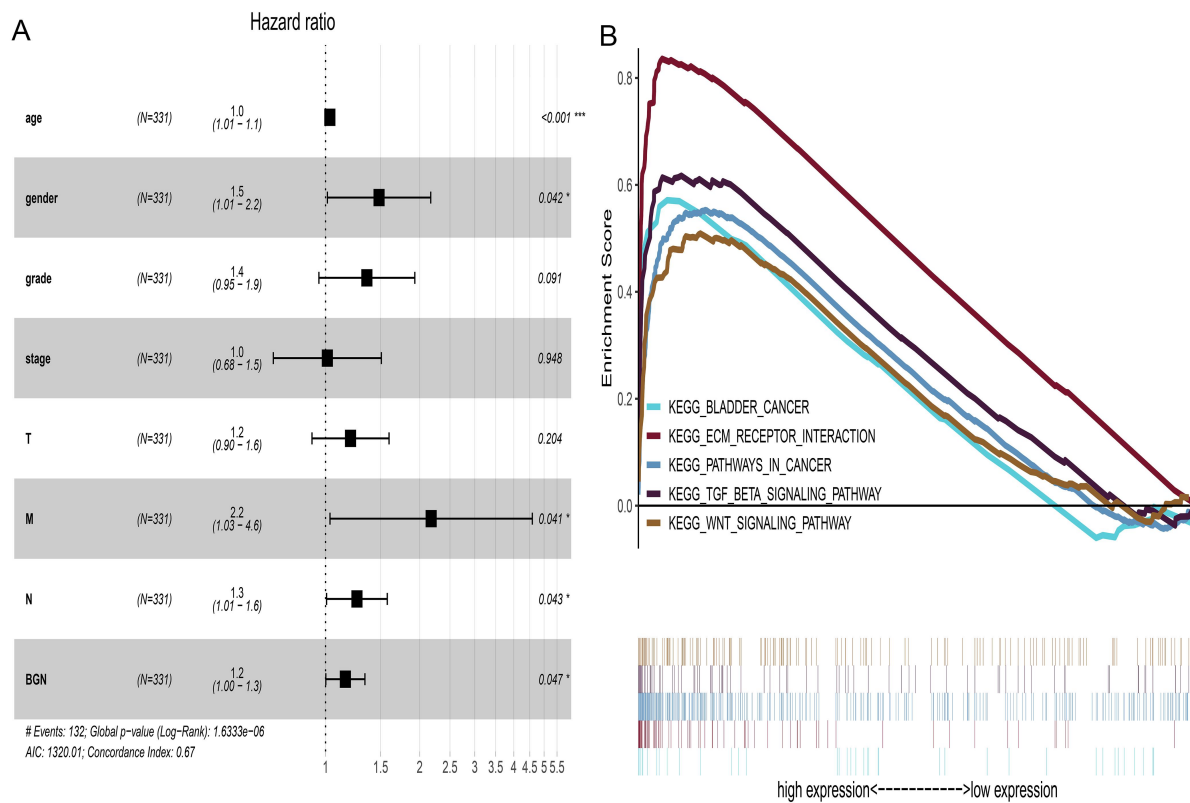


Figure 6. (A) Multivariate Cox regression analysis of risk factors associated with overall survival among gastric cancer patients. Squares represent the hazard ratio for each subject. The horizontal line represents the connection between the upper and lower limits of the 95% confidence interval for each study. Vertical line represents hazard ratio =1. (B) Enrichment plots from GSEA. Gene sets related to bladder cancer, ECM-receptor interaction, pathways in cancer, TGF-β signaling pathway and wnt-signaling pathway were enriched with the high biglycan expression phenotype in gastric cancer cases. GSEA, gene set enrichment analysis, KEGG, Kyoto Encyclopedia of Genes and Genomes.

