

High PARP-1 expression is associated with tumor invasion and poor prognosis in gastric cancer

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Received July 23, 2015; Accepted July 12, 2016

DOI: 10.3892/ol.2016.5169

Abstract. Poly (adenosine diphosphate-ribose) polymerase 1 (PARP-1) was previously demonstrated to be overexpressed in numerous malignant tumors and associated with invasiveness and poor prognosis. However, the expression of the PARP-1 protein in gastric cancer and its association with clinical outcomes requires further investigation. In the present study, the expression of PARP-1 in 564 gastric cancer tissues and 335 tumor-adjacent control tissues is investigated, using tissue microarray-based immunohistochemistry. PARP-1 expression levels were demonstrated to be significantly higher in gastric cancer tissue samples, as compared with control tissue samples. In gastric cancer, high PARP-1 expression levels were significantly associated with *Helicobacter pylori* (*H. pylori*) infection ($P=0.032$), decreased differentiation ($P<0.001$), increased depth of invasion ($P=0.037$), presence of lymphatic invasion ($P<0.001$), presence of lymph node metastasis ($P<0.001$), and advanced tumor-node-metastasis (TNM) stage ($P=0.015$). High PARP-1 expression levels were associated with a significantly shorter overall survival rate ($P<0.001$) and disease-free survival rate ($P=0.001$) in patients with gastric cancer, particularly a subset of patients with *H. pylori* infection or an advanced TNM stage. In addition, univariate analysis indicated that PARP-1 high expression levels were significantly associated with a poor prognosis in gastric cancer. These results suggest that PARP-1 expression may be involved

in the progression and prognosis of gastric cancer, particularly *H. pylori*-positive or advanced-stage gastric cancer.

Introduction

Human cancers constitute a notable burden on societies. In less developed countries, gastric cancer among males is one of the leading causes of cancer-associated mortalities (1). Due to its genetic complexity and heterogeneity, advances in the treatment of gastric cancer have been limited thus far (2). Therefore, the identification of specific biomarkers is crucial for the development of individualized treatments, which are required for the effective and precise management of gastric cancer in patients.

The poly (adenosine diphosphate-ribose) polymerase (PARP) proteins are a family of 17 enzymes involved in the regulation of transcription, DNA damage response, genome stability, cell cycle, energy metabolism, cell death and tumorigenesis (3-5). PARP-1 was the first PARP enzyme identified over 50 years ago and has been the subject of several studies (6-8). PARP-1 binds single- or double-stranded DNA breaks; its activity increases as required to maintain genomic integrity (9,10). It has previously been demonstrated that PARP-1 is overexpressed in numerous types of tumors, including malignant melanomas, colorectal cancer, breast cancer, testicular tumors and lymphangiomyomatosis, and that it is associated with invasiveness and poor clinical prognosis (11-15). Therefore, PARP-1 may be a potential anticancer target (16,17). PARP inhibitors are also currently used in combination with chemotherapeutic agents to increase tumor responses (18-20).

PARP-1 single nucleotide polymorphisms, including PARP-1 2819G, PARP-1 762Val/Ala and PARP-1 rs1136410, were previously demonstrated to be associated with gastric cancer susceptibility and lymph node metastasis in gastric cancer (21-23). Le *et al* (24) demonstrated that PARP-1 inhibitors enhance the cytotoxicity of cisplatin in human gastric cancer cells. Liu *et al* (25) previously demonstrated that the cochinchina momordica seed extract significantly inhibited the survival rate of human gastric cancer cells by downregulating PARP expression. However, the protein expression pattern of PARP-1 in gastric cancer patients requires further study. It remains to be determined whether the expression levels of PARP-1 are associated with the tumorigenesis and progression of gastric cancer.

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Abbreviations: AUCs, areas under curve; CI, confidence interval; DFS, disease-free survival; EMT, epithelial-mesenchymal transition; *H. pylori*, *Helicobacter pylori*; IRS, immunoreactivity score; OS, overall survival; PARP-1, poly (adenosine diphosphate-ribose) polymerase 1; ROC, receiver operating characteristic; RR, relative risk; TNM, tumor-node-metastasis; TMA, tissue microarray

Key words: gastric cancer, poly (adenosine diphosphate-ribose) polymerase 1, *H. pylori* infection, metastasis, prognosis

In the present study, tissue microarray-based immunohistochemistry was used to determine the expression of PARP-1 in 564 gastric cancer tissue samples and 335 tumor-adjacent tissue samples. The aim of the current study was to analyze the association between the expression levels of PARP-1 and the clinicopathological features and prognosis of gastric cancer patients.

Materials and methods

Patients and tissue samples. Human gastric cancer tissue samples were obtained from 564 patients (405 males and 159 females; age range, 29-82 years) with primary gastric tumors who underwent D1 or D2 radical gastrectomy surgery at the First Affiliated Hospital of Dalian Medical University (Dalian, China) between 2011 and 2013. The gastric tissues outside the cancer loci were selected as the tumor-adjacent tissue samples; 335 tumor-adjacent tissue samples from these patients were collected as controls. The diagnosis of gastric cancer was confirmed by pathological staining. Clinicopathological data including patient age, gender, tumor location, tumor size, histological differentiation, invasion depth, *Helicobacter pylori* (*H. pylori*) infection, ascites, lymphatic invasion, lymph node metastasis, distant metastasis and tumor-node-metastasis (TNM) stage (26) were retrospectively retrieved from the medical records. The patients did not undergo radiotherapy or chemotherapy prior to surgery. Outcomes of interest included the overall survival (OS) and disease-free survival (DFS) rates. OS was defined as the duration from diagnosis to the last follow-up, or to mortality. DFS was calculated as the time from the initial diagnosis to local recurrence, or distant metastasis.

