Potential lncRNA diagnostic biomarkers for early gastric cancer

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Abstract. Long noncoding RNAs (lncRNAs) serve important functions in many crucial biological processes; however, the effects of lncRNAs in early gastric cancer (EGC) are not entirely clear. The present study aimed to demonstrate the potential of lncRNAs to be used as biomarkers in EGC. Reverse transcription-quantitative polymerase chain reaction was used to measure the expression levels of lncRNAs, including X inactive-specific transcript (XIST), Yiya, brain cytoplasmic RNA 1 (BCYRN1), ribosomal RNA processing 1B (RRP1B), KCNQ1 opposite transcript 1 (KCNQ1OT1) and testes development related 1 (TDRG1), in EGC tissues compared with normal adjacent tissues (NATs). XIST, BCYRN1, RRP1B and TDRG1 were identified as differentially expressed in EGC tissues compared with NATs. The specificity and sensitivity of XIST, BCYRN1, RRP1B and TDRG1 were determined by receiver operating characteristic curve analysis. In addition, RRP1B expression was revealed to be significantly correlated with distal metastasis (P=0.020) and tumor-node-metastasis staging (P=0.018), and TDRG1 expression was significantly correlated with lymph node metastasis (P=0.001). Furthermore, BCYRN1, RRP1B and TDRG1 expression levels were compared between EGC tissues and plasma, and the results indicated that there were significant positive correlations of XIST, BCYRN1, RRP1B and TDRG1 expression levels between the EGC tissues and plasma. Therefore, the present study suggested that XIST, BCYRN1, RRP1B and TDRG1 may be served as potential diagnostic biomarkers for EGC.

Introduction

Gastric cancer (GC) is a high-incidence disease worldwide, particularly in Eastern Asia, although there has been a recent downward trend in morbidity (1); GC is the third major cause of cancer-related mortality in the world (2). Patients with early GC (EGC) may be cured completely though effective treatment. At present, although surgery, chemotherapy and radiotherapy are used in treatments, the prognosis of patients with GC is still very poor owing to metastasis (3-6). Therefore, early diagnosis serves an important role in reducing GC-related mortality. However, as there are no effective diagnostic signs or sensitive biomarkers for early diagnosis, most GC patients develop terminal cancer (7). Therefore, identifying specific biomarkers and effective molecular targets for GC are extremely important.

Long noncoding RNAs (lncRNAs) are a type of noncoding RNA that are >200 nucleotides, which regulate gene expression through transcription regulation, post-transcription regulation, chromatin modification and genomic imprinting (8,9). An increasing number of studies have indicated that lncRNAs participated in various biological processes, such as cell cycle and cell differentiation (10), apoptosis (11,12), epithelial-mesenchymal transition (EMT), cell migration and metastasis (13). Several previous studies have reported that lncRNAs may be closely associated with tumor genesis, including liver cancer (14), lung cancer (15), ovarian cancer (16), colorectal cancer (17) and breast cancer (18-20). Therefore, lncRNAs may be potential diagnostic biomarkers for certain diseases. A previous report using a human lncRNA microarray identified 33 differentially expressed lncRNAs associated with EGC, including 13 that were upregulated and 20 downregulated (21). The present study further validated that X inactive-specific transcript (XIST), brain cytoplasmic RNA 1 (BCYRN1), ribosomal RNA processing 1B (RRP1B) and testes development related 1 (TDRG1) were aberrantly expressed both in EGC tissues and plasma.

The present study examined the expression levels of XIST, Yiya, BCYRN1, RRP1B, KCNQ1 opposite transcript 1 (KCNQ1OT1) and TDRG1 in EGC tissues and normal adjacent tissues (NATs) by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). XIST, BCYRN1, RRP1B and TDRG1 were identified as differentially expressed in EGC tissues compared with NATs, and exhibited potential diagnostic values for the detection of EGC. The expression level of RRP1B was significantly correlated with distal metastasis and tumor-node-metastasis (TNM) staging, and the expression of TDRG1 was significantly correlated with lymph node metastasis. Furthermore, significant positive correlations for XIST, BCYRN1, RRP1B and TDRG1 expression levels were made between the EGC tissues and plasma. Therefore, XIST, BCYRN1, RRP1B and TDRG1 may serve as potential diagnostic biomarkers for EGC.

