Original Article

Expression levels and the prognostic value of long non-coding RNA PVT1 in serum of Han and Uygur gastric cancer patients in Xinjiang, China

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Background: The expression level of a long non-coding RNA (lncRNA), plasmacytoma variant translocation 1 (PVT1), was studied in serum of Han and Uygur gastric cancer (GC) patients and normal persons in Xinjiang, China, and the prognostic value of PVT1 was analyzed.

Methods: We collected serum samples from 87 GC patients (51 Han and 36 Uygur), and 95 normal persons (55 Han and 40 Uygur). Total RNA was extracted from the serum and used for real-time polymerase chain reaction (PCR) to detect the level of PVT1.The relationships between PVT1 and clinicopathological features were analyzed using the Spearman's relative analysis, rank sum test, and χ^2 test. At the same time, selection electrochemiluminescence was used to detect the concentration of serum tumor markers, including alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and carbohydrate antigen 72-4 (CA72-4). The correlation between PVT1 and the tumor markers was analyzed by Spearman's relative analysis.

Results: The serum expression level of PVT1 was higher than that of the normal group in both Han and Uygur GC patients ($P<0.05$). PVT1 expression in Uygur GC serum was higher than that of Han patients ($P<0.05$). The high level of serum PVT1 correlated with lymph node metastasis in both Han and Uygur GC patients ($P<0.05$). Compared with Han GC patients, the Uygur people were more likely to develop distant metastasis ($P<0.05$). Uygur patients were more often diagnosed at stage III or IV ($P<0.05$). In addition, serum PVT1 showed a significant correlation with CA19-9 in Han GC patients ($P<0.05$).

Conclusions: Increased serum PVT1 level may indicate an increased tendency for lymphatic metastasis, especially in Uygur GC patients. When combined with CA19-9, the PVT1 expression level may be a better diagnostic marker in Han GC patients.

Keywords: Gastric cancer (GC); long non-coding RNA (lncRNA); PVT1; tumor markers

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Introduction

Gastric cancer (GC) is the second most common cause of cancer death worldwide and GC mortality in China makes up 42% of the general mortality rate of the world (1). Xinjiang is the largest autonomous region in northwestern China. The population in this region is made up of a number of ethnic groups. The major ethnic groups are the Han (39.7%) and the Uygur (45.7%). Different ethnic groups have different characteristics regarding the morbidity of GC. The relevance ratio of GC in Uygur is 12.76% (351/2,751) and in Han people is 3.85% (92/2,568). Thus the mortality risk of Uygur people is 2.4 times higher than that of Han people (2,3).

Early symptoms of GC are not obvious. In most GC patients, the disease has progressed to middle or advanced stages when they were diagnosed. However, the prognosis of GC is closely associated with the TNM stage. The 5-year survival rates of GC patients are 90%, 50–60%, and 10–15% for GC stages I, II, and III, respectively (4). Thus, it is important to identify a diagnostic marker of GC which will help early diagnosis and thus prolong the life of GC patients. Serum detection is a convenient and easy technique for disease diagnosis, which is also simple and relatively painless for patients. Thus, identification of serum tumor biomarkers is significant for GC patients.

At present, serum carbohydrate antigen 72-4 (CA72-4), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9), are used as tumor markers for GC diagnosis (5). However, their sensitivity and specificity are limited. Long non-coding RNAs (lncRNAs) are greater than 200 nucleotides in length. They can regulate gene expression at both the transcriptional and post-transcriptional levels (6). Thus they play a significant role in the fundamental biological processes of cells and are emerging as new players in the tumorigenic process (7). LncRNAs exhibit specific expression in tissues and can be detected easily in body fluids. This advantage makes them ideal as biomarkers (8), and some, such as *H19* (9,10), *GAS5* (11), and *HOTAIRM1* (12,13), have recently attracted significant attention for the early diagnosis of cancer.

Plasmacytoma variant translocation 1 (PVT1), a new lncRNA, was found to be over expressed in GC tissues and could be a potential biomarker for diagnosis of GC (14,15). However, the expression level of PVT1 in serum is unclear, and whether there are differences between Han and Uygur GC patients is also not known.

