Clin Transl Oncol (2014) 16:668–674
DOI 10.1007/s12094-013-1133-y

RESEARCH ARTICLE

# Increased expression of α5β1-integrin is a prognostic marker for patients with gastric cancer

**J. Ren · S. Xu · D. Guo · J. Zhang · S. Liu**

Received: 15 August 2013 / Accepted: 1 November 2013 / Published online: 19 November 2013


**Abstract**
*Objective* The study was to evaluate the association of expression level of α5β1-integrin with clinicopathologic features and prognosis in gastric cancer (GC).
*Methods* The expression of α5β1-integrin in normal gastric mucosa and GC tissue was detected with immunohistochemistry. The level of α5 and β1 mRNA in GC tissues and non-neoplastic tissues was evaluated in 48 paired cases by quantitative real-time polymerase chain reaction (qRT-PCR). Survival analysis by the Kaplan–Meier method was performed to assess prognostic significance.
*Results* The α5β1-integrin expression was detected in 68.3 % (127/186) GC samples, and there was a significant difference on their positive expression rate between GC tissue and normal gastric mucosa ($P < 0.001$). The positive expression rate of α5β1-integrin in patients with poor histologic differentiation ($P = 0.001$), lymph node metastasis ($P < 0.001$), and recurrence ($P < 0.001$) group was heightened. Using Kaplan–Meier analysis, a comparison of survival curves of low versus high expresser of α5β1-integrin revealed a highly significant difference in human GC tissue ($P = 0.002$), which suggested that overexpression of α5β1-integrin is associated with a worse prognosis. Multivariate analyses showed that α5β1-integrin expression was independent risk factor predicting overall survival [Hazard ratio (HR) 1.594, 95 % confidence interval (CI) 1.236–2.408, $P = 0.006$] and disease-free survival [HR 3.952, 95 % CI 1.676–9.861, $P = 0.003$] in GC.
*Conclusions* The α5β1-integrin promotes angiogenesis and associates with lymph node metastasis, vascular invasion and poor prognosis of GC. The current study shows that α5β1-integrin may be an independent prognostic factor for GC patients.

**Keywords** Gastric cancer · α5β1-integrin · Prognosis · Survival

J. Ren · S. Xu (✉) · D. Guo
Department of Pathology, Shenzhen Futian Hospital Affiliated to Guangdong Medical College, 3025 Shennan Middle Road, Shenzhen 518029, Guangdong, People's Republic of China
e-mail: shengmeixu2008@126.com

J. Zhang · S. Liu
Department of Pathology, Shenzhen Nanshan Hospital Affiliated to Guangdong Medical College, Shenzhen 518052, People's Republic of China

## Introduction

Gastric cancer (GC) is the second most common cause of cancer deaths worldwide [1]. The survival rate of GC has steadily increased due to advances in early detection and surgical techniques, but in advanced GC, contrary to early GC, results of treatment, the quality of life, and the rate of recurrence and survival are less favorable. Therefore, many biomarkers are urgently needed to identify the latent molecular pathogenesis and predict prognosis in GC.

Tumor metastasis is a complex, multi-step process involving alterations in cell–cell or cell-extracellular matrix (ECM) interactions mediated by specific receptors. The interaction between transformed cells and the basement membrane is an important step in the development of invasion and metastasis. Integrins are the major receptors for cell adhesion to extracellular matrix proteins, which play critical roles in many physiological and pathological processes including morphogenesis and tumorigenesis [2]. The integrin family of cell adhesion receptors regulates a

diverse array of cellular functions crucial to the initiation, progression and metastasis of solid tumors [3]. Much of the mass of a solid tumor is comprised of the stroma which is richly invested with ECM. The ECM is a complex structure formed by distinct molecular networks that interact with specific cell receptors, such as collagens and fibronectin (FN). The classical fibronectin receptor (FnR), the α5β1 integrin, binds to FN and has a well-defined role in cell adhesion, migration, matrix formation and angiogenesis. Several previous studies demonstrated that α5β1-integrin expression levels are altered in many types of cancer [4, 5]. However, little data are available on the alterations of α5β1-integrin and the correlation between clinicopathologic characteristics and prognosis of GC patients. In the present study, we undertook to clarify relationships between the expression of α5β1-integrin and clinicopathological parameters, including prognosis, using an immunohistochemical approach.