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Materials and methods

Clinical specimens. The present study was approved by the Ethics committee of Zhongda Hospital, School of Medicine, Southeast University (Nanjing, China), and informed consent was received from each patient. A total of 76 pairs of EGC tissues and paired NATs were collected from Zhongda Hospital between May 2014 and November 2016. Among them, 10 pairs of EGC tissues and paired NATs were used to preliminarily detect the XIST, Yiya, BCYRN1, RRP1B, KCNQ1OT1 and TDRG1 expression levels. The 10 patients included 5 males and 5 female patients, and the average age was 63.4 and 64.2 years old. None of the patients in the study received radiotherapy or chemotherapy prior to surgical resection. All collected tissue samples were frozen at -80˚C for total RNA extraction. Peripheral blood (5 ml) was collected from the 76 fasting patients prior to endoscopy, and controls (76 healthy patients, including 32 males and 44 female patients, and the average age was 65.4 and 61.2 years old) were done at the same time; serum was separated by centrifugation (3,500 x g; 10 min; 10˚C) and the serum supernatant was frozen at -80˚C until further analysis.

RT-qPCR. Total RNA was extracted from EGC tissues (100 mg for every organization) and paired NATs using the RNeasy Plus Mini kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol. Total RNA was extracted from serum (800 μl) using the QIAamp Circulating Nucleic Acid kit (QiagenKK, Tokyo, Japan). RNA purity was measured using the NanoDrop (Peqlab Biotechnologie GmbH, Erlangen, Germany). The $OD_{260/280}$ ratio was used as indicator for RNA purity. A ratio higher than 1.8 was regarded as suitable for gene expression measurements. The RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to synthesize cDNA according to the manufacturer's protocol. XIST, Yiya, BCYRN1, RRP1B, KCNQ1OT1 and TDRG1 expression were examined in EGC tissues (paired NATs were used as control) and EGC plasma (healthy patient serum were used as control) by RT-qPCR using the SYBRGreen Master Mix kit (Takara Bio, Inc., Otsu Japan) and PRISM 7900HT sequence detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.) as described previously (22). The quantitative PCRs were carried out in 20-μl reaction volume containing 2 μl cDNA products. Reaction steps were as follows: 95˚C for 30 sec (predegeneration) as the first step in a loop; 95˚C for 5 sec (degeneration), 60˚C for 34 sec (extension) as the second step, a total of 40 cycles. The data was analyzed using SDS 2.3 software (Applied Biosystems; Thermo Fisher Scientific, Inc.). The specificity of primer sequences was measured according to the dissociation curve, and the relative gene expression levels were analyzed using the $2^{-\Delta\Delta Cq}$ (quantitation threshold) method (23). All data are presented as the mean ± standard deviation of three independent experiments. The following qPCR primers were used: XIST, forward 5'-AACCAC CTACACTTGAGCCA-3', reverse 5'-AGGACAATGACG AAGCCACT-3'; Yiya, forward 5'-TTGAGTCGGATCCTC TCAGC-3', reverse 5'-CTCTCTGAGTTGCCCTTGGA-3'; BCYRN1, forward 5'-TCATGAAGCTTGCCTCTGGA-3', reverse 5'-AACATGGAGAGGGAAGGTGG-3'; RRP1B, forward 5'-CACAGCACAAACACGAGTCA-3', reverse 5'-TGCCTTCTACTTGGTGAGGG-3'; KCNQ1OT1, forward 5'-TGGTAAGTTACAGGGCAGGG-3', reverse 5'-TGAACA TCCATCCCCAAGCT-3'; TDRG1, forward 5'-GGTGCAGTC TTCAGGGATCT-3', reverse 5'-GCCTCCCTCCTCTTCATT GT-3'; GAPDH, forward 5'-TGTTCGTCATGGGTGTGA AC-3', reverse 5'-ATGGCATGGACTGTGGTCAT-3'. Samples were normalized to GAPDH.

Statistical analysis. All data and calculations were analyzed using Prism6 (GraphPad Software Inc., La Jolla, CA, USA) and SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). The area under the receiver operating characteristic (ROC) curve (AUC) was used to assess the predictive power and to determine the cut-off scores for XIST, BCYRN1, RRP1B and TDRG1 expression levels between patients with EGC and controls. The differences in lncRNA expressions (XIST, BCYRN1, RRP1B and TDRG1) in tissues among the patients were analyzed using the χ^2 test concerning clinical parameters such as age (>60 vs. <60 years), sex (male vs. female), pathological node (pN status; N0 vs. N1-N2), pathological metastasis (pM status; M0 vs. M1), and clinical stage (I and II vs. III and IV). For paracarcinoma-carcinoma paired tissues, the difference in lncRNA expression was evaluated using paired Student's t-test. The relationship of lncRNA expression in EGC tissue and plasma was analyzed using Mantel-Haenszel statistics. All results are presented as the mean ± standard deviation (SD). $P<0.05$ was considered to indicate a statistically significant difference.