In the present study, we investigated and compared the expression level of PVT1 in the serum of Han and Uygur GC patients, and explored the relationship between the expression of PVT1 and clinicopathological features. We analyzed the correlation of PVT1 and AFP, CEA, CA19-9, and CA72-4, in an attempt to reveal the clinical value of PVT1 as a serum biomarker for diagnosis of GC in Han and Uygur patients.

Methods

Sample collection

We collected serum samples from 87 GC patients, comprising 51 Han and 36 Uygur patients, and from 95 normal persons, comprising 55 Han and 40 Uygur. The samples were collected at the First Affiliated Hospital of Shihezi, Xinjiang, China and the First People Hospital of Kashi, Xinjiang, China. Whole venous blood samples (10 mL) were incubated for 30 minutes at room temperature, and centrifuged at low speed (3,000 rpm, 5 min). The supernatant was transferred to new tubes and centrifuged at high speed (12,000 rpm, 10 min) at 4 ℃, then the supernatant serum was collected and stored at –80 ℃. Clinical data for GC patients and normal controls were obtained by medical record review, from patient records and information, which was anonymized and de-identified prior to analysis. Details of the investigation and the required informed consent were examined and certified by the Ethics Committee of the First Affiliated Hospital School of Medicine, Shihezi University (No. 2016-035-01).

Serum RNA extraction and cDNA synthesis

Total mRNA from serum of GC patients and normal controls was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). A 750 μL aliquot of TRIzol Reagent was added directly to 250 μL of serum, then the RNA was extracted following the manufacturer's instructions. At the same time, 1.5 mL of fresh blood was collected from each patient, then added to five volumes of 1× erythrocyte lysis buffer, centrifuged at 12,000 rpm for 10 min, and the supernatant was discarded. Two volumes of 1× erythrocyte lysis buffer were added, centrifugation was repeated, and the supernatant was discarded. TRIzol was then used to obtain total RNA from respective blood samples. The integrity of RNA was checked using 1.2% agarose gel electrophoresis (*Figure S1A*). The purity and concentration of RNA were assessed by measuring the absorbance at 260 and 280 nm

using a spectrophotometer. Total RNA from all samples was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol and 1.0 μg of RNA was taken from each sample for cDNA synthesis.

Real-time polymerase chain reaction (PCR)

The PVT1 expression level was quantified using a SYBR Green PCR kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol, with the following primers: forward, 5'-GGAAGGTGGAGCGTAAGGA-3' and reverse, 5'-CAATGCCGCCAATCTTGTA-3'. The length of the quantitative PCR product was 92 base pairs. The expression level of PVT1 in each serum sample was normalized to the respective β-actin expression level from blood total RNA, using the following primers: forward, 5'-CCCAGCACAATGAAGATCAAGATCAT-3', and reverse, 5'-ATCTGCTGGAAGGTGGACAGCGA-3' (product length, 101 base pairs). The amplification protocol included an initial heat activation step at 95 ℃ for 5 min, followed by 40 cycles of denaturation at 95 ℃ for 30 s and combined annealing/extension at 55 ℃ for 30 s. The expression of PVT1 in serum was calculated using the $2^{(-\Delta CT)}$ value and the specificity of each PCR reaction was confirmed by melting curve analysis (*Figure S1B,C,D*).

Electrochemiluminescence

Samples of venous serum (4 mL) were collected in the morning after overnight fast, and centrifuged at 3,000 rpm for 10 min. The content of tumor markers in serum was detected using AFP, CEA, CA19-9, or CA72-4 specific kits (Roche Diagnostics GmbH Production, Basel, Switzerland) with an automatic immunology analyzer (Roche E170). The tumor markers were defined as positive when above the normal range, and defined as negative when within the range.

Statistical analysis

The rank sum test was used to compare differences between GC patients and normal controls. The correlation between PVT1 and clinicopathological characteristics or tumor markers of GC patients was analyzed by χ^2 and Spearman's test. All statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS, version 20.0, IBM SPSS Statistics for Windows, version 19.0. Armonk, NY, USA). Values of P<0.05 were considered statistically significant.