## Materials and methods

### Patients and tissue samples

GC tissue was obtained from 186 patients in the Department of Pathology, Shenzhen Futian Hospital Affiliated to Guangdong Medical College, between January 2002 and December 2006. Clinicopathological patient characteristics are summarized in Table 1. The study protocol was approved by Ethics Committee of Shenzhen Futian Hospital Affiliated to Guangdong Medical College, and all participants signed an informed consent form. No patient had received radiotherapy, chemotherapy, or other treatment prior to surgery. Following surgical removal, the tissue sample was immediately frozen in liquid nitrogen until used, and was formalin-fixed and paraffin-embedded for histopathologic diagnosis and immunohistochemical examination. The 72 non-tumor parts were taken from the grossly normal gastric mucosa more than 5 cm away from the tumor in resected gastric specimen. During the follow-up period from the date of surgery until December 31, 2011, 32 patients died and 154 were alive (median follow-up time, 51.9 mo, range: 4–72 mo).

### Immunohistochemistry analysis

The paraffin-embedded GC tissues and distal normal mucosa tissues were cut at 4 μm and mounted on glass slides. Then the slides were dewaxed in xylene and rehydrated in ethanol, and treated with a solution of peroxidase-blocking reagent (Dako, Glostrup, Denmark) to exhaust endogenous peroxidase activity. They were put in 0.01 mol/L citrate buffer at pH 6.0 for 15 min in an 800 W microwave oven and then left at room temperature for 20 min to expose antigen hidden inside the tissue due to formalin fixation. To inhibit non-specific antigen–antibody reactions possible in immunohistochemical staining, protein blocker (Research Genetics, Huntsville, AL, USA) was used for 5 min and the slides were washed thoroughly with PBS buffer. Then the slides were incubated overnight with the primary antibodies against α5β1-integrin (1:100; mouse polyclonal antibody, ECM410, Tianyuan Huida Bio-engineering Limited Company, Wuhan, China) at 4 °C. Biotinylated goat anti-rabbit secondary antibody (1:200; BA1003, Boster Bio-engineering Limited Company, Wuhan, China) was applied for 20 min at room temperature, followed by further washing with buffer to remove

**Table 1** The relationship between α5β1-integrin expression levels of tumors and clinicopathological feature

| Variable | Cases | α5β1-integrin | | | |
|---|---|---|---|---|---|
| | | N | L | H | *P* value |
| Age | | | | | |
| <60 years | 74 | 25 | 40 | 9 | 0.270 |
| ≥60 years | 112 | 34 | 54 | 24 | |
| Gender | | | | | |
| Male | 107 | 36 | 42 | 29 | 0.734 |
| Female | 79 | 23 | 31 | 25 | |
| Tumor size | | | | | |
| <5 cm | 85 | 27 | 43 | 15 | 0.941 |
| ≥5 cm | 101 | 32 | 53 | 16 | |
| Depth of invasion | | | | | |
| Mucosa and submucous | 133 | 41 | 73 | 19 | 0.236 |
| Muscular layer | 30 | 11 | 12 | 7 | |
| Under infiltrate serosa | 23 | 7 | 9 | 7 | |
| Histologic differentiation | | | | | |
| Well | 62 | 27 | 21 | 14 | 0.001** |
| Moderate | 51 | 22 | 20 | 9 | |
| Poor and unknown | 73 | 10 | 45 | 18 | |
| TNM stage | | | | | |
| I | 34 | 11 | 17 | 6 | 0.192 |
| II | 109 | 27 | 61 | 21 | |
| III | 33 | 16 | 13 | 4 | |
| IV | 10 | 5 | 3 | 2 | |
| Metastasis of lymph node | | | | | |
| N0 | 105 | 20 | 75 | 10 | 0.000** |
| N(+) | 81 | 39 | 21 | 21 | |
| Follow-up | | | | | |
| Live without recurrence | 140 | 50 | 80 | 10 | 0.000** |
| Died of recurrence | 32 | 7 | 6 | 19 | |
| Live with recurrence | 14 | 2 | 7 | 5 | |

*N* negative, *L* low expression, *H* high expression. Statistically significant * $P < 0.05$, ** $P < 0.01$

unbound antibody. A complex of avidin with horseradish peroxidase was then applied for 20 min at room temperature. For color development, the slides were stained with 3,3′-Diaminobenzidine (DAB, Sigma–Aldrich, St Louis, MO, USA) and then were counterstained with hematoxylin. A reddish brown precipitate in the cytoplasm and membrane of cells indicated a positive reaction. In each immunohistochemistry run, the positive section provided by reagent company served as positive control and omission of the primary antibody served as negative control.

