Int J Clin Exp Pathol 2018;11(10):4836-4844
www.ijcep.com /ISSN:1936-2625/IJCEP0080281

*Original Article*

# A study on the correlation of MyD88 expression with gastric cancer

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Received May 25, 2018; Accepted August 28, 2018; Epub October 1, 2018; Published October 15, 2018

**Abstract:** Objective: The myeloid differentiation factor-88 (MyD88) plays a key role in mediating the innate immune signal transduction of toll-like receptor (TLR) and interleukin-1 (IL-1) family members, and it also participates in the regulation of tumorigenesis in various cancer models Our study sought to determine whether there is any correlation with MyD88 and the development of gastric cancer and, if such a correlation exists, to find out whether it can be used to improve the prognosis of gastric cancer patients. Patients and methods: The expression of MyD88 in 108 cases of gastric cancer specimens, 15 cases of adenoma, and 15 cases of normal mucosa was detected by immunohistochemistry, and the correlations of the MyD88 expression with clinicopathologic changes (including disease-free survival [DFS] and overall survival [OS] were analyzed. The level of MyD88 was detected in well-differentiated MGC-803 and poorly-differentiated BGC-823 cell lines by qPCR and western blot. The expression of MyD88 was then measured by western blot after the treatment of an MyD88 overexpression vector or MyD88 inhibitor. Cell proliferation was determined by overexpression or suppression of MyD88. Results: In clinical cases, MyD88 was highly expressed in 23% of patients with gastric cancer as compared to those in normal mucosa and adenoma. There was a significant correlation of MyD88 overexpression with gastric metastasis ($P<0.01$). The overexpression of MyD88 significantly promoted the proliferation of MGC-803 and BGC-823 cell lines in gastric cancer. According to the single factor analysis, a high expression of MyD88 was strongly associated with poor DFS and OS ($P<0.01$), and MyD88 was an independent prognostic factor of OS. Conclusion: This study demonstrates that a high expression of MyD88 is associated with the gastric cancer patients with liver metastasis, and facilitates the proliferation of gastric cancer cells.. MyD88 is an independent predictive factor for the poor prognosis of gastric cancer patients, which provides a potential tool for future clinical diagnosis.

**Keywords:** MyD88, gastric cancer, correlation

## Introduction

Gastric cancer is the second-leading cause of cancer death worldwide, and its major risk factor is persistent gastritis due to the colonization of *Helicobacter pylori*. Though all the individuals with the colonization of Helicobacter pylori suffer from a certain degree of gastritis, gastritis in only 2.9% of patients will develop into gastric cancer, suggesting that the additional factors contribute to the development of gastritis into gastric cancer [1-3]. In this context, the role of pathogen recognition receptors, especially those in the TRL family, has attracted much attention and has been investigated as a potential promoter of gastrointestinal cancer in recent years [4].

Myeloid differentiation factor-88 (MyD88) is regarded as a kind of key adaptor molecule which links IL-1R1 to IL-1 receptor associated kinase (IRAK) complex [5]. The module component of MyD88 consists of the death domain (DD) at the end of NH2 and the Toll/IL-1R (TIR) domain at the end of COOH. The adaptor molecule modulates the signal transduction of innate immunity (such as TLR members and IL-1 family) and thus affects cancer promotion and antitumor response [6]. The MyD88 protein directly triggers the protein signal transduction of TLR/IL-1R and the signal transduction of the interferon (IFN)-γ receptor in the innate immune responses [7, 8].

The inflammatory responses promote the occurrence of cancer through multiple mecha-

**Table 1.** Clinicopathological statistical results of 108 patients

| Clinicopathological factor | *p* | High expression of MyD88 (n=25) | Low expression of MyD88 (n=83) |
|---|---|---|---|
| Age | | | |
| 65 | 0.915 | 11 | 37 |
| <65 | | 14 | 46 |
| Gender | | | |
| Male | 0.5054 | 13 | 49 |
| Female | | 12 | 34 |
| Tumor site | | | |
| Distant | 0.3113 | 23 | 68 |
| Proximal | | 2 | 15 |
| Histology | | | |
| Good | 0.0076 | 4 | 36 |
| Medium | | 18 | 43 |
| Poor | | 3 | 4 |
| Vascular invasion | | | |
| Yes | 0.0987 | 15 | 32 |
| No | | 10 | 51 |
| Lymphatic invasion | | | |
| Yes | 0.3038 | 20 | 59 |
| No | | 5 | 24 |
| Liver metastasis | | | |
| Yes | 0.0001 | 10 | 12 |
| No | | 15 | 71 |
| Peritoneal metastasis | | | |
| Yes | 0.0611 | 2 | 7 |
| No | | 23 | 76 |
| TNM staging | | | |
| I, II | 0.0197 | 7 | 31 |
| III | | 7 | 35 |
| IV | | 11 | 17 |