Results

Serum PVT1 levels were higher in GC patients than in normal controls and PVT1 levels in the serum of Uygur GC patients were higher than those of Han

PVT1 expression levels in serum of 51 Han and 36 Uygur GC patients, together with 55 Han and 40 Uygur normal controls were examined by real-time PCR. According to the rank sum test, PVT1 expression in GC serum was significantly higher than that of normal controls in both Han and Uygur ethnic groups (P<0.05) (*Figure 1A,B*). However, compared with Uygur GC patients, PVT1 expression level of Han GC patients was significantly lower (P<0.05) (*Figure 1C*).

High serum PVT1 level was associated with lymph node metastasis in both Han and Uygur GC patients

Integrated clinical information was available for 28 Han and 31 Uygur GC patients (*Table S1*). The relationship between serum PVT1 expression level and clinicopathological features was examined in these patients. First, the Han and Uygur GC serum samples were divided into a high PVT1 expression group and a low PVT1 expression group according to the median. The associations between PVT1 expression levels and clinicopathological features of the patients are summarized in *Tables 1,2*. A significant relationship was found between PVT1 expression and lymph node metastasis in both Han and Uygur patients (P<0.05). However, PVT1 expression level showed no correlation with age, sex, or primary tumor site. Analysis showed that Uygur GC patients were more likely to develop distant metastasis (P<0.05) and more likely to be diagnosed at a late clinical stage (P<0.05) (*Table 3*).

Serum PVT1 expression level was correlated with serum CA19-9 level in Han GC patients

Electrochemiluminescence was used to measure serum levels of the tumor markers AFP, CEA, CA19-9, and CA72-4 and the correlation with serum PVT1 level was analyzed in Han and Uygur GC patients by Spearman's relative analysis. A significant association was found between PVT1expression and CA19-9 in Han GC patients (P<0.05). However, no