### Quantitative real-time PCR

Total RNA extraction was performed using RNAiso Plus (Takara, Shiga, Japan) according to the manufacturer's protocol. The cDNAs from total RNA were synthesized using with PrimeScript® RT Reagent Kit (Takara, Shiga, Japan) from 48 self-pairs of GC specimens and non-neoplastic tissues. The sequences of the primers used for the amplification of integrin alpha5 mRNA were: (sense) 5′-TGCATCAACCTTAGCTTCTGCCT-3′ and (anti-sense) 5′-ACCAGCAGGCGGCTCTGGT -TCAC-3′(591 bp). The sequences of the primers used for the amplification of integrin beta1 mRNA were: (sense) 5′-GGAAAACGGCAAA TTGTCAGAAGG-3′ and (anti-sense) 5′-TGGACCAGT GGGACACTCTGGATT-3′ (1,030 bp). The sequences of the primers used for the amplification of GAPDH mRNA were: (sense) 5′-ACCACAGTCCATGCCATCAC-3′ and (antisense) 5′-TCCACCACCCTGTTGCTGTA-3′ (451 bp) [6]. RT-PCR was performed using ABI Step One Plus (Applied Biosystems, Singapore, Singapore). The threshold cycle values for each gene amplification cycle were normalized by subtracting the threshold cycle value calculated for the GAPDH gene. Normalized gene expression values were expressed as the relative quantity of gene-specific mRNA. All standards and samples were analyzed in triplicate.

### Quantification of IHC staining

Assessment of the staining was scored independently by two investigators (JQY and DBW) who were blinded to all clinical data. The allocation of tumors and scoring staining by the two investigators was similar. In cases of disagreement, slides were reevaluated and discussed until a consensus was achieved. α5β1-integrin staining was considered positive if there was cytoplasm and membrane expression. Staining was graded (0: negative, 1: weak, 2: moderate, 3: strong) and percentage of positive staining cells was counted (0: <10 %, 1: 11–50 %, 2: 51–75 %, 3: >76 %). The final score was determined by the combined staining score and proportion score (intensity score × proportion score). The total score ranged from 0 to 9. The immunoreactivity was divided into three levels on the basis of the final score: negative immunoreactivity was defined as a total score of 0; low immunoreactivity, as a total score of 1–4; and high immunoreactivity, as a total score higher than 4. The final results were subjected to statistical analysis.

### Statistical analysis

Associations among categorical variables were assessed using Fisher's exact probability test or the $\chi^2$ test. Overall survival (OS) and disease-free survival (DFS) were measured by the Kaplan–Meier method. The prognostic value of the nine variables was tested by univariate analysis using the log-rank test. Multivariate Cox proportional hazard models were used to define the potential prognostic significance of individual parameter. A $P$ value less than 0.05 was considered significant. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

## Results

### Pattern of α5β1-integrin expression in gastric cancer and normal mucosa

The α5β1-integrin staining was as performed in 186 GC patients and 72 cases of normal tissues by immunohistochemistry. The α5β1-integrin expression was detected in 68.3 % (127/186) GC (Fig. 1a), and in 22.2 % (16/72) distal normal mucosa (Fig. 1b). The expression of α5β1-integrin was found in cytoplasm and membrane. The difference in α5β1-integrin expression between GC and normal mucosa was statistically significant ($\chi^2 = 44.569$, $P < 0.001$).

### Correlation of α5β1-integrin expression and clinicopathological features in GC

When comparing the α5β1-integrin status with clinicopathological variables, we found significant positive correlations between α5β1-integrin expression and histologic differentiation ($P = 0.001$), lymph node metastasis ($P = 0.000$), and recurrence ($P = 0.000$) (Table 1).