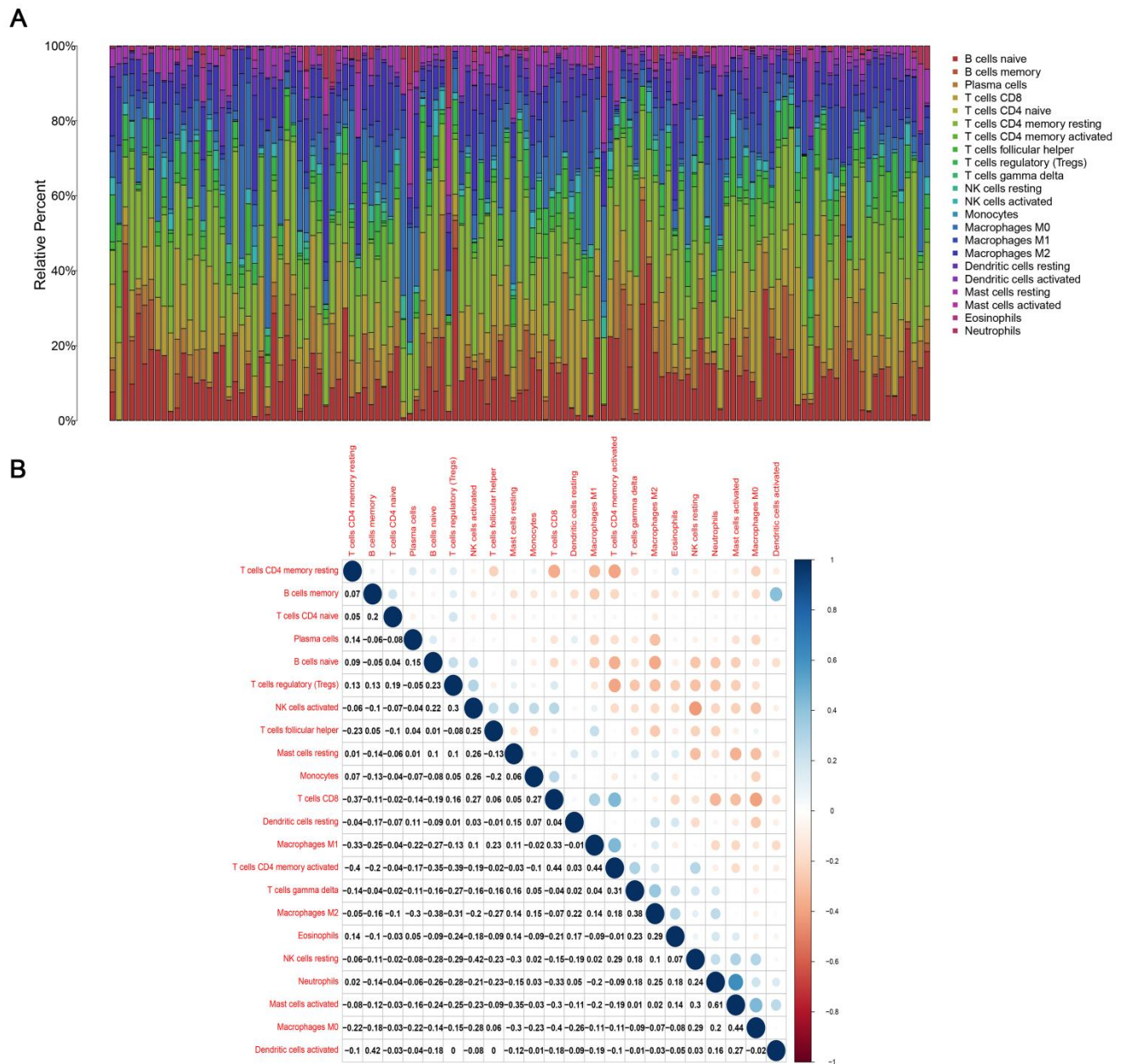


Figure 7. TIC profile in tumor samples and correlation analysis. (A) Barplot showing the distribution of 22 TICs in gastric cancer and normal samples. Column names of plot were sample ID. (B) Heatmap showing the correlation between 22 kinds of TICs and numeric in each tiny box indicating the p value of correlation between two kinds of cells. Blue represents a positive correlation, red represents a negative correlation. The darker the color, the stronger the correlation. TIC: Tumor-infiltrating Immune Cell.