nisms [9]. The nuclear factor-κB (NF-κB) is the key factor in inflammation, and it plays an essential role in regulating tumors caused by chronic inflammation or exogenous mutagen [10]. NF-κB is activated by a variety of stimuli [11]. However, it is not yet clear which pathway is potentially involved in the cancer-related inflammation and tissue repair responses. The roles of inflammation and tissue repair responses other than chronic inflammation or the use of exogenous carcinogen in the spontaneous cancer are not yet fully understood. Recent study indicated that MyD88 (the signal transducer of TLRs) employs the toll-like receptor (TLR) in the innate immune system during the control of the tissue regeneration responses [12].

The classic TLR2 signal model involves the adaptor protein MyD88 and Mal (also known as TIR domain-containing adaptor protein [TIRAP]), thus activating the downstream pathways of NF-κB, p38, extracellular regulated kinase, c-Jun N-terminal kinase and mitogen-activated protein kinase (MAPK) [13]. However, a recent in vitro study on macrophages indicated that the important role of MyD88 in TLR2-induced activation is different from activation in the signal pathways of NF-κB and MAPK, and it highlighted the necessity that TLR2 relies on Mal to activate the signal cascades in response to the low concentration of ligand.

In this study, the expression of MyD88 in normal gastric mucosa, adenoma and gastric cancer was systematically analyzed. We aim to determine the possible correlation of MyD88 with DFS and OS in clinical diagnosis.

## Materials and methods

### *Information resource and cell line*

Human gastric cancer well-differentiated cell line MGC-803 and poorly-differentiated cell line BGC-823 were purchased from Genomeditech (Shanghai) Co., Ltd., and they were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 2 mM glutamine and cultured under 10% $CO_2$ at 37°C. A total of 108 patients with gastric cancer (including 62 males and 46 females) subjected to surgery in the Cancer Hospital of Wuhan University from 2010 to 2015 were investigated in this study. Demographic information (gender and age) and tumor features (anatomic site, histology, vascular invasion, lymphatic vessel invasion, lymph node metastasis, liver metastasis, peritoneal metastasis and TNM stage) of the patients was obtained from clinical and pathological records (**Table 1**). The tumor locations were classified into proximal or distal positions. The disease stage was

classified on the basis of the standard proposed by the American Joint Committee on Cancer (AJCC) [14]. The disease-free survival (DFS) was defined as the time interval between the day of surgery and the day of recurrence detected for the first time. If the recurrence was not detected, the date of death or of the last follow-up was used instead. The overall survival (OS) was defined as the time interval between the surgery and the patient's death. The follow-up period of DFS and OS after the first surgery was 5 years. Patients with cancer in relation to gastritis ulcers, Crohn's disease, and familial adenomatous polyposis were excluded. The study was approved by the Ethics Committee of Beijing First Hospital of Integrated Chinese And Western Medicine. All patients enrolled in this study signed written informed consent after a detailed explanation.

*Main reagents*

MyD88 antibody (HFL-296, Santa Cruz Biotech, Santa Cruz, CA, USA), streptavidin (Dako-Cytomation, Glostrup, Denmark), Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA), MyD88 inhibitor (NBP2-29328) (Novus, Littleton, CO, USA), MyD88 over expression vector (Genomics-online, Beijing, China), CCK8 kit (Kumamoto, Japan), RNeasy mini kit (Qiagen, Chatsworth, CA, USA), quantitative reverse transcription-polymerase chain reaction (qRT-PCR) micro ribonucleic acid (miRNA) detection kit (Ambion, USA), RT-buffer (Toyobo, Osaka, Japan), SYBR Green real-time PCR mix (Toyobo, Osaka, Japan), primer synthesis (Invitrogen), nitrocellulose membrane (Millipore, Billerica, MA, USA), anti-MyD88 primary antibody (Santa Cruz Biotechnology, CA, USA), β-actin (Sigma), StatView software (Abacus Concepts, Berkeley, CA, USA), and ABI PRISM 7700 sequence detection system (Applied Biosystems, Hamilton, New Zealand).

*Immunohistochemical detection*

Formalin-fixed and paraffin-embedded tissue specimens of 15 cases of normal mucosa, 15 cases of adenoma, 108 cases of gastric cancer, and 14 cases of liver metastatic lesion were used in the immunohistochemical detection.