### Analysis of α5β1-integrin mRNA expression

Level of α5β1-integrin transcripts in 48 pairs of resected specimens (tumor tissue samples and non-neoplastic tissue samples) from patients with GC was determined using qRT-PCR. We found that the 48 specimens had higher α5β1-integrin (70.8 %) mRNA expression in GC tissue than in the corresponding non-neoplastic tissue (at least a 2.4-fold increase). In addition, the relative expression of

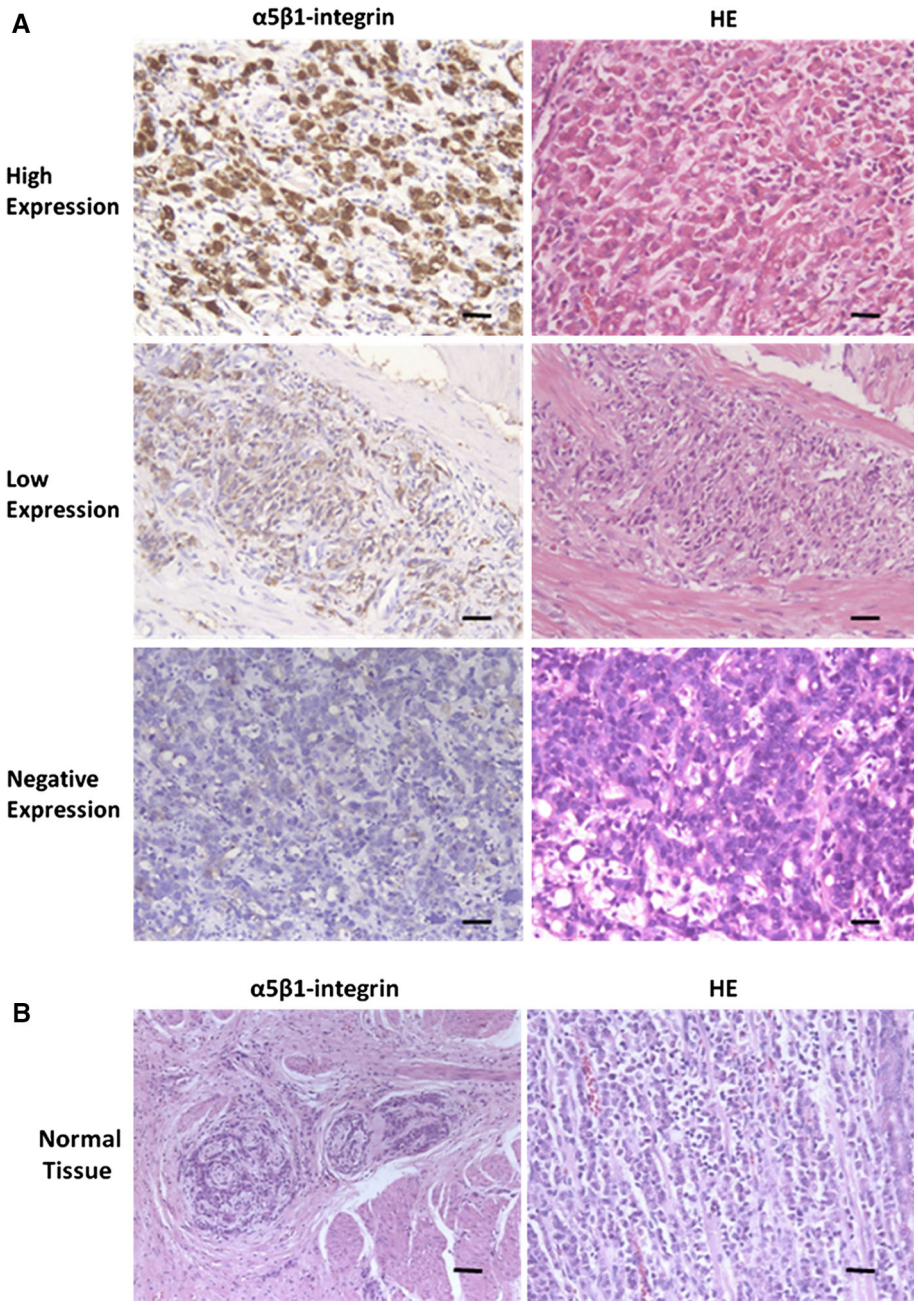


**Fig. 1** **a** The *left panels* show expression of α5β1-integrin by immunohistochemistry in the GC tissues with high, low, and negative expression. The *right panels* show the sections stained with H&E. The scores for α5β1-integrin in tissue with high, low, and negative expression were 9, 3, and 0, respectively. **b** The *left* picture shows expression of α5β1-integrin by immunohistochemistry in normal tissues with negative expression. The *right* picture shows the sections stained with H&E. *Bar* indicates 20 μm

α5β1-integrin mRNA in GC specimens was significantly higher than in the corresponding non-neoplastic tissues ($P < 0.001$; Fig. 2a). Following agarose gel electrophoresis and staining with ethidium bromide, the intensities of the visualized PCR products were evaluated by densitometric scanning (Fig. 2b).

## Relationship between α5β1-integrin and OS and DFS of GC patients

At the time of the last follow-up, 140 (75.3 %) of 186 patients were alive and disease-free, 14 (7.5 %) were alive with recurrent disease, and 32 (17.2 %) died of recurrent tumor. In the univariate Cox proportional hazard regression model analysis shown in Table 2, histologic differentiation ($P = 0.011$), TNM stage ($P = 0.013$), node status ($P = 0.003$), recurrence ($P = 0.002$), and expression intensity of α5β1-integrin ($P < 0.001$; Fig. 3a) were significantly associated with OS. The histologic differentiation ($P = 0.003$), TNM stage ($P = 0.009$), node status ($P = 0.025$), recurrence ($P = 0.005$), and expression intensity of α5β1-integrin ($P < 0.001$; Fig. 3b) were significantly associated with DFS. Consequently, patients with tumors with negative or low expression of α5β1-integrin

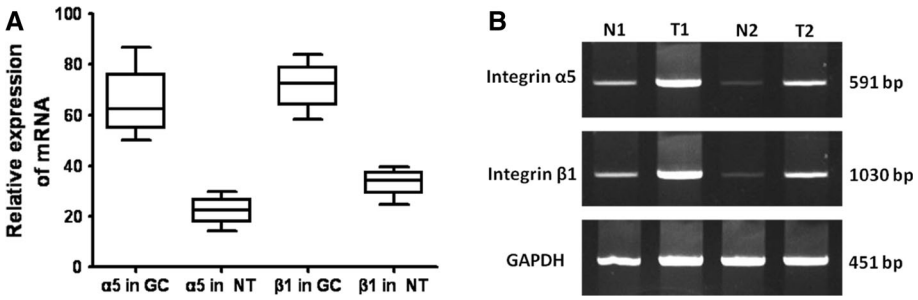


**Fig. 2** **a** α5 and β1 relative expression in GC and non-neoplastic tissues (*NT*) were analyzed by qRT-PCR. The relative amount of α5 and β1 mRNA in GC was higher than in the corresponding non-neoplastic tissue, the difference was statistically significant ($P < 0.001$). **b** The electrophoretogram of RT-PCR of α5 and β1 from paired gastric cancer tissues (*T*) and non-neoplastic tissues (*N*), GAPDH was used as an internal control

**Table 2** Univariate cox regression analysis of OS and DFS

| Parameter | Overall survival | | Disease-free survival | |
|---|---|---|---|---|
| | Log-rank test | *P* value | Log-rank test | *P* value |
| Age (<60, ≥60 years) | 0.387 | 0.534 | 1.304 | 0.309 |
| Gender | 0.651 | 0.420 | 2.501 | 0.475 |
| Tumor size(<5, ≥5 cm) | 0.344 | 0.558 | 0.136 | 0.712 |
| Depth of invasion | 1.899 | 0.594 | 2.457 | 0.116 |
| Histologic differentiation (well, moderate, poor and unknown) | 6.502 | 0.011* | 8.545 | 0.003** |
| TNM stage (I, II, III, IV) | 6.041 | 0.013* | 6.875 | 0.009** |
| Nodal metastasis [N0, N(+)] | 8.683 | 0.003** | 5.029 | 0.025* |
| Recurrence | 9.24 | 0.002** | 8.001 | 0.005** |
| α5β1-integrin expression (negative, low, high) | 26.582 | 0.000** | 21.67 | 0.000** |