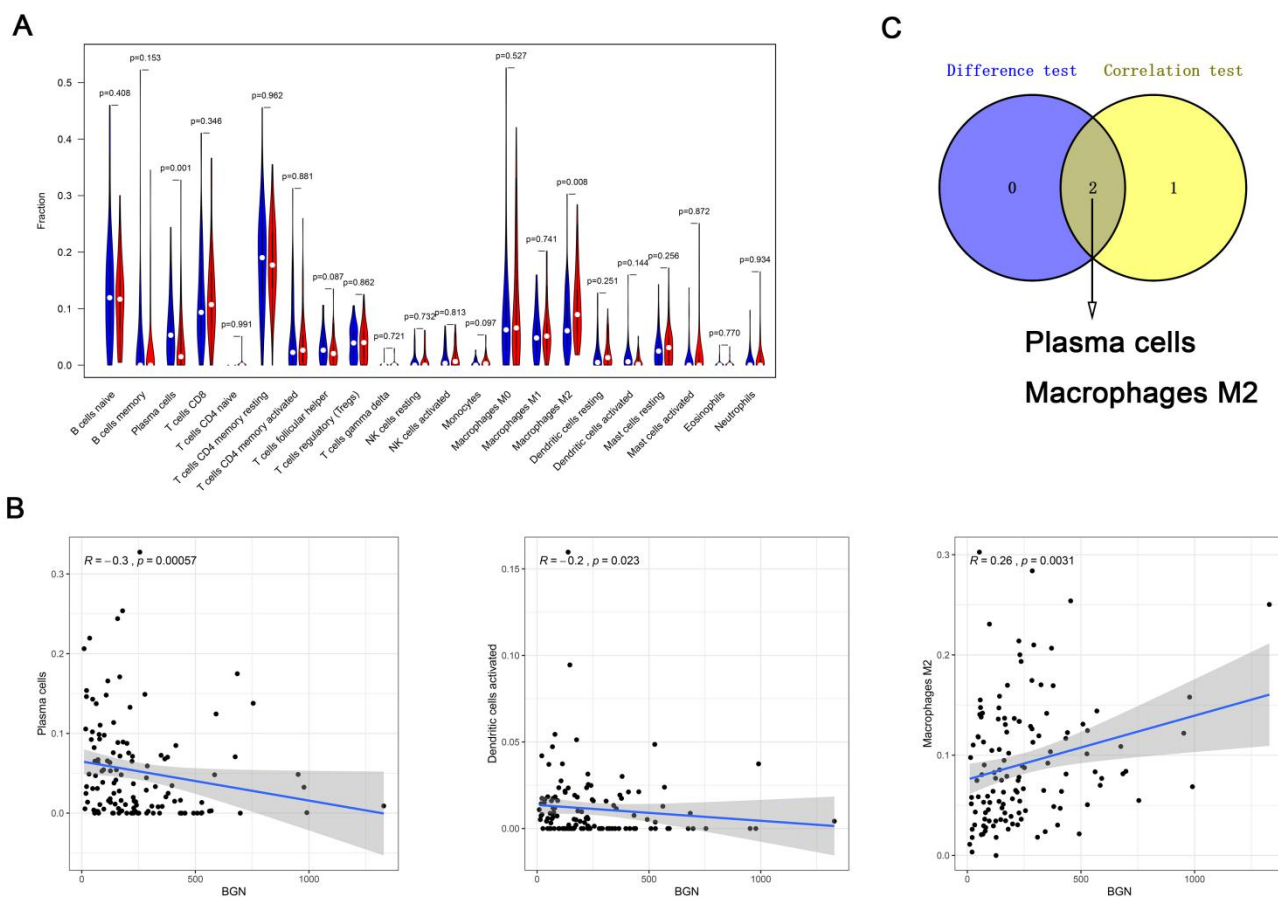


Figure 8. Correlation of TICs proportion with biglycan expression. (A) Violin plot showed the ratio differentiation of 22 kinds of immune cells between gastric cancer tumor samples with low (blue color) or high (red color) biglycan expression relative to the median of biglycan expression level. (B) Scatter plot showed the correlation of three kinds of TICs proportion with the biglycan expression ($p < 0.05$). The blue line in each plot was fitted linear model indicating the proportion tropism of the immune cell along with biglycan expression. (C) Venn plot displayed two kinds of TICs correlated with biglycan expression codetermined by difference and correlation tests displayed in violin and scatter plots, respectively.

1. Discussion

We found that BGN overexpression is positively correlated with the progression and worse survival of GC patients. BGN encodes a proteoglycan, which belongs to an ECM protein (20). ECM proteins play an important role in the tumor microenvironment of GC (21). Interestingly, BGN may influence the progression of GC by promoting neovascularization (22). Although Wang et al. (23) considered that BGN was related to the prognosis of GC, but did not provide specific survival analysis, and did not elaborate on the content of immune cell infiltration in the tumor microenvironment of GC. Based on the literature

search, the impact of BGN expression on GC prognosis has not been fully studied yet. For the first time, our study comprehensively analyzed the transcriptional level and prognostic value of BGN in GC. Our results showed that the mRNA level of BGN in GC tissue was increased compared with normal tissue, and the protein expression of BGN was also higher than normal tissue. Therefore, this study provided the first evidence that BGN overexpression predicts poor prognosis of GC and BGN is a potential biomarker for GC prognosis. The finding may have significant implication for the diagnosis and treatment of GC and the evaluation of patients' prognosis.