The patients consented to have tissue samples collected at the time of admission for surgery, according to protocols authorized by the Regional Human Ethics Committee of Dalian Medical University. The Medical Ethics Committee of Dalian Medical University approved the present study. Due to the retrospective nature of the present study, the Ethics Committees waived the requirement for written informed consent from the patients.

Tissue microarray (TMA)-based immunohistochemistry. Tissue cores were extracted from formalin-fixed and paraffin-embedded tissue blocks containing the tumor tissue samples and the tumor-adjacent tissue samples and arrayed into a paraffin recipient block. Sections (4 μ m thick) were obtained from the TMA blocks using a microtome, mounted on poly-L-lysine-coated glass slides and used for immunohistochemistry.

TMA sections were washed in xylene, rehydrated in a graded ethanol series and washed in tap water. The tissue sections were then heated in 100 ml 10 mM sodium citrate buffer (pH 6.0) in a microwave oven (high power, 700 W for 3 min; medium power, 400 W for 3 min; low power, 100 W for 3 min; a total of 9 min) to retrieve antigen. The sections were then incubated at 37°C for 30 min in 3% H₂O₂/methanol to block endogenous peroxidase activity. Nonspecific protein binding sites were blocked by 10% normal goat serum (Boster Biological Technology, Ltd., Wuhan, China) at 37°C for 30 min. The sections were incubated in a primary polyclonal rabbit anti-human antibody against PARP-1 (cat. ab6079;

dilution 1:200; Abcam, Cambridge, UK) overnight at 4°C, and subsequently incubated with a biotinylated goat anti-rabbit secondary antibody (cat. no. SAEP031; dilution, 1:150; Wuhan Elabscience Biotechnology Co., Ltd., Wuhan, China) for 30 min and streptavidin horseradish peroxidase (LSAB kit; Dako, Glostrup, Denmark) for an additional 30 min. Sections were stained with 3,3-diaminobenzidine at room temperature for 50-60 sec, counterstained with hematoxylin, dehydrated with graded ethanol and mounted with neutral resin. For the negative controls, the primary antibodies were replaced with phosphate-buffered saline.

Evaluation of immunohistochemistry. Two pathologists blinded to the experimental conditions examined the final effective immunostaining under a light microscope (Eclipse 50i; Nikon Corporation, Tokyo, Japan). The intensity of immunoreactivity was graded on a scale of 0-3, as follows: 0) For no visible staining, i) for low staining, ii) for moderate staining and iii) for high staining. The percentage of the stained nuclei in gastric cancer gland cells and normal gland cells was assigned using 5% increments. Five random, non-overlapping fields were defined and 40 cells for each field (a total of 200 cells) were selected in order to calculate the percentage of stained cells present in each sample. The immunoreactivity score (IRS) was determined by multiplying the intensity score and the percentage of stained nuclei, giving a minimum IRS score of 0 and a maximum of 300%. Receiver operating characteristic (ROC) curve analysis was performed to determine an optimal cutoff IRS for PARP-1 expression. Plotting the sensitivity and specificity for each outcome under study generated the ROC curves.

Statistical analysis. The Pearson's χ^2 test and the Fisher's exact probability test were used to determine significant differences between the categorical data. The Mann-Whitney U test was used to detect differences in the IRS or lymph node metastasis number between various groups. The Wilcoxon rank-sum test was used to compare the IRS of gastric cancer tissues with tumor-adjacent tissues, for the paired tissue samples. Kaplan-Meier survival plots were generated and comparisons between the survival curves were determined with a log-rank test. Cox's proportional hazards regression model was used to evaluate the association between the potential confounding variables and the prognosis (OS or DFS). Only those cases with complete data on all the variables (n=153) were included in the multivariate analyses. The data were processed using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate statistical significance.

Results

Clinicopathological characteristics of patients with gastric cancer. The clinicopathological characteristics of 564 patients with gastric cancer are summarized in Table I. For these 564 patients, the features of tumor location of 498 patients (88.3%), tumor size of 522 patients (92.6%), histological differentiation of 555 patients (98.4%), depth of tumor invasion of 529 patients (93.8%), status of *H. pylori* infection of 297 patients (52.7%), presence of ascites of 479 patients (84.9%), lymphatic invasion of 540 patients (95.7%), lymph

node metastasis of 553 patients (98.0%), distant metastasis of 434 patients (77.0%) and TNM stage of 481 patients (85.3%) were recorded. The average age (mean ± standard deviation) of gastric cancer patients in the present study was 60.1±10.4 years (range, 29-82 years). The histological differentiation of the cancers was determined in 555 patients as follows: 14.6% high differentiation (n=81), 21.4% moderate differentiation, (n=119) and 64.0% low differentiation (n=355). The depth of tumor invasion was evaluated in 529 patients as follows: 10.8% T1 (n=57, tumor invades the mucosa or submucosa), 15.9% T2 (n=84, tumor invades the muscularis propria), 64.1% T3 (n=339, tumor invasion of the serosa), and 9.3% T4 (n=49, tumor invades the adjacent organs and structures). The status of *H. pylori* infection was defined by a ¹³C-urea breath test. Of the total group, 208 patients (70.0%) were diagnosed with *H. pylori* infection while 89 patients (30.0%) were negative for *H. pylori* infection. The stage of the cancer was evaluated in 481 patients according to the TNM staging system as follows: 13.7% Stage I (n=66), 24.9% stage II (n=120), 35.3% stage III (n=170) and 26.0% stage IV (n=125). Ascites, lymphatic invasion, lymph node metastasis and distant metastasis occurred in 176 (36.7%) of 479 patients, 203 (37.6%) of 540 patients, 316 (57.1%) of 553 patients and 148 (34.1%) of 434 patients, respectively.