*N0* no nodal metastasis, *N(+)* nodal metastasis. Statistically significant * $P < 0.05$, ** $P < 0.01$

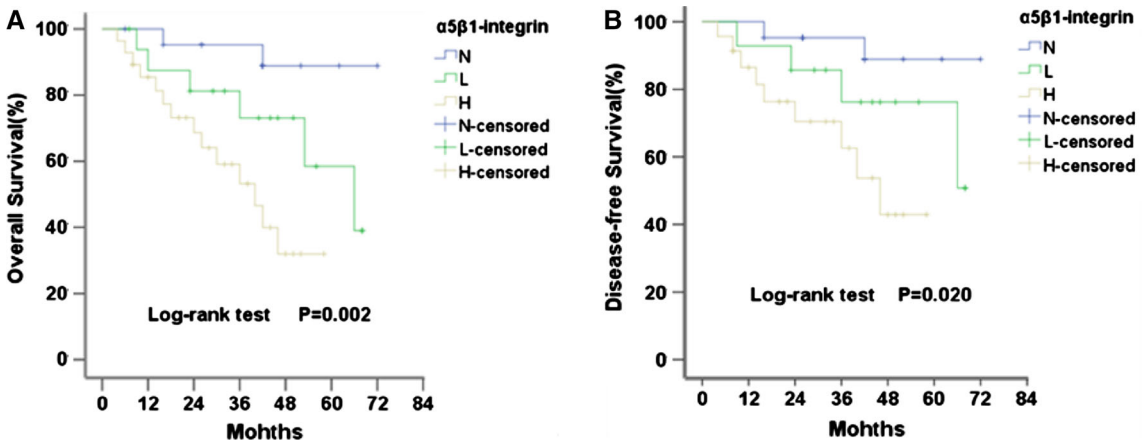


**Fig. 3** Survival curves of 186 patients with GC. **a** Overall survival, **b** disease-free survival of patients with tumors lacking (*N*) or expressing low (*L*) or high (*H*) levels of α5β1-integrin

had a better prognosis than those with tumors having high α5β1-integrin expression.

In a multivariate Cox regression analysis (Table 3), the expression intensity of α5β1-integrin ($P = 0.006$), histologic differentiation ($P = 0.013$), TNM stage ($P = 0.026$), node status ($P = 0.036$), and recurrence ($P < 0.001$) showed a significant association with OS. The expression intensity of α5β1-integrin ($P = 0.003$), histologic differentiation ($P = 0.011$), TNM stage ($P = 0.030$), node status ($P = 0.022$), and recurrence ($P = 0.027$) showed a significant association with DFS.

**Table 3** Multivariate Cox regression analysis of OS and DFS in GC

| Parameter | Overall survival | | Disease-free survival | |
|---|---|---|---|---|
| | HR (95 % CI) | *P* value | HR (95 % CI) | *P* value |
| Histologic differentiation (well, moderate, poor and unknown) | 1.847 (1.336–2.936) | 0.013* | 1.326 (0.958–2.187) | 0.011* |
| TNM stage (I, II, III, IV) | 1.696 (1.431–2.467) | 0.026* | 1.612 (1.048–2.444) | 0.030* |
| Nodal metastasis [N0, N(+)] | 1.417 (1.012–2.358) | 0.036* | 2.023 (1.107–3.695) | 0.022* |
| Recurrence | 16.479 (7.839–35.441) | 0.000** | 4.871 (1.106–5.458) | 0.027* |
| α5β1-integrin expression (negative, low, high) | 1.594 (1.236–2.408) | 0.006** | 3.952 (1.676–9.861) | 0.003** |

*N0* no nodal metastasis, *N(+)* nodal metastasis, *HR* hazard ratio, *CI* confidence interval. Statistically significant * $P < 0.05$, ** $P < 0.01$

## Discussion

Numerous reports suggested that integrins play critical roles in the cell adhesion, migration, proliferation, and survival [2, 7, 8]. The altered integrins can change affinity and avidity for their ECM, and cancer cells become more adhesive and invasive, and lead to increased metastatic potential and enhanced angiogenic potential [9]. In previous studies, robust relationships between altered α5β1-integrin expression and highly metastatic potential were uncovered in human lung adenocarcinoma cell line [10]. In our study, we found that α5β1-integrin expression in GC tissues (127/186, 68.3 %) was conspicuously higher than the paired normal mucosa (16/72, 22.2 %), and there was statistically significant difference ($\chi^2 = 44.569$, $P < 0.001$). Our results demonstrated that α5β1-integrin was closely correlated to GC metastasis.