At present, the diagnosis and prognosis of GC are based on clinical classification, pathological staging, and histological grading. We found that the expression of BGN statistically significantly increased gradually with histological grades T1/T2/T3/T4 and G1/G2/G3. The expression of BGN in stage I/II was also relatively high. The univariate and multivariate Cox analysis provides evidence that BGN may be an independent biomarker for the prognosis of GC patients. Sun et al. (24) and Jacobsen et al. (25) revealed that BGN expression was up-regulated in endometrial and prostate cancer respectively. Li et al. demonstrated that over-expression of BGN activated the TGF- β /Snail signaling pathway leading to poor survival in patients with colon cancer (26). These reports are consistent with our findings.

To understand the role and mechanisms of BGN in GC, we use GSEA to enrich and analyze the signaling pathways. We found that Wnt signaling pathway, TGF- β signaling pathway, ECM-receptor interaction, and the bladder cancer pathways were significantly enriched in the BGN over-expression tissues. Previous studies suggest that BGN was involved in the transformation, progression and metastasis of many tumors. Sun et al. found that BGN in vitro promoted endothelial cell proliferation of endometrial cancer (23). Hu et al. revealed that BGN could enhance angiogenesis in the TLR signaling pathway (27). Subbarayan et al. showed that BGN was involved in the immune escape mechanism of breast cancer cells under the regulation of HER-2/neu (8). Liu et al. proved that in colon cancer SW-480 cells, overexpression of BGN activates NF- κ B signaling pathway to promote the development of multidrug resistant colon cancer (28). Hakur et al. demonstrated that TAP73 regulated the activation of TGF- β signaling pathway by BGN and has a carcinogenic effect (29). This report is consistent with our identification of TGF- β signaling pathway in GC. Recent studies have shown that EREG gene can be used as a marker of independent prognosis of GC (30). There is no report of Wnt signaling pathway in relation to

BGN in any cancers. Further mechanistic studies are needed.