First, 4 μm-thick tissue sections were labeled with streptavidin-biotin, dewaxed in xylene and rehydrated in ethanol, followed by treatment with 0.3% hydrogen peroxide to block the endogenous peroxidase activity for 30 min. Then the tissue sections were incubated with the serum-free protein blocker and then incubated with the mouse anti-rabbit MyD88 polyclonal antibody (1:100) at 4°C. Biotinylated secondary antibody and peroxidase-labeled streptavidin were applied at room temperature for 50 min, respectively. The antigen-antibody complex was developed using 3,3'-diaminobenzidine. After slight counterstaining with hematoxylin, the glass slides were examined under a microscope. Human tonsil tissues were used as the positive control for MyD88 immunoreactivity, while phosphate buffered saline without primary antibody was used as the negative control.

*Evaluation of immunohistochemical results*

Each section was evaluated independently by two pathologists who were blinded to the clinical data. MyD88 in most specimens displayed a similar staining intensity (moderate-strong), so the staining intensity was not evaluated in this study. Membrane and/or cytoplasm staining in any intensity indicated the positive staining of MyD88. Several high-power fields (×200) were selected from regions with different staining densities (high, medium, low, and negative) using a digital camera. Photos were printed on plain paper, on which a grid was drawn. An average of 2000 (1500-2500) tumor cells were selected in each tumor, and the percentage of tumor cells with positive staining was calculated. After that, the percentage of MyD88-positive tumor cells was scored based on the 0-4-point scale (0 point: no staining, 1 point: ≥10%, 2 points: 11-30%, 3 points: 31-50%, and 4 points: >50%). In addition, the expression level of MyD88 was divided into the following two groups according to the score: the low expression group (0, 1 and 2 points) and the high expression group (3 and 4 points). Also, the sum of the MyD88 staining scores were divided into the following two groups: the <5 group and the =5 group.

*Over expression or inhibition of MyD88 in cells*

Cells were passaged once every 2 to 3 days and cells at logarithmic growth were used in the experiment. Cell transfection was performed 24-72 h after passage. Cells in 6-well plates

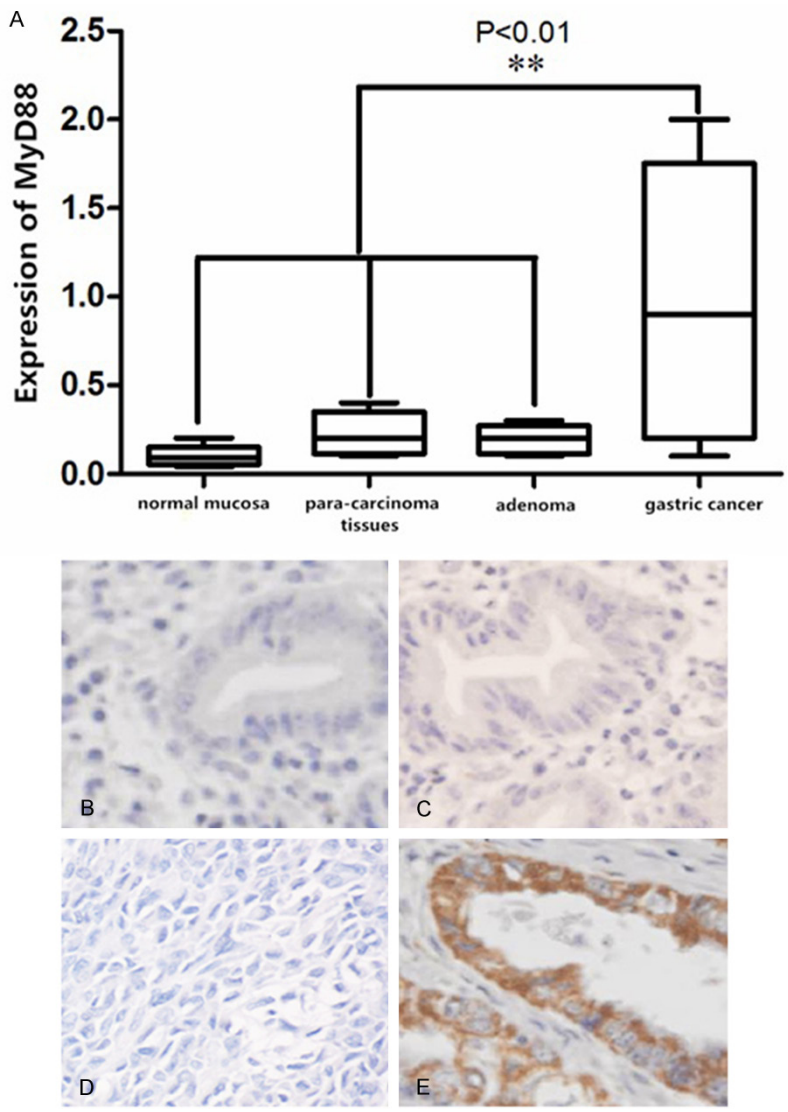


**Figure 1.** Differences in the MyD88 expression among normal gastric mucosa, para-carcinoma tissues, adenoma and gastric cancer. (A) The expression of MyD88 in different groups. The representative images of MyD88 expression in normal gastric mucosa (B), para-carcinoma tissues (C), adenoma (D) and gastric cancer (E) by immunohistochemical detection (×200). The level of MyD88 in gastric cancer was remarkably higher than that in normal gastric mucosa, para-carcinoma tissues, and adenoma. **The difference is extremely significant, $P<0.01$.

were transfected with the overexpression vector (1 μg) Lipofectamine 2000 or treated with the MyD88 inhibitor (100 μM). The growth and proliferation of cells were then observed.