Follow-up information was available for 523 patients with gastric cancer. During the follow-up period of 0-95 months, relapses occurred in 246 cases and mortality occurred in 208 cases. The 5-year survival rate was determined to be 46.5%. The mean OS time was 65.8 months (95% confidence interval (CI); 62.6-68.9 months) and the mean DFS time was 60.5 months (95% CI; 57.3-63.7 months) respectively.

PARP-1 overexpression in breast cancer. Using immunohistochemistry, the expression levels of PARP-1 in 564 gastric cancer tissue samples and 335 tumor-adjacent control tissue samples were studied. Positive PARP-1 staining in gastric tissues appeared as brown particles that were primarily nuclear (Fig. 1). The gastric cancer tissue samples exhibited significantly more intense staining for PARP-1 expression, as compared with tumor-adjacent tissue samples (P<0.001; Fig. 2A). There was also a significant increase in the IRS of PARP-1 expression among paired samples (P<0.001; Fig. 2B).

The ROC analysis was performed to determine an optimal cutoff score for PARP-1 expression; the ROC curves for the clinicopathological features with statistical significance are indicated in Fig. 3. The area under the curve for the lymph node metastasis status had the largest relative area (Fig. 3A). Based on this outcome, a cutoff score of 175% was selected for PARP-1 expression levels: The low and high expression levels of PARP-1 were defined as IRS≤175% and IRS>175%, respectively. A total of 298 (52.8%) tumors exhibited low expression levels of PARP-1 and 266 (47.2%) tumors exhibited high expression levels of PARP-1.

Association of PARP-1 expression with clinicopathological characteristics of gastric cancer. The association between PARP-1 expression levels and the clinicopathological characteristics of gastric cancer were investigated (Table II). Statistical analysis indicated that PARP-1 expression levels were increased with the following: Decreased differentiation

Table I. Clinicopathological characteristics of patients with gastric cancer.

Features	Total	Number	%
Age (years)	564		
≤60		296	52.5
>60		268	47.5
Gender	564		
Male		405	71.8
Female		159	28.2
Tumor location	498		
Lower		257	51.6
Middle		199	40.0
Upper		42	8.4
Tumor size (cm)	522		
≤5.0		240	46.0
>5.0		282	54.0
Histological differentiation	555		
High		81	14.6
Moderate		119	21.4
Low		355	64.0
Invasion depth	529		
T1		57	10.8
T2		84	15.9
T3		339	64.1
T4		49	9.3
<i>H. pylori</i> infection	297		
Negative		89	30.0
Positive		208	70.0
Ascites	479		
Negative		303	63.3
Positive		176	36.7
Lymphatic invasion	540		
Negative		337	62.4
Positive		203	37.6
Lymph node metastasis	553		
Negative		237	42.9
Positive		316	57.1
Distant metastasis	434		
Negative		286	65.9
Positive		148	34.1
TNM stage	481		
I		66	13.7
II		120	24.9
III		170	35.3
IV		125	26.0

H. pylori, *Helicobacter pylori*; TNM, tumor-node-metastasis.

(P<0.001), increased depth of invasion (P=0.037), presence of lymphatic invasion (P<0.001), presence of lymph node metastasis (P<0.001) and advanced TNM stage (P=0.015).

Table II. Association of the expression of PARP-1 with clinicopathological features of gastric cancer.

Features	High expression		P-value ^a
	n	%	
Age at diagnosis (years)			0.105
≤60	130	43.9	
>60	136	50.7	
Gender			0.575
Male	194	47.9	
Female	72	45.3	
Tumor location			0.057
Lower	111	43.2	
Middle	104	52.3	
Upper	15	35.7	
Tumor size (cm)			0.123
≤5.0	108	45.0	
>5.0	146	51.8	
Histologic differentiation			<0.001 ^b
High	18	22.2	
Moderate	53	44.5	
Low	190	53.5	
Invasion depth			0.037 ^b
T1	20	35.1	
T2	36	42.9	
T3	163	48.1	
T4	31	63.3	
<i>H. pylori</i>			0.032 ^b
Negative	38	42.7	
Positive	117	56.2	
Ascites			0.253
Negative	142	46.9	
Positive	73	41.5	
Lymphatic invasion			<0.001 ^b
Negative	136	40.4	
Positive	118	58.1	
Lymph node metastasis			<0.001 ^b
Negative	84	35.4	
Positive	180	57.0	
Distant metastasis			0.263
Negative	123	43.0	
Positive	72	48.6	
TNM stage			0.015 ^b
I~II	76	40.9	
III~IV	154	52.2	

^aP-value obtained from pearson chi-square or Fisher's exact test; ^bstatistically significant (P<0.05); *H. pylori*, *Helicobacter pylori*; TNM, tumor-node-metastasis; PARP-1, poly (adenosine diphosphate-ribose) polymerase 1.

In addition, high PARP-1 expression levels were increased among patients with *H. pylori* infection (P=0.032). The expression levels of PARP-1 were not identified to be

significantly associated with age, gender, tumor location, tumor size, distal metastasis or presence of ascites in the patients (P>0.05).

Table III. Univariate Cox regression analysis of clinicopathological data associated with OS and DFS in gastric cancer.