Nowadays, an increasing interest in tumor immune infiltrates was paid in tumor immunotherapy. To further explore the role of immune cell infiltration in GC, we used CIBERSORT to conduct a comprehensive evaluation of GC immune infiltration. In this study, the CIBERSORT analysis for the proportion of tumor-infiltrating immune cell revealed that macrophages M2 was positively correlated with BGN expression in GC patients. In addition, there was a negative correlation between plasma cells and BGN expression in GC patients. We suggest that an increased infiltration of macrophages M2 and a decreased infiltration of plasma cells may be related to the occurrence and development of BGN high expression tumors in GC. Recent studies have found that macrophage M2 polarization in tumor infiltrating macrophages is positively correlated with tumor invasion, tumor angiogenesis and poor prognosis (31). Macrophages M2 are usually induced by IL-4 and IL-13, inhibit the activation and proliferation of lymphocytes, secrete matrix metalloproteinases (MMPs), and promote the invasion of cancer cells. Studies have shown that the more macrophages M2 infiltration in tumor tissues, the worse the clinical prognosis of patients (32). Zhang Yu et al. found that down regulation of mir-130b-3p in macrophages M2 inhibited tumor formation and angiogenesis of GC (33). The expression of macrophages M2 may be related to the prognosis of GC, and the prognosis of GC with high tumor-associated macrophages expression is poor. Many scholars have shown that the survival rate of macrophages M2 high expression patients is significantly lower than that of low expression patients ($p < 0.05$) (34, 35). It can be speculated that understanding the expression of macrophages M2 in GC tissues has a certain clinical value in predicting the prognosis of GC. This study found that the positive correlation between the amounts of macrophages M2 and BGN expression in GC patients. These results revealed the possibility that

BGN may be closely involved in the tumor immunity in GC. BGN may promote the immune escape of cancer cells of GC by promoting M2 polarization of macrophages, thus affecting tumor growth and prognosis.

Declarations

1) Consent to publication

All authors agreed to publish the manuscript at this journal based on the signed Copyright Transfer Agreement, and followed publication ethics.

2) **Ethical approval and consent to participants**
Not applicable.

3) **Disclosure of conflict of interests**
We declare that no conflict of interest exists.

4) **Funding**
This work was supported by Joint Foundation of the Health Commission of Hubei Province (WJ2018H173), Central Special Foundation for Guiding Local Science and Technology Development of Hubei Province (2019ZYD066) and National Natural Science Foundation of China (81271872 and 81872412).

5) **Availability of data and material**
Our data used to support the findings of this study are all included within the article.

6) **Authors' Contributions**
QG organized the article. XH, HC and WS perform bioinformatics analyses and wrote the draft. YW, MZ, HZ, NL and BZ edited the language, figure, table and discussion of the article. QG, KL and HX designed the experiment, and supervised the project. XH, HC and YW contributed equally to the manuscript.

7) **Acknowledgement**
We thank Dr. Zhaowu Ma for critically editing the manuscript.

References

1. Muhammad D, Saeed A, Mukhtiar A, Hafiz Muhammad A, Muhammad A, Saif UR, et al. Risk Factors and Epidemiology of Gastric Cancer in

Pakistan. *Asian Pacific Journal of Cancer Prevention Apjcp*. 2015;16(12):4821-4.

2. Zhang XY, Zhang PY. Gastric cancer: somatic genetics as a guide to therapy. *Journal of medical genetics*. 2017;54(5):305-12.

3. Zhu C, Xia Q, Gu B, Cui M, Zhang X, Yan W, et al. Esophageal Cancer Associated Immune Genes as Biomarkers for Predicting Outcome in Upper Gastrointestinal Tumors. *Frontiers in genetics*. 2021;12:707299.