*Cell proliferation assay*

Cells were seeded in 96-well plates and cultured at 37°C and 5% $CO_2$. A cell proliferation assay was performed at 48 h after transfection or treatment. Cells were washed with PBS 3 times. 100 μL CCK8 mixture (CCK8 Reagents: medium =1:10) was added to each well and incubated at 37°C for 2 h in the dark. Absorbance at the 450 nm wavelength was measured by a microplate reader. Each group has 5 wells.

*RNA extraction and qRT-PCR*

RNA extraction and qRT-PCR were performed as described previously. Total RNA was isolated from the fixed paraffin specimens or cells [16]. RNA was extracted from cells using the RNeasy mini kit, and reversely transcribed using RT-buffer according to manufacturer's instructions, followed by qPCR using SYBR Green real-time PCR mix. Primers were designed as follows: MyD88 (sense: CTCCTCCACATCCCTTCC, antisense: CCGCACGTTCAAGAACAGAGA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sense: TGAAGCAGGCATCTGAGGG, antisense: CGAAGGTGGAAGAGTGGGAG). PCR was performed using the ABI PRISM 7700 sequence detection system, and reaction conditions are as follows: at 95°C for 10 min, at 95°C for 30 s, at 60°C for 1 min, a total of 40 cycles. The relative quantitative value of MyD88 expression was normalized based on the expression level of the reference gene GAPDH in each case.

*Western blot*

Tissues were washed with Hanks balanced salt solution twice and directly lysed in a radioimmunoprecipitation assay buffer. Cell or tissue

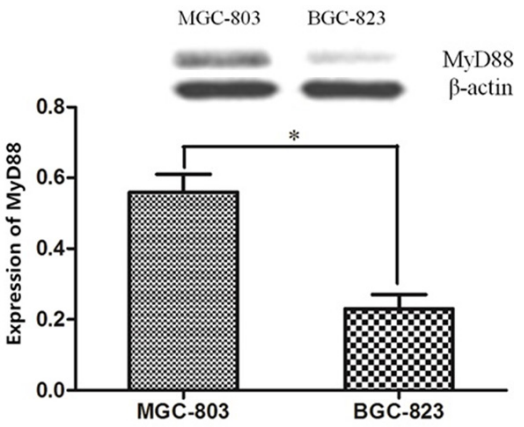


**Figure 2.** MyD88 expressions in MGC-803 and BGC-823 cell lines. It was shown that the level of MyD88 in MGC-803 was significantly higher than that in BGC-823 by the detection of qPCR and western blot. *, $P<0.05$.

lysates (60 mg) were isolated via sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane, incubated with anti-MyD88 primary antibody or mouse β-actin monoclonal antibody at room temperature for 2 h, fully washed with 0.1% phosphate buffered saline with Tween-20 and incubated with the horseradish peroxidase-labeled secondary antibody (diluted at 1:3000). Finally, the signal was enhanced via chemiluminescence, thus enabling visualizing.

*Statistical analysis*

GraphPad Prism 5.0 software was used for the analysis. DFS and OS measured on the day of each operation were the main statistical results. DFS and OS were estimated using the Kaplan-Meier curve, and the curve was compared using a log-rank test. Recurrence and date of death were analyzed using the univariate and multivariate Cox proportional hazard model. In addition, the hazard ratio (HR) and 95% confidence interval were calculated. The probability value ($p$) <0.05 suggested that the difference was statistically significant.

## Results

*MyD88 expressions in normal gastric mucosa, para-carcinoma tissues, adenoma and gastric cancer*

The immunolocalization of the MyD88 protein was observed in the cytoplasms. Basically, there was no or very weak MyD88 expression in the normal mucosa, para-carcinoma tissues and adenoma. MyD88 expression was displayed in 93 out of 108 (86%) gastric cancer specimens, and the high-level expression was detected in 25 kinds of cancer (23%). The MyD88 expression was positive in all 14 cases of liver metastatic lesions, 12 cases of which (86%) showed high expression (**Figure 1**).