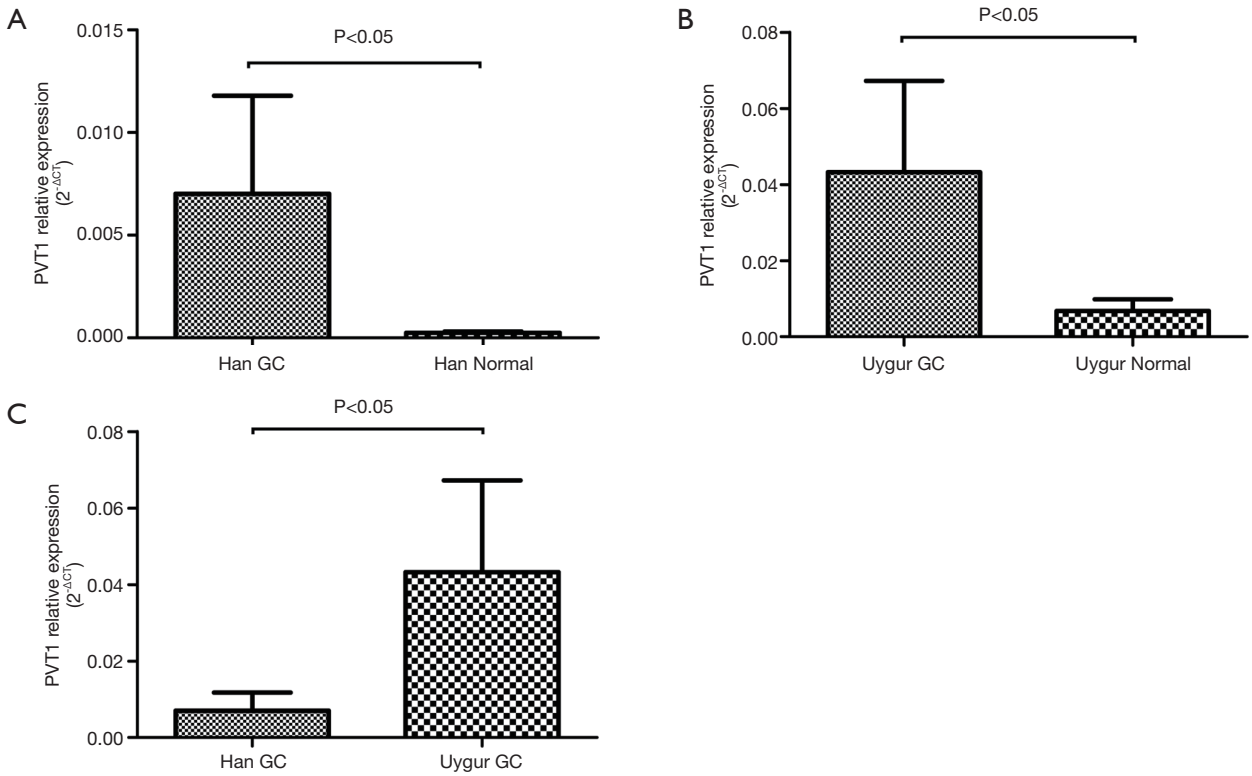

Figure 1 Comparison of PVT1 level in serum of Han and Uygur GC patients and normal controls. (A) Real-time PCR analysis showed that the level of PVT1 expression in GC serum was significantly higher than that of normal controls in the Han population (P<0.05). (B) Real-time PCR analysis showed that PVT1 expression in GC serum was significantly higher than that of normal controls in the Uygur population (P<0.05). (C) The PVT1 expression level in the serum of Uygur GC patients was higher than that of Han patients (P<0.05). PVT1, plasmacytoma variant translocation 1; GC, gastric cancer; PCR, polymerase chain reaction.

correlation was found with other tumor markers in Han GC patients (*Table 4*). In addition, in Uygur people, PVT1 expression levels showed no relationship with any of the four tumor markers (*Table 5*).

Discussion

GC is a major cause of cancer-related mortality in China (16). Host genetics, bacterial virulence, environmental, and many other factors have all been implicated in affecting the gastric oncogenic process, but the underlying molecular mechanism remains poorly understood.

Han and Uygur people have different genetic backgrounds and their characteristics of morbidity and mortality in GC are also different. For Han and Uygur GC cases, the histopathological features maybe similar, but whether the diagnostic parameters, especially some genetic and molecular biology biomarkers, are suitable for both of these ethnic groups is not known.

In recent years, the results of transcriptomics have indicated that only approximately 2% of genes making up the human genome are protein coding genes. The remaining 98% are transcribed into non-coding RNAs (ncRNAs). Among the non-coding RNAs, 80% are lncRNAs. Interestingly, these lncRNAs could be important biomarkers for clinical diagnosis as well as drug targets for cancer.

Cao *et al.* (14) found that the lncRNA PVT1 is upregulated in the tumor tissues of GC based on highly significant microarray results. Kong *et al.* (17) further confirmed that PVT1 is upregulated in GC tumor tissues, and showed that PVT1 silencing can block the G1 phase of the cell cycle, thus halting the proliferation of GC cells. Zhang *et al.* (18) found that PVT1 promotes the multidrug resistance of GC cells. In addition, PVT1 is located in chromosome 8q24 (19), exactly upstream of the oncogene MYC. All of these results indicate that PVT1 is closely

Table 1 Correlation analysis of PVT1 expression level and clinicopathological factors in serum from Han GC patients

Variable	Category	PVT1		P
		High expression	Low expression	
Age (year)		55.29±10.908	59.50±11.621	0.311
Sex	Male	12	8	0.209
	Female	2	6	
T	T1–2	3	4	1.000
	T3–4	11	10	
N	N0	2	8	0.046*
	N1–3	12	6	
M	M0	13	14	1.000
	M1	1	0	
Stage	I, II	3	6	0.420
	III, IV	11	8	
Histopathological grade	Poorly	10	7	0.440
	Well + moderately	4	7	

Note: Fisher's exact probability test. T, the primary tumor site; N, the involvement of regional lymph node; M, the presence of distant metastatic; *, P<0.05.