4. Wu ZJ, Tang FR, Ma ZW, Peng XC, Xiang Y, Zhang Y, et al. Oncolytic Viruses for Tumor Precision Imaging and Radiotherapy. *Human gene therapy*. 2018;29(2):204-22.

5. Liu CT, Hong CQ, Huang XC, Li EM, Xu YW, Peng YH. Blood-based Markers in the Prognostic Prediction of Esophagogastric Junction Cancer. *Journal of Cancer*. 2020;11(15):4332-42.

6. Yang LS, Hartley I, Thompson AJ, Desmond P, Taylor ACF, Moss A, et al. Evaluation of Endoscopic Practices and Outcomes in Follow-up of Gastric Ulcers. *Journal of clinical gastroenterology*. 2021.

7. Cho SY, Bae JS, Kim ND, Forzano F, Girisha KM, Baldo C, et al. BGN Mutations in X-Linked Spondyloepimetaphyseal Dysplasia. *American Journal of Human Genetics*. 2016;98(6):1243-8.

8. Subbarayan K, Leisz S, Wickenhauser C, Bethmann D, Massa C, Steven A, et al. Biglycan-mediated upregulation of MHC class I expression in HER-2/neu-transformed cells. *Oncoimmunology*. 2018;7(4):00-.

9. Poluzzi C, Nastase MV, Zeng-Brouwers J, Roedig H, Hsieh LT, Michaelis JB, et al. Biglycan evokes autophagy in macrophages via a novel CD44/Toll-like receptor 4 signaling axis in ischemia/reperfusion injury. *Kidney international*. 2019;95(3):540-62.

10. Di Y, Chen D, Yu W, Yan L. Bladder cancer stage-associated hub genes revealed by WGCNA co-expression network analysis. *Hereditas*. 2019;156(1):7.