*MyD88 expressions in MGC-803 and BGC-823 cell lines*

We then measured the MyD88 expressions in human gastric cancer well-differentiated MGC-803 cell lines and poorly-differentiated BGC-823 cell lines via qPCR and western blotting. Results revealed that the expression of MyD88 in MGC-803 cells at both the mRNA and protein levels was significantly higher than it was in the BGC-823 cells ($P<0.05$), suggesting that the MyD88 expression is correlated with the differentiation degree of gastric cancer cells (**Figure 2**).

*Effects of MyD88 on the proliferation of different gastric cancer cell lines*

Based on the results above that MyD88 was related to the development of gastric cancer, we then evaluated the effect of MyD88 on the proliferation of gastric cancer cells. The expression of MyD88 was initially upregulated or downregulated by the overexpression vector and inhibitor. Of note, the level of MyD88 was obviously mediated in both cell lines, respectively (**Figure 3A**, **3B**). Afterwards, our data showed that the over expression of MyD88 significantly increased the proliferation of both MGC-803 and BGC-823 cells ($P<0.05$). By contrast, the inhibition of MyD88 statistically suppressed the cell proliferation (**Figure 3C**, **3D**).

*Clinicopathological significance of MyD88 expression*

The high expression of MyD88 was related to the histology ($P=0.0076$), liver metastasis ($P=0.001$), and TNM staging ($P=0.0197$) of the cancer (**Table 1**). In addition, the MyD88 expression level was higher in gastric cancer accompanied with liver metastasis than that in gastric cancer without liver metastasis ($P<0.05$) (**Figure 4**).

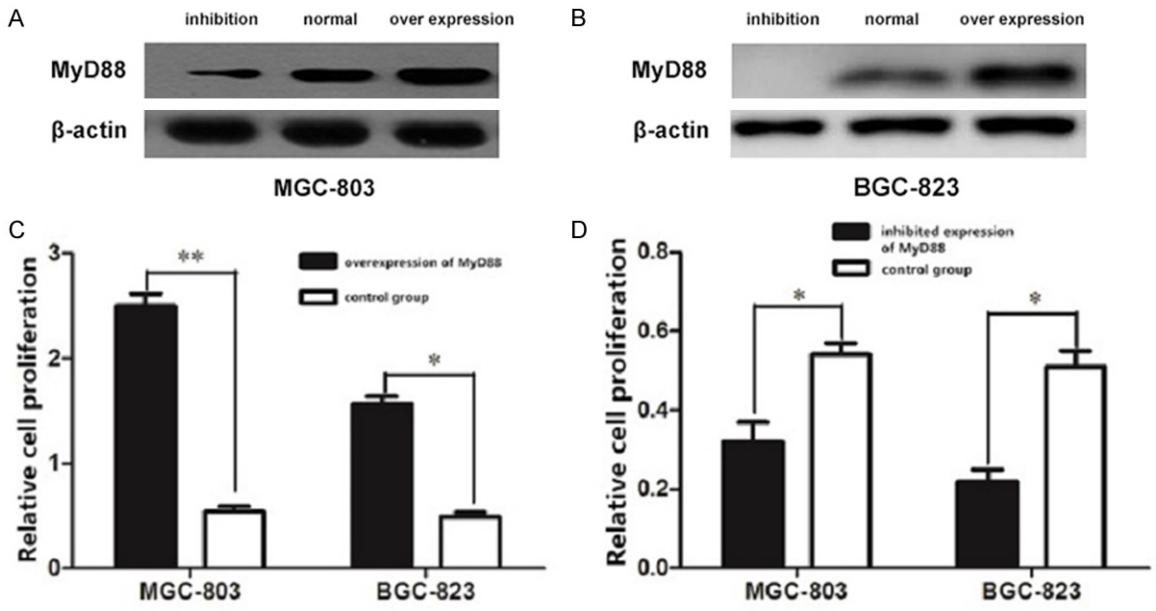


**Figure 3.** Effects of overexpression and inhibition of MyD88 on proliferation of gastric cancer cells. The expression of MyD88 was regulated by the overexpression vector or inhibitor in MGC-803 (A) and BGC-823 (B) cell lines by western blotting. (C) Over expression of MyD88 significantly elevated the proliferation of both cell lines (D) while the inhibition of MyD88 impeded the cell growth. *, $P<0.05$. **, $P<0.01$.

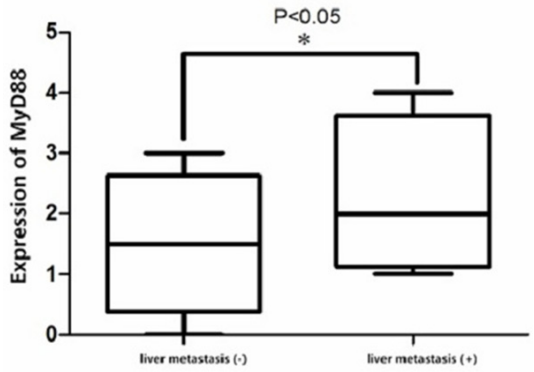


**Figure 4.** Difference in the immunostaining score of MyD88 between gastric cancer with liver metastasis (+) (n=22) and gastric cancer with liver metastasis (-) (n=86). The expression level of MyD88 in gastric cancer with liver metastasis (+) was significantly higher than it was in gastric cancer with liver metastasis (-) ($P<0.05$).