Table 2 Correlation analysis of PVT1 expression level and clinicopathological factors in serum from Uygur GC patients

Variable	Category	PVT1		P
		High expression	Low expression	
Age (year)		51.38±14.245	59.80±10.936	0.078
Gender	Male	11	11	1.000
	Female	5	4	
T	T1–2	1	1	1.000
	T3–4	15	14	
N	N0	0	4	0.043*
	N1–3	16	11	
M	M0	8	11	0.273
	M1	8	4	
Stage	I, II	2	2	1.000
	III, IV	14	13	

Note: Fisher's exact probability test; T, the primary tumor site; N, the involvement of regional lymph node; M, the presence of distant metastatic; *, P<0.05.

related to the mechanism and treatment of GC.

In addition to the tissues, abnormal expression of lncRNAs can also be detected in body fluid samples, such as serum and saliva (20,21). The expression level of PVT1 in serum is unclear, and whether or not there are difference between Han and Uygur GC patients is also not known.

Table 3 Comparison of the clinicopathological features between Han and Uygur GC patients

Variable	Category	Han	Uygur	P
Age (year)		57.39±11.266	55.45±13.251	0.471
Gender	Male	20	22	1.000
	Female	8	9	
T	T1–2	7	2	0.071
	T3–4	21	29	
N	N0	10	4	0.065
	N1–3	18	27	
M	M0	27	19	0.001*
	M1	1	12	
Stage	I, II	9	2	0.018*
	III, IV	19	29	

Note: Fisher's exact probability test. T, the primary tumor site; N, the involvement of regional lymph node; M, the presence of distant metastatic; *, P<0.05.

Table 4 Correlation between PVT1 and serum tumor markers in Han GC patients

Tumor markers	n	PVT1	
		r	P
AFP	51	0.013	0.926
CEA	51	–0.060	0.678
CA19-9	51	0.429	0.002*
CA72-4	51	0.000	1.000

Note: Spearman correlation analysis; r, correlation coefficient. *, P<0.05. PVT1, plasmacytoma variant translocation 1; GC, gastric cancer; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

Table 5 Correlation between PVT1 and serum tumor markers in Uygur GC patients

Tumor markers	n	PVT1	
		r	P
AFP	25	0.045	0.829
CEA	25	–0.073	0.729
CA19-9	25	–0.097	0.643
CA72-4	25	–0.095	0.651

Note: Spearman correlation analysis; r, correlation coefficient. PVT1, plasmacytoma variant translocation 1; GC, gastric cancer; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

In this study, we collected samples of serum from Han and Uygur GC patients and normal controls. We analyzed the serum expression level of PVT1 and found that the PVT1 expression level was higher in GC patients than in normal individuals both in the Han and Uygur populations. Furthermore, when comparing the Han and Uygur GC patients, we found that the serum PVT1 level in Uygur GC patients was higher than that in Han GC patients. The result of the relationship analysis between serum expression of PVT1 and clinical characteristics suggested that PVT1 could be a marker to identify patients with a tendency for lymphatic metastasis. In a clinical test, if GC patients were found to have a high serum level of PVT1, they would need to be vigilant for the incidence of lymphatic metastasis.

Changes of tumor markers in serum are likely to appear earlier than clinical symptoms. Thus, combined detection of a multiterm tumor marker will be effective in evaluating both the diagnosis and prognosis of GC patients. In one study, patients with an elevated CA19-9 level in serum were found to have more nodal metastases in intrahepatic cholangiocarcinoma (22). Zhou *et al.* (23) found that CA199 ≥14.06 U/mL and CA125 ≥14.30 U/mL were predictors of endometrial carcinogenesis when entered into the risk mode. The variation of CEA and CA19-9 levels in serum can accurately predict the efficacy of first-line chemotherapy in advanced GC (24). In our research, serum expression

levels of PVT1 and CA19-9 showed a correlation in Han GC patients. PVT1 and CA19-9 can thus be combined to diagnose Han GC patients. However, in Uygur GC patients, PVT1 serum expression showed no correlation with tumor markers in serum.