11. Morimoto H, Hida Y. Biglycan, tumor endothelial cell secreting proteoglycan, as possible biomarker for lung cancer. 2021;12(9):1347-57.
12. Wen-Bin C, Wolfgang L, Karen T, Fischer JW, Holger K, Hendrik U. Smad4/DPC4-dependent regulation of biglycan gene expression by transforming growth factor-beta in pancreatic tumor cells. *Journal of Biological Chemistry*. 2002;277(39):36118-28.
13. Xiaojie Z, Soo Jung L, Young MF, Wang MM. The small leucine-rich proteoglycan BGN accumulates in CADASIL and binds to NOTCH3. *Translational Stroke Research*. 2015;6(2):148-55.
14. Pinto F, Santos-Ferreira L, Pinto M, Gomes C, Reis C. The Extracellular Small Leucine-Rich Proteoglycan Biglycan Is a Key Player in Gastric Cancer Aggressiveness. *Cancers*. 2021;13(6).
15. Cui Q, Xing J, Yu M, Wang Y, Xu J, Gu Y, et al. Mmu-miR-185 depletion promotes osteogenic differentiation and suppresses bone loss in osteoporosis through the Bgn-mediated BMP/Smad pathway. *Cell Death Dis*. 2019;10(3):172.
16. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia*. 2004;6(1):1-6.
17. Zhou Q, Zhang F, He Z, Zuo MZ. E2F2/5/8 Serve as Potential Prognostic Biomarkers and Targets for Human Ovarian Cancer. *Frontiers in oncology*. 2019;9:161.
18. Morgan AP. argyle: An R Package for Analysis of Illumina Genotyping Arrays. G3 (Bethesda, Md). 2015;6(2).
19. Croken MK, Qiu W, White MW, Kim K. Gene Set Enrichment Analysis (GSEA) of *Toxoplasma gondii* expression datasets links cell cycle progression and the bradyzoite developmental program. *BMC Genomics*,15,1(2014-06-24). 2014;15(1):515.
20. Ito M, Ohno K. Protein-anchoring therapy to target extracellular matrix proteins to their physiological destinations. *Matrix biology : journal of the International Society for Matrix Biology*. 2018;68-69:628-36.
21. Lu Y, Kong X, Zhong W, Hu M, Li C. Diagnostic, Therapeutic, and Prognostic Value of the Thrombospondin Family in Gastric Cancer. *Frontiers in molecular biosciences*. 2021;8:647095.
22. Pinto F, Santos-Ferreira L. The Extracellular Small Leucine-Rich Proteoglycan Biglycan Is a Key Player in Gastric Cancer Aggressiveness. 2021;13(6).
23. Wang B, Li GX, Zhang SG, Wang Q, Wen YG, Tang HM, et al. Biglycan expression correlates with aggressiveness and poor prognosis of gastric cancer. *Experimental Biology & Medicine*. 2011;236(11):1247-53.
24. Sun H, Wang X, Zhang Y, Che X, Liu Z, Zhang L, et al. Biglycan enhances the ability of migration and invasion in endometrial cancer. *Archives of Gynecology & Obstetrics*. 2015;293(2):429.
25. Jacobsen F, Kraft J, Schroeder C, Hube-Magg C, Kluth M, Lang DS, et al. Up-regulation of Biglycan is Associated with Poor Prognosis and PTEN Deletion in Patients with Prostate Cancer. *Neoplasia*. 2017;19(9):707-15.
26. Li H, Zhong A, Li S, Meng X, Wang X, Xu F, et al. The integrated pathway of TGF β /Snail with TNF α /NF κ B may facilitate the tumor-stroma interaction in the EMT process and colorectal cancer prognosis. *Sci Rep*. 2017;7(1):4915.
27. Lei H, Zang MD, Wang HX, Li JF, Su LP, Min Y, et al. Biglycan stimulates VEGF expression in endothelial cells by activating the TLR signaling pathway. *Molecular Oncology*. 2016;10(9):1473-84.
28. Liu B, Xu T, Xu X, Cui Y, Xing X. Biglycan promotes the chemotherapy resistance of colon cancer by activating NF- κ B signal transduction. *Molecular & Cellular Biochemistry*. 2018;449(21):1-10.
29. Thakur AK, Nigri J, Lac S, Leca J, Bressy C, Berthezene P, et al. TAp73 loss favors Smad-independent TGF- β signaling that drives EMT in pancreatic ductal adenocarcinoma. *Cell Death & Differentiation*. 2016;23(8):1358-70.

30. Xia Q, Zhou Y, Yong H, Wang X, Zhao W, Ding G, et al. Elevated epiregulin expression predicts poor prognosis in gastric cancer. *Pathol Res Pract*. 2019;215(5):873-9.
31. Lu Z, Zheng S, Liu C, Wang X, Zhang G, Wang F, et al. S100A7 as a potential diagnostic and prognostic biomarker of esophageal squamous cell carcinoma promotes M2 macrophage infiltration and angiogenesis. 2021;11(7):e459.
32. Yan X, Feng J, Hong B, Qian Y. The Expression of PD-L1 and B7-H4 in Thymic Epithelial Tumor and Its Relationship With Tumor Immune-Infiltrating Cells. *Frontiers in oncology*. 2021;11:662010.
33. Zhang Y, Meng W, Yue P, Li X. M2 macrophage-derived extracellular vesicles promote gastric cancer progression via a microRNA-130b-3p/MLL3/GRHL2 signaling cascade. *Journal of experimental & clinical cancer research : CR*. 2020;39(1):134.
34. Liu J, Ji C, Wang Y, Zhang C, Zhu H. Identification of methylation-driven genes prognosis signature and immune microenvironment in uterus corpus endometrial cancer. 2021;21(1):365.
35. Liu L, Wang C, Li S, Qu Y, Xue P, Ma Z, et al. ERO1L Is a Novel and Potential Biomarker in Lung Adenocarcinoma and Shapes the Immune-Suppressive Tumor Microenvironment. *Frontiers in immunology*. 2021;12:677169.