*Clinicopathological parameters and survival rate of patients with gastric cancer*

During the 5-year follow-up, there were 53 cases of tumor recurrence (DFS=49%) and 46 deaths (OS=43%). According to the univariate analysis, histology (DFS: $P=0.0322$, OS: $P=0.0084$), TNM staging (DFS: $P<0.0001$, OS: $P=0.0001$), vascular invasion (DFS: $P<0.0001$, OS: $P=0.0001$), lymphatic invasion (DFS: $P=0.001$, OS: $P=0.0121$), lymph node metastasis (DFS: $P=0.0001$, OS: $P=0.0017$), liver metastasis (DFS: $P<0.0001$, OS: $P<0.0001$) and peritoneal metastasis (DFS: $P=0.0386$, OS: $P=0.007$) were important factors related to DFS and OS (**Table 2**). The patient's age, gender and tumor site were not associated with DFS or OS (**Table 2**). Independent prognostic factors were determined via multivariate analysis. All factors with significant prognostic value in the univariate analysis, including histopathological variables and tumor markers, are shown in **Table 2**.

*MyD88 expression and survival rate of patients with gastric cancer*

The effect of high expression of MyD88 on the survival of patients with gastric cancer was evaluated. The high expression of MyD88 was significantly associated with low DFS ($P=0.0018$) and low OS ($P=0.015$) (**Figure 5**).

## Discussion

According to a recent study, the MyD88 signal drives the tumor growth in a variety of organs [8]. Moreover, it has been revealed that MyD88 is crucial for promoting the diethylnitrosamine-induced hepatocellular neoplasm [7]. In the spontaneous (Apcmin/+) and carcinogen (azo-

**Table 2.** Statistical results of clinicopathological parameters of patients with gastric cancer

| Variable | 5-year DFS HR | *p* | 5-year OS HR | *p* |
|---|---|---|---|---|
| Age (65 years old vs. <65 years old) | 0.84 (0.48-1.45) | 0.5355 | 1.17 (0.65-2.08) | 0.5934 |
| Gender (male vs. female) | 0.95 (0.55-1.63) | 0.8568 | 0.95 (0.53-1.71) | 0.8781 |
| Tumor site (distal end vs. proximal end) | 2.89 (1.16-8.97) | 0.0656 | 3.17 (0.98-10.23) | 0.0534 |
| Histology (poor vs. medium vs. good) | 1.95 (1.05-3.6) | 0.0322 | 2.57 (1.27-5.19) | 0.0084 |
| TNM staging (III/IV vs. I/II) | 8.07 (3.2-20.33) | <0.0001 | 6.26 (2.46-15.88) | 0.0001 |
| Vascular invasion (yes vs. no) | 3.28 (1.86-5.78) | <0.0001 | 3.35 (1.82-6.16) | 0.0001 |
| Lymphatic invasion (yes vs. no) | 4.68 (1.86-11.79) | 0.001 | 3 (1.27-7.09) | 0.0121 |
| Lymph node metastasis (yes vs. no) | 3.64 (1.87-7.09) | 0.0001 | 3.07 (1.52-6.2) | 0.0017 |
| Liver metastasis (yes vs. no) | 5.04 (2.85-8.9) | <0.0001 | 5.35 (2.94-9.74) | <0.0001 |
| Peritoneal metastasis (yes vs. no) | 2.32 (1.05-5.18) | 0.0386 | 3.06 (1.35-6.91) | 0.007 |
| MyD88 (high vs. low) | 2.33 (1.31-4.13) | 0.0038 | 3.03 (1.67-5.48) | 0.0002 |