Factors	n	OS		DFS	
		RR (95% CI)	P-value	RR (95% CI)	P-value
Age, years ≤60/>60	523	1.412 (1.075-1.856)	0.013 ^a	1.428 (1.111-1.835)	0.005 ^a
Gender Female/male	523	0.673 (0.486-0.932)	0.017 ^a	0.715 (0.532-0.959)	0.025 ^a
Tumor location Upper/middle/lower	457	1.041 (0.828-1.307)	0.732	1.013 (0.822-1.247)	0.905
Tumor size, cm >5.0/≤5.0	484	1.958 (1.460-2.624)	<0.001 ^a	1.896 (1.453-2.475)	<0.001 ^a
Histologic differentiation High/moderate/low	514	0.779 (0.633-0.959)	0.018 ^a	0.785 (0.650-0.947)	0.011 ^a
Invasion depth T4/T3/T2/T1	495	1.317 (1.088-1.596)	0.005 ^a	1.309 (1.097-1.561)	0.003 ^a
<i>H. pylori</i> Positive/negative	268	1.341 (0.880-2.045)	0.172	1.492 (1.00-2.218)	0.048 ^a
Ascites Yes/no	439	1.622 (1.221-2.155)	0.001 ^a	1.623 (1.249-2.107)	<0.001 ^a
Lymphatic invasion Yes/no	500	1.164 (0.879-1.541)	0.290	1.240 (0.958-1.605)	0.102
Lymph node metastasis Yes/no	512	4.991 (3.476-7.165)	<0.001 ^a	4.979 (3.594-6.898)	<0.001 ^a
Distant metastasis Yes/no	400	1.192 (0.876-1.623)	0.263	1.257 (0.945-1.671)	0.116
TNM stage IV/III/II/I	442	1.524 (1.309-1.775)	<0.001 ^a	1.493 (1.299-1.715)	<0.001 ^a
PARP-1 expression Positive/negative	523	1.685 (1.280-2.218)	<0.001 ^a	1.507 (1.172-1.936)	0.001 ^a

RR and 95% CI were assessed using univariate Cox regression analysis; ^astatistically significant (P<0.05); OS, overall survival; DFS, disease-free survival; RR, relative risk; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; TNM, tumor-node-metastasis; PARP-1, poly (adenosine diphosphate-ribose) polymerase 1.

The association between PARP-1 expression levels and lymph node metastasis in gastric cancer patients was also investigated (Fig. 4). The numbers of lymph nodes positive for metastasis were recorded from 553 gastric cancer patients, and ranged from 0 to 33 lymph nodes among the 553 patients. Compared with PARP-1 low expression levels, PARP-1 high expression levels were associated with a larger number of positive lymph nodes (P<0.001; Fig. 4A). Furthermore, lymph node metastasis-positive tumors exhibited a higher IRS of PARP-1 expression (P<0.001; Fig. 4B).

Association of PARP-1 expression with the survival of patients with gastric cancer. Kaplan-Meier analysis and a log-rank test were used to evaluate the association between the expression levels of PARP-1 protein and patient mortality. High expression levels of PARP-1 were associated with significantly shorter OS time (P<0.001; Fig. 5A) and DFS time (P=0.001; Fig. 5B).

Univariate analysis of the potential prognostic impact of the clinicopathological parameters identified age, gender, tumor size, histological differentiation, invasion depth, presence of ascites, lymph node metastasis, TNM stage and PARP-1 expression as significantly associated with OS and DFS in gastric cancer patients (Table III). Subsequently, multivariate Cox regression models (using the same clinicopathological features) revealed that only histological differentiation, presence of ascites, lymph node metastasis and TNM stage remained as independent prognostic factors (Table IV).

Subgroup analysis of the association between PARP-1 expression levels and the survival of gastric cancer patients. The present study used Kaplan-Meier analysis to investigate the association of PARP-1 expression levels with OS and DFS in subgroups of gastric cancer patients, which were categorized according to clinicopathological parameters. The results of the

Table IV. Multivariate Cox regression analysis of OS and DFS in gastric cancer.

Factors	OS		DFS	
	RR (95% CI)	P	RR (95% CI)	P
Age, years ($\leq 60 / > 60$)	1.063 (0.676-1.669)	0.792	1.144 (0.753-1.738)	0.528
Gender (female/male)	0.855 (0.516-1.417)	0.543	0.904 (0.568-1.438)	0.670
Tumor size, cm ($> 5.0 / \leq 5.0$)	1.447 (0.838-2.499)	0.185	1.346 (0.815-2.222)	0.246
Histological differentiation	1.435 (1.012-2.033)	0.042 ^a	1.376 (1.001-1.891)	0.049 ^a
Invasion depth (T4/T3/T2/T1)	1.136 (0.796-1.621)	0.483	1.218 (0.879-1.689)	0.236
<i>H. pylori</i> (positive/negative)	1.260 (0.740-2.144)	0.395	1.366 (0.838-2.225)	0.211
Ascites (yes/no)	1.614 (1.011-2.576)	0.045 ^a	1.686 (1.098-2.588)	0.017 ^a
Lymph node metastasis (yes/no)	3.432 (1.784-6.603)	$< 0.001^a$	4.010 (2.204-7.295)	$< 0.001^a$
TNM stage (IV/III/II/I)	1.411 (1.057-1.883)	0.019 ^a	1.402 (1.080-1.821)	0.011 ^a
PARP-1 expression	1.642 (0.994-2.712)	0.053	1.354 (0.863-2.122)	0.187

n=153; RR and 95% CI were assessed using multivariate Cox regression analysis; ^astatistically significant (P<0.05); OS, overall survival; DFS, disease free survival; RR, relative risk; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; TNM, tumor-node-metastasis; PARP-1, poly (adenosine diphosphate-ribose) polymerase 1.