Furthermore, the OS and DFS of patients with high α5β1-integrin expression were significantly worse than that of patients with low or lacking expression. The univariate survival analysis revealed α5β1-integrin expression as well as histologic differentiation, TNM Stage, lymph node metastasis, and recurrence, was a significant prognostic factor. The status of α5β1-integrin expression might be dependent on the status of lymph node metastasis or other variables. So the multivariate Cox regression analysis for OS and DFS was undertaken, and multivariate analysis found that α5β1-integrin expression was picked up for its independent level of prognostic significance. The α5β1-integrin expression level plays important functions in the biology of GC and defines a more aggressive tumor phenotype of GC. Preoperative adjuvant therapy in GC is designed to improve survival and reduce local recurrence. Our results also showed that the tumors with a strong expression of α5β1-integrin were associated with an increased recurrence, which suggests that patients with high α5β1-integrin expression may be prone to metastasis. So, α5β1-integrin overexpression was closely related to poor prognosis, and α5β1-integrin may serve as a marker for poor prognosis.

The conceivable mechanisms responsible for these correlations maybe as follows. First, α5β1-integrin may be involved in promoting tumor angiogenesis [11, 12]. It is well known that the growth and spread of neoplasms depend on the establishment of an adequate blood supply [13]. Angiogenesis depends on endothelial cell interactions with the extracellular matrix [14]. The α5β1-integrin and its ligand, FN, are clearly proangiogenic [15]. Global deletion of the α5 integrin gene results in an embryonic lethal phenotype, with aberrant blood vessel formation in the embryo [16]. Similar vascular defects are also apparent in α5 integrin-null embryoid bodies and teratoma cells [17]. Recently reported that α5β1-integrin plays an important role in stimulating endothelial cell proliferation at an early step in the angiogenic process [11]. Second, α5β1-integrin may involve in activating MMP-2 [18, 19]. Invasion and metastasis of cancer have a close relationship with basement membrane adhesion and extracellular matrix degradation. Recently, accumulating evidences show α5β1-integrin mediated modulation of MMP-2 activity and suggest a direct interaction between MMP-2 and α5 integrin in melanoma and breast cancer cells [4, 20]. Migrating astrocytes show co-localization of MMP-2 with β1 integrin at the cell periphery, indicating its significance in pericellular proteolysis [21]. Galina Morozevich's study showed that α5β1-integrin controls invasion of the breast cancer cells via regulation of MMP-2 collagenase expression which can occur either through signaling pathways involving PI-3K, Akt and Erk protein kinases and the c-Jun or via direct recruitment of MMP-2 to the cell surface [4]. Third, α5β1-integrin is also implicated to trigger Ras, MAP kinase, focal adhesion kinase (FAK), Src, Rac/Rho/cdc42 GTPases, PKC and PI3K (phosphatidylinositol 3-kinase) signaling pathways [22–24]. Integrins are widely recognized as important molecules for the transduction of positional cues from the ECM to the intracellular signaling machinery. Many of the signaling pathways and effectors activated by integrin ligation are also activated following growth-factor stimulation.

Taken together, our present study has underlined the importance of α5β1-integrin in tumor initiation, progression and metastasis process, and the possible rationale underlying the relationship between α5β1-integrin and

angiogenesis. Further work is required to define the molecular mechanisms. The expression of α5β1-integrin may serve as a valuable tool of clinical assessment of tumor biological behavior and prognosis in patients with GC.