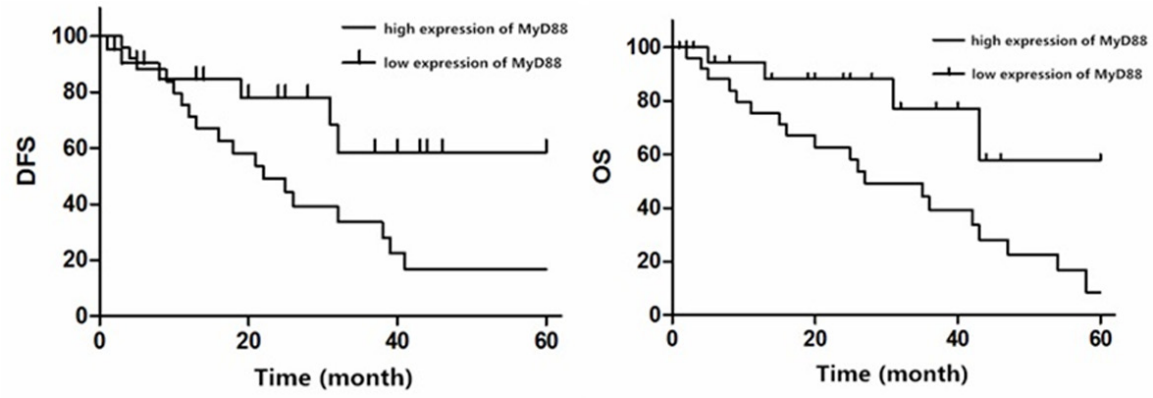


**Figure 5.** Kaplan-Meier survival curves of DFS and OS in gastric cancer patients with different expressions of MyD88. A high level of MyD88 was associated with low DFS and OS.

xymethane)-induced intestinal tumor model, it has also been proved that MyD88 is important in the development of tumors [8]. In addition, MyD88 is a chemical-induced positive regulator of skin and connective tissues [10]. It was observed in this study that the MyD88 signal was often upregulated in gastric cancer compared with those in normal mucosa and adenoma, and the correlation between MyD88 expression and gastric cancer was further validated. The above findings suggest that the MyD88 signal transduction, as a carcinogenic factor, favors the development of gastric cancer.

Whether MyD88 is involved in the occurrence of tumors remains unclear [15]. Chronic infection and inflammation in humans are regarded as the two most important factors resulting in the occurrence and progression of tumors [16]. TLR signal transduction possibly plays an important role in various cancers, including gastric cancer, ovarian cancer, lung cancer, pancreatic cancer and liver cancer. It has been proved to be associated with local chronic inflammation [17-20]. Upregulation of TLR may lead to genomic DNA damage, mutation, and abnormal chromosomal translocation [8]. The activation of TLR4 signal seemingly promotes the development of gastritis-related cancers through such mechanisms as enhanced cyclooxygenase-2 (Cox-2) expression and increased epidermal growth factor receptor (EGFR) signal transduction [18]. Besides gastritis-related cancer [21], symbiotic bacteria are also involved in the development of sporadic gastric cancer and may promote gastric cancer by inducing chromosomal instability [22]. Results of this study manifested that MyD88 signal transduction contributed to tumorigenesis in both gastritis-related cancer and sporadic gastric cancer.

Results of this study clearly demonstrated that overexpression of MyD88 was an independent and significant prognostic factor for the 5-year DFS and OS. Previous evidence demonstrated that the increase in downstream signals of the TLR4/MyD88 pathway, such as Cox-2 and nuclear factor-κB (NF-κB), was associated with the survival of gastric cancer patients. Cox-2 is also considered to play an important role in the occurrence of rectal cancer. According to a recent study by Ogino et al., Cox-2 overexpression is associated with the poor survival rate of colon cancer patients [23]. NF-κB is the terminal point of the MyD88 signal transduction pathway. Evidence suggests a link between NF-κB activation and cancer development, and the constitutive NF-κB activation has also been detected in most cancer cell lines and various tumor tissues [24]. Scartozzi et al. reported that the NF-κB nuclear expression may indicate the response and survival in irinotecan-refractory metastatic gastric cancer treated with cetuximab-irinotecan therapy [25]. These findings illustrate that the MyD88 signal pathway in tumor cells plays a crucial role in the prognosis of gastric cancer patients, and blocking this pathway can greatly benefit patients with gastric cancer.

In conclusion, this study demonstrates that MyD88 is associated with an increased risk of liver metastasis and a decreases survival rate, which suggests a possible method by targeting the MyD88 signal in the treatment of gastric cancer.

## Disclosure of conflict of interest

None.

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## References

[1] Milne AN, Carneiro F, O'Morain C, Offerhaus GJ. Nature meets nurture: molecular genetics of gastric cancer. Hum Genet 2009; 126: 615-628.

[2] Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med 2001; 345: 784-789.

[3] Tye H, Kennedy CL, Najdovska M, McLeod L, McCormack W, Hughes N, Dev A, Sievert W, Ooi CH, Ishikawa TO, Oshima H, Bhathal PS, Parker AE, Oshima M, Tan P, Jenkins BJ. STAT3-driven upregulation of TLR2 promotes gastric tumorigenesis independent of tumor inflammation. Cancer Cell 2012; 22: 466-478.

[4] Uno K, Kato K, Atsumi T, Suzuki T, Yoshitake J, Morita H, Ohara S, Kotake Y, Shimosegawa T, Yoshimura T. Toll-like receptor (TLR) 2 induced through TLR4 signaling initiated by Helicobacter pylori cooperatively amplifies iNOS induction in gastric epithelial cells. Am J Physiol Gastrointest Liver Physiol 2007; 293: 1004-12.