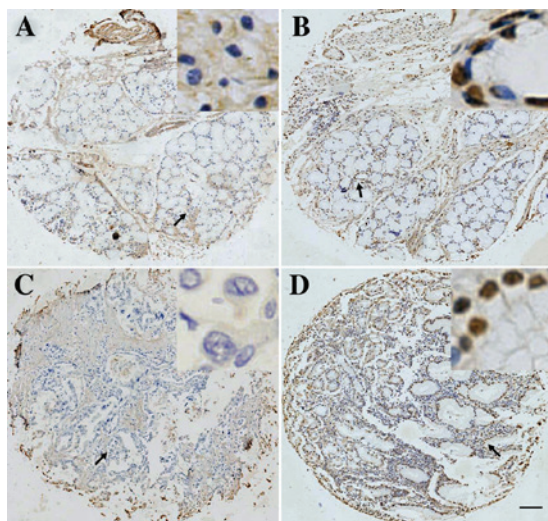


Figure 1. Representative micrographs indicating the immunohistochemical staining of PARP-1 proteins in gastric cancer tissue samples and in tumor-adjacent tissue samples. (A) Negative staining of PARP-1 in tumor-adjacent tissue samples and (B) gastric cancer tissue samples. (C) Positive staining of PARP-1 in tumor-adjacent tissue samples and (D) gastric cancer tissue samples. Magnification, x100. Scale bar, 100 μ m. Arrows indicate the magnified regions in the insert (x1,000). PARP-1, poly (adenosine diphosphate-ribose) polymerase 1.

current study indicated that high expression levels of PARP-1 were associated with shorter OS (P<0.001; Fig. 6A) and DFS (P<0.001; Fig. 6B) in the subset of patients with *H. pylori* infection. However, in those patients without *H. pylori* infection, the expression levels of PARP-1 were not significantly associated with OS or DFS (OS, P=0.338; DFS, P=0.999; Fig. 6C and D). In addition, high expression levels of PARP-1 were associated with shorter OS (P=0.001; Fig. 7A) and DFS (P=0.006; Fig. 7B) in the subset of patients with an advanced TNM stage, but not with an early TNM stage (OS, P=0.834; DFS, P=0.955; Fig. 7C and D). The impact of PARP-1 expression levels on the prognosis was not significantly different

between the subgroups of gastric cancer patients categorized according to age, gender, tumor size, tumor location, invasion depth, histological differentiation, presence of ascites, distant metastasis, lymphatic invasion or lymph node metastasis.

Discussion

PARP-1 is the most abundant and best characterized nuclear enzyme of the PARP superfamily (27). PARP-1 binds single- or double-stranded DNA breaks in response to stresses and functions to maintain genomic integrity (10). This role has been the focus of a number of studies in the field of oncology (28,29). However, the role of PARP-1 in gastric cancer tumorigenesis remains to be determined. The present study investigated PARP-1 expression in gastric cancer for the first time. The present study has demonstrated that gastric cancer tissues exhibit significantly higher immunoreactivity of PARP-1, compared with tumor-adjacent tissues, indicating that PARP-1 overexpression may contribute to gastric cancer malignancy. These results are consistent with those of previous studies, which demonstrated that PARP-1 was upregulated in numerous types of tumor (11,15,30-32).

The present study analyzed the association between PARP-1 expression levels and various clinicopathological features in patients with gastric cancer. It was demonstrated that increased PARP-1 expression levels are associated with increased depth of invasion, lymphatic invasion, lymph node metastasis and advanced TNM stage. Furthermore, high PARP-1 expression levels were associated with a larger number of lymph node metastases, suggesting that PARP-1 over-expression increased invasion and metastasis in gastric cancer. Concordant with these results, Rodríguez *et al* (33) previously demonstrated that inhibition of PARP-1 expression suppressed the invasion and colonization of distal organs in melanoma cells. In addition, Li *et al* (34) demonstrated that inhibition of PARP expression attenuated the adhesion of mouse colon carcinoma cells to the extracellular matrix and decreased their

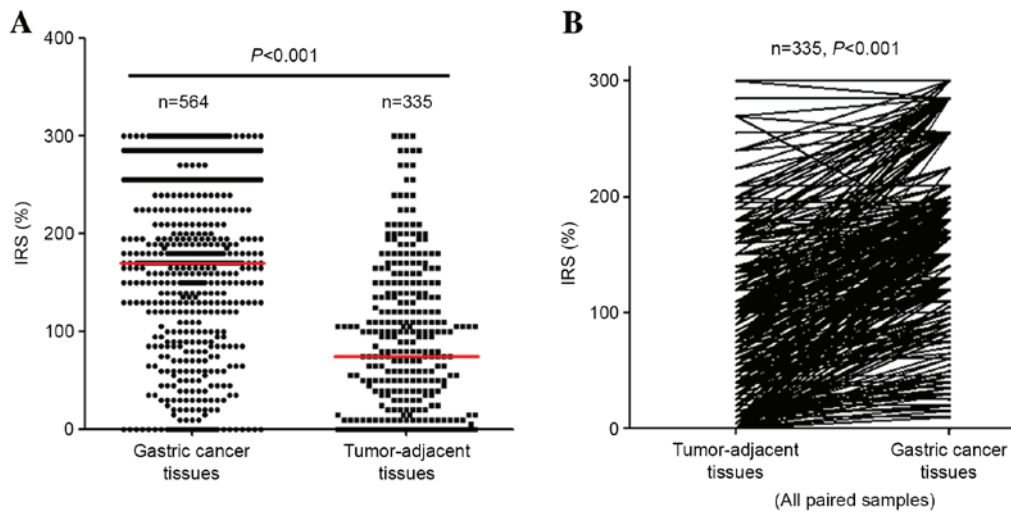


Figure 2. The difference in PARP-1 IRS between gastric cancer tissue samples and tumor-adjacent tissue samples. (A) The distribution of staining by IRS; the red line denotes the median value; the P-value was obtained using a Mann-Whitney U test. (B) Each line represents the IRS for an individual gastric cancer case with tumor-adjacent tissue samples and cancer tissue samples; P-values were obtained from a Wilcoxon rank-sum test. PARP-1, poly (adenosine diphosphate-ribose) polymerase 1; IRS, immunoreactivity score.