In conclusion, our results suggested that an increase in the serum PVT1 level could be an ideal tumor biomarker for GC diagnosis both in Han and Uygur GC patients. PVT1 level in serum can help to judge the tendency of lymphatic metastasis in GC patients. PVT1 and CA19-9 can be combined as serum tumor markers in Han GC patients. However, whether PVT1 and tumor markers can be combined in Uygur GC patients still needs to be explored. In our future research, we will study the mechanism underlying the high PVT1 level in the serum and the function of PVT1 in GC cells. We plan to analyze the PVT1-protein interaction networks in an attempt to identify the transcription factors or polymerases that are involved in the mechanism of PVT1 in GC cells. We will also test the tumorigenic capacity of PVT1 in gastric cells.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.12.29). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the First Affiliated Hospital School of Medicine, Shihezi University (No. 2016-035-01), and written informed consent was obtained from the patient for publication of this manuscript and any accompanying images.

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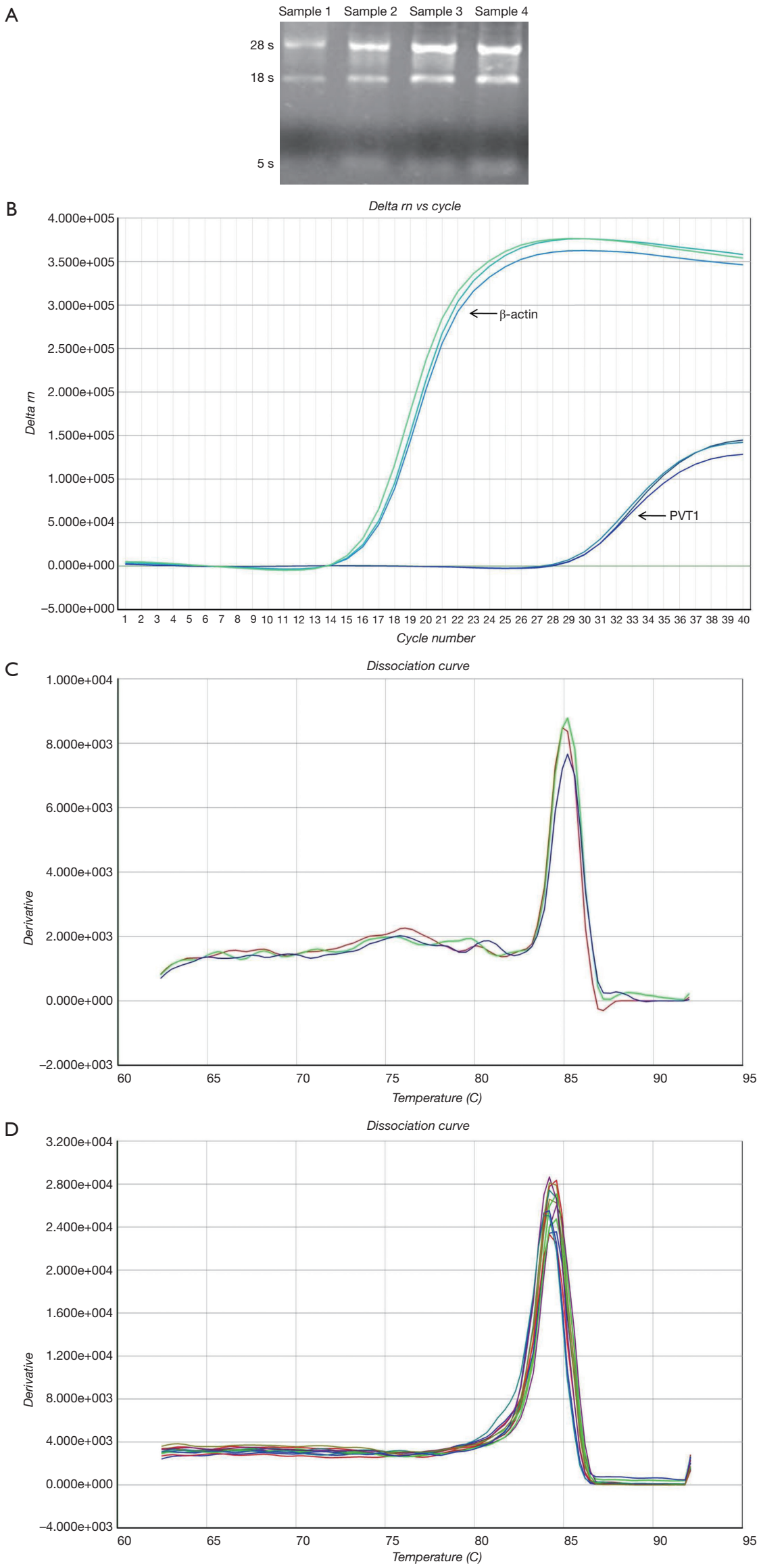