**Acknowledgments** We are very grateful to Juan Zhang and Jiangqiao Shu for excellent technical support.

**Conflict of interest** The authors declare that they have no conflict of interests.

## References

1. Price P, Sikore K. Stomach. In: Price P, Sikore K, editors. Treatment of Cancer, 4th ed. London: Arnold Press, 2002. pp. 583–99.
2. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell. 2002;110:673–87.
3. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer. 2010;10:9–22.
4. Morozevich G, Kozlova N, Cheglakov I, Ushakova N, Berman A. Integrin α5β1 controls invasion of human breast carcinoma cells by direct and indirect modulation of MMP-2 collagenase activity. Cell Cycle. 2009;8(14):2219–25.
5. Chi F, Fu D, Zhang X, Lv Z, Wang Z. Expression of the c-Met proto-oncogene and Integrin α5β1 in human gastric cardia adenocarcinoma. Biosci Biotechnol Biochem. 2012;76(8):1471–6.
6. Weston BS, Wahab NA, Mason RM. CTGF mediates TGF-beta-induced fibronectin matrix deposition by upregulating active alpha5beta1 integrin in human mesangial cells. J Am Soc Nephrol. 2003;14(3):601–10.
7. Hynes RO. Cell-matrix adhesion in vascular development. J Thromb Haemost. 2007;5(S1):32–40.
8. Serini G, Valdembri D, Bussolino F. Integrins and angiogenesis: a sticky business. Exp Cell Res. 2006;312:651–8.
9. Hood JD, Cheresh DA. Role of integrins in cell invasion and migration. Nat Rev Cancer. 2002;2(2):91–100.
10. Takenaka K, Shibuya M, Takeda Y, Hibino S, Gemma A, Ono Y, et al. Altered expression and function of beta1 integrins in a highly metastatic human lung adenocarcinoma cell line. Int J Oncol. 2000;17(6):1187–94.
11. Li LX, Welser-Alves J, van der Flier A, Boroujerdi A, Hynes RO, Milner R. An angiogenic role for the α5β1-integrin in promoting endothelial cell proliferation during cerebral hypoxia. Exp Neurol. 2012;237:46–54.
12. Caiado F, Dias S. Endothelial progenitor cells and integrins: adhesive needs. Fibrogenesis Tissue Repair. 2012;5:4.
13. Fidler IJ. Angiogenesis and cancer metastasis. Cancer J. 2000;2:S134–41.
14. Eliceiri BP, Cheresh DA. Adhesion events in angiogenesis. Curr Opin Cell Biol. 2001;13:563–8.
15. Hynes RO. A reevaluation of integrins as regulators of angiogenesis. Nat Med. 2002;8(9):918–21.
16. Yang JT, Rayburn H, Hynes RO. Embryonic mesodermal defects in α5 integrin-deficient mice. Development. 1993;19:1093–105.
17. Taverna D, Hynes RO. Reduced blood vessel formation and tumor growth in alpha5-integrin-negative teratocarcinomas and embryoid bodies. Cancer Res. 2001;61:5255–61.
18. Kesanakurti D, Chetty C, Dinh DH, Gujrati M, Rao JS. Role of MMP-2 in the regulation of IL-6/Stat3 survival signaling via interaction with a5β1 integrin in glioma. Oncogene. 2012. pp. 1–14.
19. Mitra A, Chakrabarti J, Chatterjee A. Binding of alpha5 monoclonal antibody to cell surface alpha5beta1 integrin modulates MMP-2 and MMP-7 activity in B16F10 melanoma cells. J Environ Pathol Toxicol Oncol. 2003;22:167–78.
20. Ogier C, Bernard A, Chollet AM, LE Diguardher T, Hanessian S, Charton G, et al. Matrix metalloproteinase-2 (MMP-2) regulates astrocyte motility in connection with the actin cytoskeleton and integrins. Glia. 2006;54:272–284.
21. Aplin AE, Howe A, Alahari SK, Juliano RL. Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. Pharmacol Rev. 1998;50:197–263.
22. Miyamoto S, Teramoto H, Gutkind JS, Yamada KM. Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors. J Cell Biol. 1996;135:1633–42.
23. Schwartz MA, Shattil SJ. Signaling networks linking integrins and rho family GTPases. Trends Biochem Sci. 2000;25:388–91.
24. Ridley A. Rho GTPases. Integrating integrin signaling. J Cell Biol. 2000;150:F107–9.