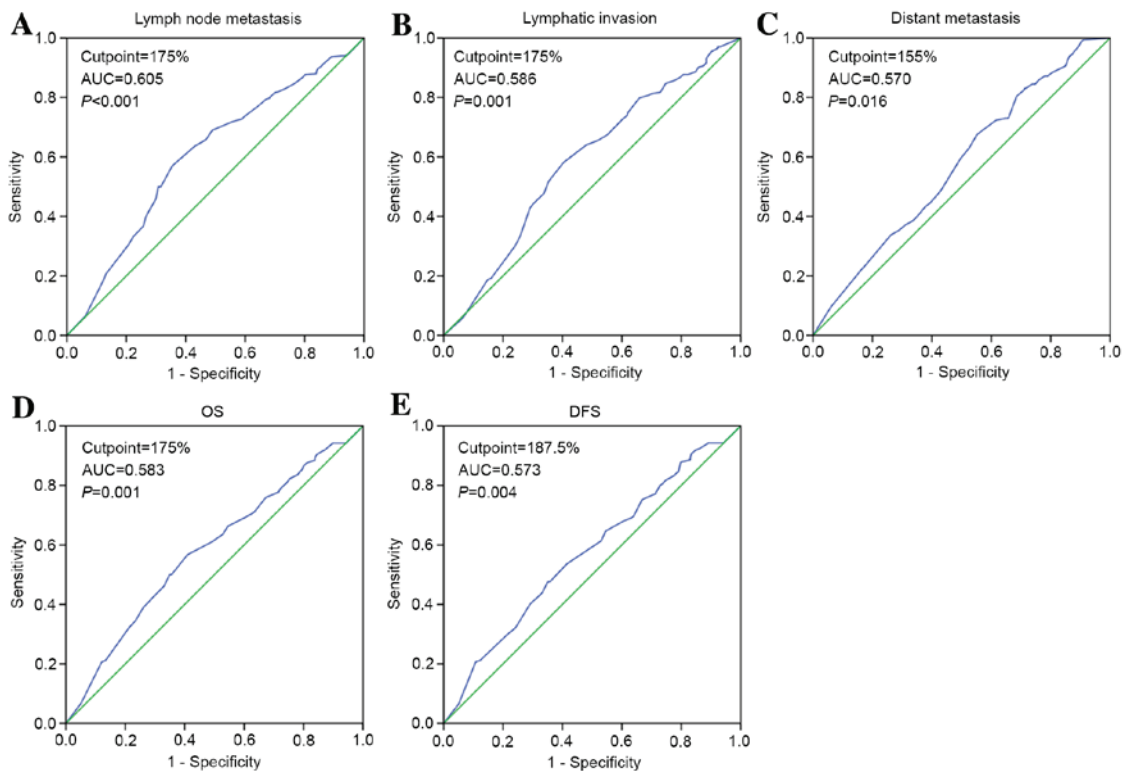


Figure 3. ROC curves were used to determine the cutoff for the IRS of PARP-1 expression based on the (A) lymph node metastasis status, (B) lymphatic invasion status, (C) distant metastasis status, (D) OS status and (E) DFS status of breast cancer patients. The sensitivity and specificity for each outcome were plotted and the AUCs and P-values were indicated. PARP-1, poly (adenosine diphosphate-ribose) polymerase 1; ROC, receiver operating characteristic; IRS, immunoreactivity score; AUCs, areas under curve.

migration and invasion through Matrigel, suggesting that PARP-1 is important in controlling the migration and invasion of certain cancers. PARP-1 has been previously demonstrated to regulate cell invasion and metastasis through the modulation of epithelial-mesenchymal transition-induced malignant transformation or the regulation of the activity of the nuclear factor kappa-light-chain-enhancer of activated B cells (33-36).

Further studies are required to determine whether a similar mechanism of PARP-1 occurs in gastric cancer.

The present study demonstrated that increased PARP-1 expression levels were associated with lower histological differentiation in gastric cancer. This result is concordant with the previously demonstrated inverse correlation between the degree of cell differentiation and PARP-1 activity (37).