Figure S1 Analysis of the quality of the isolated RNA and real time PCR. (A) Samples 1–4 are results of 1.2% agarose gel electrophoresis to evaluate the integrity of RNA from blood samples. Bands of 28 s, 18 s, and 5 s RNA can be seen and the RNA was not degraded; (B) amplification curve of β-actin and PVT1 from real-time PCR to observe the Ct value of different genes. The lines are from one sample and made triplicate to check the assay; (C) dissociation curve of PVT1 from real-time PCR to detect the specificity of the PVT1 primer. The lines are from one sample and made triplicate to check the assay; (D) dissociation curve of β-actin from real time PCR to detect the specificity of the β-actin primer. PVT1, plasmacytoma variant translocation 1; PCR, polymerase chain reaction.

Table S1 Clinicopathological factors of Han and Uygur GC patients

No.	Pathological No.	Age	Gender	Nationality	TNM
1	C8	47	Male	Han	III
2	C40	48	Male	Han	III
3	C75	70	Male	Han	IV
4	C120	63	Male	Han	III–IV
5	C124	63	Male	Han	III
6	C127	40	Male	Han	IV
7	C57	58	Male	Han	II
8	C95	45	Male	Han	I
9	C122	61	Male	Han	III
10	C129	55	Male	Han	II
11	C135	65	Male	Han	III
12	C138	73	Male	Han	III
13	C34	43	Female	Han	III
14	C60	58	Female	Han	III
15	C47	45	Female	Han	I
16	C55	43	Female	Han	III
17	C72	54	Male	Han	III
18	C73	63	Male	Han	III
19	C80	77	Female	Han	III
20	C81	65	Male	Han	III
21	C82	44	Female	Han	III
22	C99	64	Female	Han	II
23	C125	47	Female	Han	II
24	C128	73	Male	Han	I
25	C131	78	Male	Han	III
26	C137	55	Male	Han	IV
27	C143	46	Male	Han	I
28	C104	64	Male	Han	I
29	K1	66	Male	Uygur	IV
30	K47	49	Female	Uygur	IV
31	K13	67	Female	Uygur	IV
32	K28	38	Male	Uygur	IV
33	K81	56	Male	Uygur	II
34	K45	40	Female	Uygur	IV
35	K49	64	Male	Uygur	IV
36	K50	57	Male	Uygur	IV
37	K52	83	Male	Uygur	IV
38	K55	76	Male	Uygur	IV
39	K56	42	Female	Uygur	II
40	K69	41	Male	Uygur	IV
41	K78	40	Male	Uygur	IV
42	K84	43	Male	Uygur	IV
43	K19	42	Female	Uygur	IV
44	K21	40	Male	Uygur	IV
45	K3	64	Male	Uygur	IV
46	K5	72	Male	Uygur	IV
47	K29	68	Male	Uygur	IV
48	K68	71	Male	Uygur	IV
49	K36	55	Female	Uygur	IV
50	K44	50	Female	Uygur	IV
51	K61	58	Female	Uygur	IV
52	K63	52	Male	Uygur	IV
53	K64	39	Male	Uygur	IV
54	K76	40	Female	Uygur	IV
55	K8	56	Male	Uygur	IV
56	K60	72	Male	Uygur	IV
57	K65	66	Male	Uygur	III
58	K72	72	Male	Uygur	II
59	K74	57	Male	Uygur	II

GC, gastric cancer.