[5] Kenny EF, Talbot S, Gong M, Golenbock DT, Bryant CE, O'Neill LA. MyD88 Adaptor-Like is not essential for TLR2 signaling and inhibits signaling by TLR3. J Immunol 2009; 183: 3642-3651.

[6] Shen Y, Kawamura I, Nomura T, Tsuchiya K, Hara H, Dewamitta SR, Sakai S, Qu H, Daim S, Yamamoto T, Mitsuyama M. Toll-like receptor 2- and MyD88-dependent phosphatidylinositol 3-kinase and rac1 activation facilitates the phagocytosis of listeria monocytogenes by murine macrophages. Infect Immun 2010; 78: 2857-2867.

[7] Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007; 317: 121-124.

[8] Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science 2007; 317: 124-127.

[9] Kfoury A. Dual function of MyD88 in RAS signaling and inflammation, leading to mouse and human cell transformation. J Clin Invest 2010; 120: 3663-3667.

[10] Swann JB, Vesely MD, Silva A, Sharkey J, Akira S, Schreiber RD, Smyth MJ. Demonstration of inflammation-induced cancer and cancer immunoediting during primary tumorigenesis. Proc Natl Acad Sci U S A 2008; 105: 652-656.

[11] Haslett PA, Corral LG, Matthew A, Kaplan G. Thalidomide costimulates primary human t lymphocytes, preferentially inducing proliferation, cytokine production, and cytotoxic responses in the CD8+ subset. J Exp Med 1998; 187: 1885.

[12] Paul SC, Lv P, Xiao YJ, An P, Liu SQ, Luo HS. Thalidomide in rat liver cirrhosis: blockade of tumor necrosis factor-alpha via inhibition of degradation of an inhibitor of nuclear factor-kappaB. Pathobiology 2006; 73: 82-92.

[13] Keifer JA, Guttridge DC, Ashburner BP, Baldwin AS Jr. Inhibition of NF-kappa B activity by thalidomide through suppression of IkappaB kinase activity. J Biol Chem 2001; 276: 22382-22387.
[14] Schootman M, Lian M, Deshpande AD, Baker EA, Pruitt SL, Aft R, Jeffe DB. Temporal trends in area socioeconomic disparities in breast-cancer incidence and mortality, 1988-2005. Breast Cancer Res Treat 2010; 122: 533-543.
[15] Zhang Y, Zhuang R, Geng C, Cai X, Lei W, Tian N, Gao F. Insulin promotes T cell recovery in a murine model of autoimmune myocarditis. Clin Exp Immunol 2013; 171: 46-53.
[16] Balkwill F, Coussens LM. Cancer: an inflammatory link. Nature 2004; 431: 405-406.
[17] Kelly MG, Alvero AB, Chen R, Silasi DA, Abrahams VM, Chan S, Visintin I, Rutherford T, Mor G. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res 2006; 66: 3859.
[18] Fukata M, Abreu MT. Role of toll-like receptors in gastrointestinal malignancies. Oncogene 2008; 27: 234.
[19] He W, Liu Q, Wang L, Chen W, Li N, Cao X. TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Mol Immunol 2007; 44: 2850-2859.
[20] Schwartz A, Malgor R, Dickerson E, et al. Phenylmethimazole decreases toll-like receptor 3 and non-canonical Wnt5a expression in pancreatic cancer & melanoma together with tumor cell growth & migration. Clinical Cancer Research An Official Journal of The American Association for Cancer Research 2009; 15: 4114.
[21] Hope ME, Hold GL, Kain R, El-Omar EM. Sporadic colorectal cancer-role of the commensal microbiota. FEMS Microbiol Lett 2010; 244: 1-7.
[22] Wang X, Huycke MM. Extracellular superoxide production by Enterococcus faecalis promotes chromosomal instability in mammalian cells. Gastroenterology 2007; 132: 551-561.
[23] Ogino S, Kirkner GJ, Nosho K, Irahara N, Kure S, Shima K, Hazra A, Chan AT, Dehari R, Giovannucci EL, Fuchs CS. Cyclooxygenase-2 expression is an independent predictor of poor prognosis in colon cancer. Clin Cancer Res 2008; 14: 8221.
[24] Shishodia S, Aggarwal BB. Nuclear factor-κB: a friend or a foe in cancer? Biochem Pharmacol 2004; 68: 1071-1080.
[25] Scartozzi M. Nuclear factor-kB tumor expression predicts response and survival in irinotecan-refractory metastatic colorectal cancer treated with cetuximab-irinotecan therapy. J Clin Oncol 2007; 25: 3930-5.