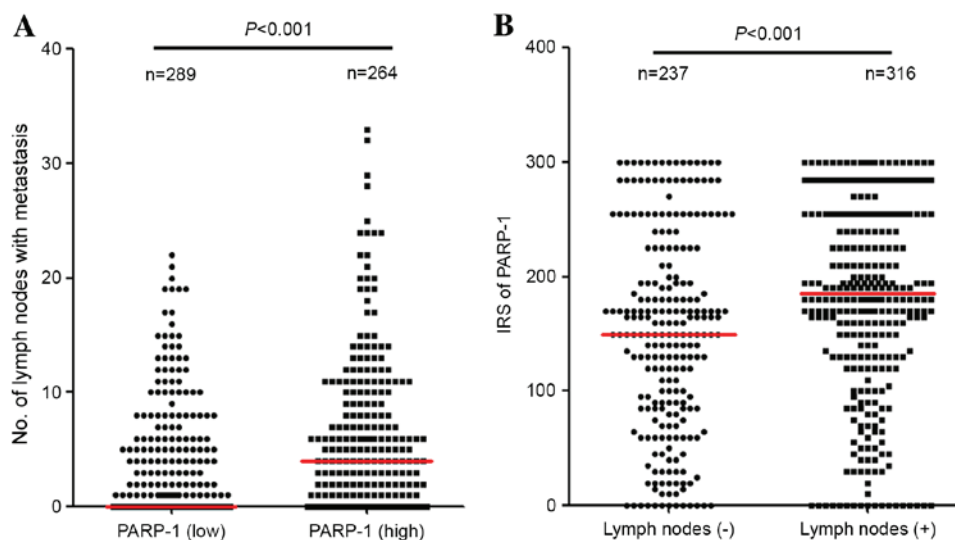


Figure 4. The association of PARP-1 expression with lymph node metastasis in patients with gastric cancer. (A) The number of metastasis-positive lymph nodes in cases of gastric cancer with high PARP-1 expression was increased significantly compared with those with low PARP-1 expression; (B) The IRS of cases of gastric cancer with positive lymph nodes was increased significantly compared with cases without positive lymph nodes. The red line denotes the median value. The P-values indicated in A and B were obtained using a Mann-Whitney U test. PARP-1, poly (adenosine diphosphate-ribose) polymerase 1; IRS, immunoreactivity score.

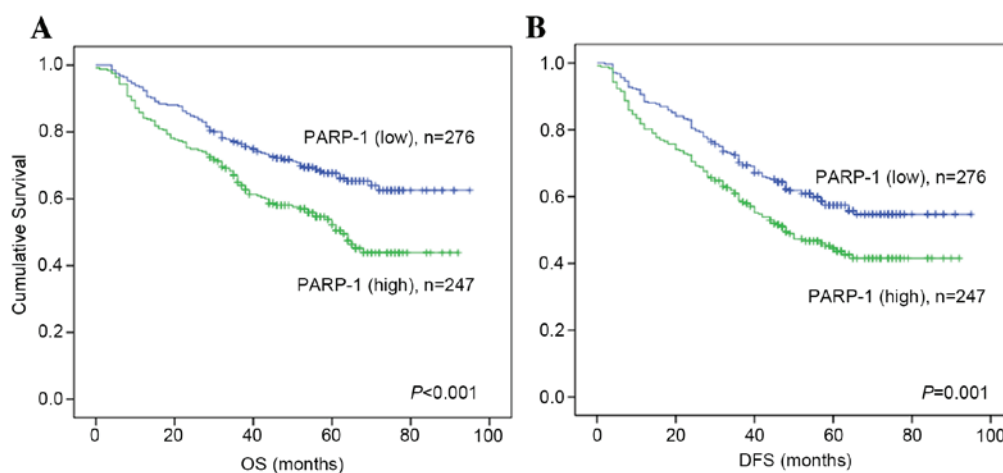


Figure 5. Kaplan-Meier estimates of gastric cancer patients stratified by the expression of PARP-1. Survival rate curves indicating that PARP-1 expression levels were significantly associated with a shorter (A) OS and (B) DFS. The log-rank test was performed to test the statistical significance. PARP-1, poly (adenosine diphosphate-ribose) polymerase 1; OS, overall survival; DFS, disease-free survival.

Barboro *et al* (38) demonstrated that higher PARP expression levels were detected in a less-differentiated PC3 cell line, as compared with a more-differentiated LNCaP prostate carcinoma cell line. It is possible that PARP-1 upregulation is involved in maintaining the stemness of cells, therefore exhibiting an association with lower differentiation in certain cancer cells. Concordant with this hypothesis, Chiou *et al* (39) previously demonstrated that the activation of PARP-1 promoted induced pluripotent stem cell production and helped to maintain a pluripotent state.

PARP-1 expression has been previously demonstrated to be associated with the poor prognosis of numerous tumor types, including early breast cancer and non-small cell lung cancer (13,40-42). However, Aiad *et al* (43) demonstrated that high nuclear PARP-1 expression levels were significantly associated with improved OS in locally advanced breast cancer;

Klauschen *et al* (44) demonstrated that low nuclear expression levels of PARP were associated with a poor prognosis in pancreatic cancer. These previous studies indicated that PARP-1 expression had differing roles in between different tumor types and stages of the tumors. The present study demonstrated that high PARP-1 expression levels are associated with significantly reduced DFS and OS in gastric cancer patients. Furthermore, high expression levels of PARP-1 were demonstrated to be associated with a poor prognosis in a subset of patients with an advanced TNM stage (III-IV), but not early TNM stage (I-II). In addition, a univariate Cox regression analysis identified that high PARP-1 expression levels are associated with a poor prognosis for gastric cancer patients. Therefore, PARP-1 expression levels may have a prognostic value in gastric cancer, particularly for those patients with an advanced TNM stage. However, a multivariate analysis

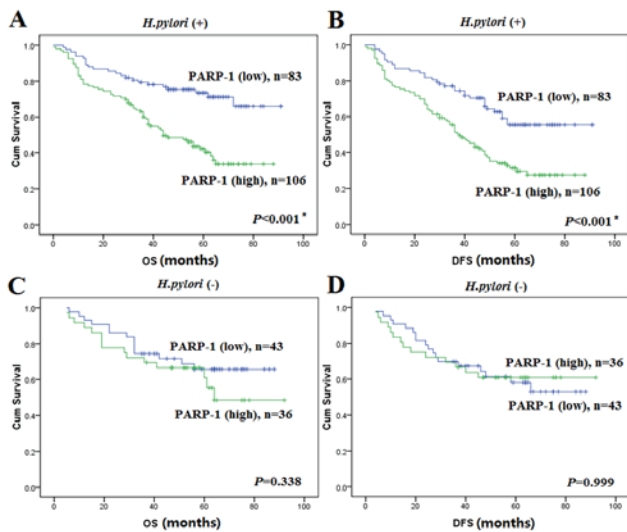


Figure 6. Analysis of the prognostic significance of PARP-1 expression levels in specific subgroups of breast cancer patients with or without *H. pylori* infection. Survival rate curves demonstrate that PARP-1 expression levels were significantly associated with (A) OS and (B) DFS in patients with *H. pylori* infection but were not significantly associated with shorter (C) OS or (D) DFS in patients without *H. pylori* infection. A log-rank test was performed to determine the statistical significance. *Indicated statistical significance. PARP-1, poly (adenosine diphosphate-ribose) polymerase 1; *H. pylori*, *Helicobacter pylori*; OS, overall survival; DFS, disease-free survival.

determined that PARP-1 expression levels were not independent prognostic factors in gastric cancer, which may be due to a significant association between PARP-1 overexpression and tumor invasion and metastasis of gastric cancer.

H. pylori may induce apoptosis of gastric epithelial cells (45,46), and *H. pylori* infection is a risk factor associated with gastric cancer (47,48). Chen *et al* (49) previously demonstrated that incubating BGC-823 gastric cancer cells with *H. pylori* extract induced a breakdown of caspase-1 and caspase-3, but not of PARP. Nossa *et al* (50) subsequently demonstrated that PARP-1 became activated in *H. pylori* infected gastric epithelial cells. Notably, the present study similarly demonstrated that PARP-1 expression levels were significantly increased in *H. pylori* infected gastric cancer cells. Regarding pathogenic infection, Hassumi-Fukasawa *et al* (51) demonstrated a significant positive association between PARP-1 expression levels and human papilloma virus positivity, in high-grade squamous intraepithelial lesions of the uterine cervix. Therefore, PARP-1 expression levels may be involved in host cell responses to pathogen infection. Furthermore, the current study demonstrated that the expression of PARP-1 is associated with significantly shorter OS and DFS in gastric cancer patients with *H. pylori* infection, but not in patients without *H. pylori* infection. These results suggest that the upregulation or activation of PARP-1 in response to *H. pylori* infection may be one mechanism underlying the association of PARP-1 expression levels and poor prognosis in gastric cancer patients. Concordant with a previous study (52), the results of the present study indicate the potential applications of PARP-1 targeted therapy for treating *H. pylori*-mediated gastric cancer.

In conclusion, the present study was a novel investigation into PARP-1 expression patterns in gastric cancer, and the

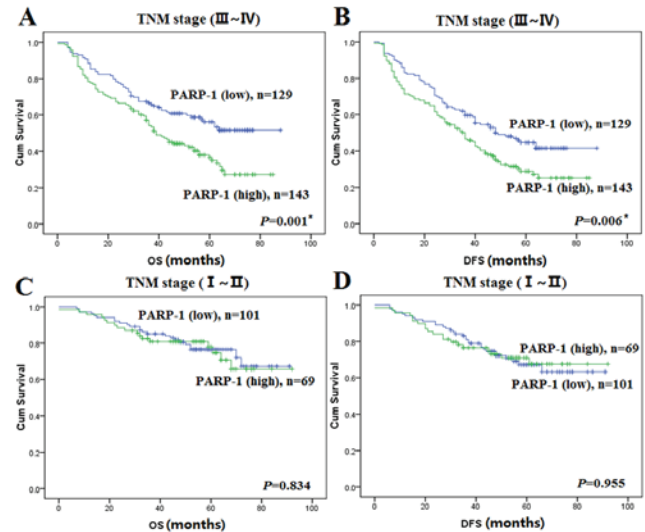


Figure 7. Analysis of the prognostic significance of PARP-1 in specific subgroups of breast cancer patients with various TNM stages. Survival rate curves show that high PARP-1 expression levels were significantly associated with shorter (A) OS or (B) DFS in patients with advanced TNM stage (III-IV) but not (C and D) early TNM stage (I-II). A log-rank test was performed to evaluate the statistical significance. Asterisks (*) indicated statistical significance. PARP-1, poly (adenosine diphosphate-ribose) polymerase 1; OS, overall survival; DFS, disease-free survival; TNM, tumor-node-metastasis.

association between PARP-1 expression levels and the clinicopathological features and prognosis of gastric cancer patients. The results of the current study demonstrated that PARP-1 expression levels are significantly higher in gastric cancer tissues as compared with tumor-adjacent tissues; high PARP-1 expression levels are associated with *H. pylori* infection, lower histological differentiation, tumor invasion and metastasis, and poor prognosis in gastric cancer. Furthermore, it was demonstrated that high PARP-1 expression levels are associated with increased mortality in the subgroups of patients with *H. pylori* infection and an advanced TNM stage. The results of the present study suggest that the inhibition of PARP-1 may suppress tumor invasion and metastasis and improve histological differentiation and the survival rate in gastric cancer. The targeting PARP-1 may be an effective therapeutic strategy for the treatment of gastric cancer, particularly of *H. pylori*-positive or advanced-stage gastric cancer.

Acknowledgements

This study was supported by the Science and Technology Department Foundation of Liaoning Province (grant no. 2013225021).

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