Results

XIST, Yiya, BCYRN1, RRP1B, KCNQ1OT1 and TDRG1 expression levels in EGC tissues and NATs. A previous study analyzed lncRNA expression profiles including XIST, Yiya, BCYRN1, RRP1B, KCNQ1OT1 and TDRG1 in GC tissues and paired NATs by a human lncRNA microarray (21). This previous study identified 68 lncRNAs that were associated with diseases, of which the top 33 were demonstrated to be differentially expressed, including 13 upregulated and 20 downregulated lncRNAs. As hypoxia inducible factor 1α-antisense RNA 1 (HIF1α-AS1), plasmacytoma variant translocation 1 (PVT1), carbonyl reductase 3-antisense RNA 1 (CBR3-AS1) and urothelial cancer associated 1 (UCA1) have been identified previously, the present study further examined the expression levels of XIST, Yiya, BCYRN1, RRP1B, KCNQ1OT1 and TDRG1 by RT-qPCR in EGC tissues and NATs (21). Initially, we identified six abnormally expressed lncRNAs in 10 tissues as a preliminary screening. The results demonstrate that the expression levels of XIST (Fig. 1A) and BCYRN1 (Fig. 1B) were significantly increased in EGC tissues compared with NATs (n=10; $^{**}P<0.01$); Yiya (Fig. 1C) and KCNQ1OT1 (Fig. 1D) exhibited no significant changes in expression levels in EGC tissues compared with NATs (n=10); and the expression levels of RRP1B (Fig. 1E) and TDRG1 (Fig. 1F) were significantly decreased in EGC tissues compared with NATs (n=10; $^{*}P<0.05$ and $^{**}P<0.01$, respectively).

XIST, BCYRN1, RRP1B and TDRG1 expression levels were validated in EGC tissues and NATs. In the preliminary study

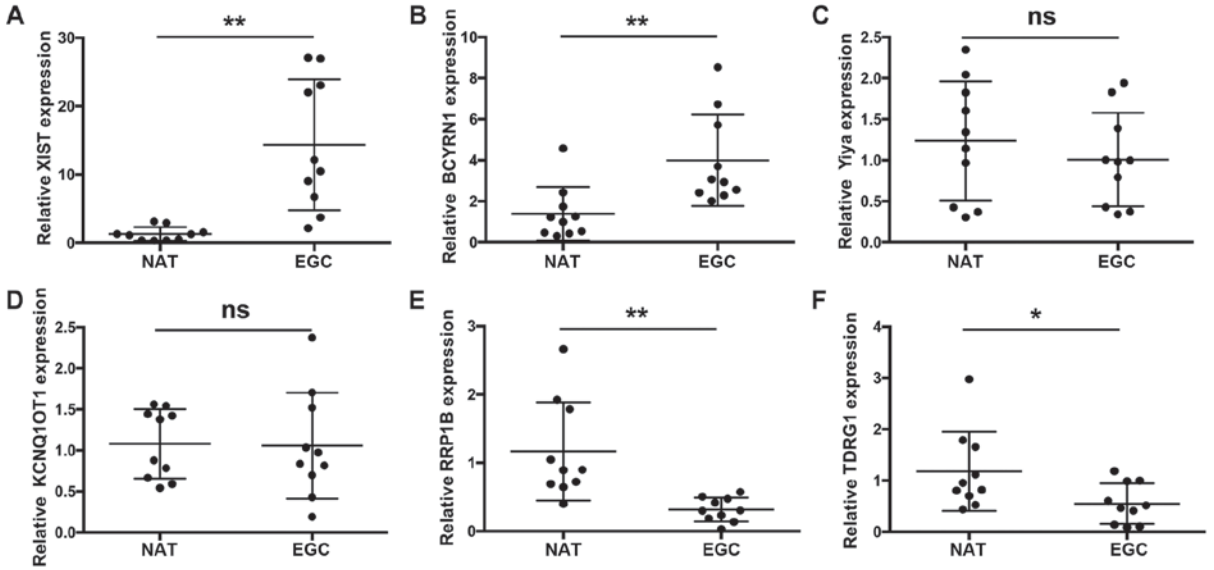

Figure 1. XIST, Yiya, BCYRN1, RRP1B, KCNQ1OT1 and TDRG1 expression levels in EGC tissues and NATs. Reverse transcription-quantitative polymerase chain reaction was used to detect the expression levels of (A) XIST, (B) BCYRN1, (C) Yiya, (D) KCNQ1OT1, (E) RRP1Band (F) TDRG1 in EGC tissues and paired NATs. Relative expression levels were normalized to GAPDH using the $2^{-\Delta\Delta Cq}$ method. n=10; *P<0.05; **P<0.01. BCYRN1, brain cytoplasmic RNA 1; EGC, early gastric cancer; KCNQ1OT1, KCNQ1 opposite transcript 1; NAT, normal adjacent tissue; ns, no statistically significant difference; RRP1B, ribosomal RNA processing 1B; TDRG1, testes development related 1; XIST, X inactive-specific transcript.

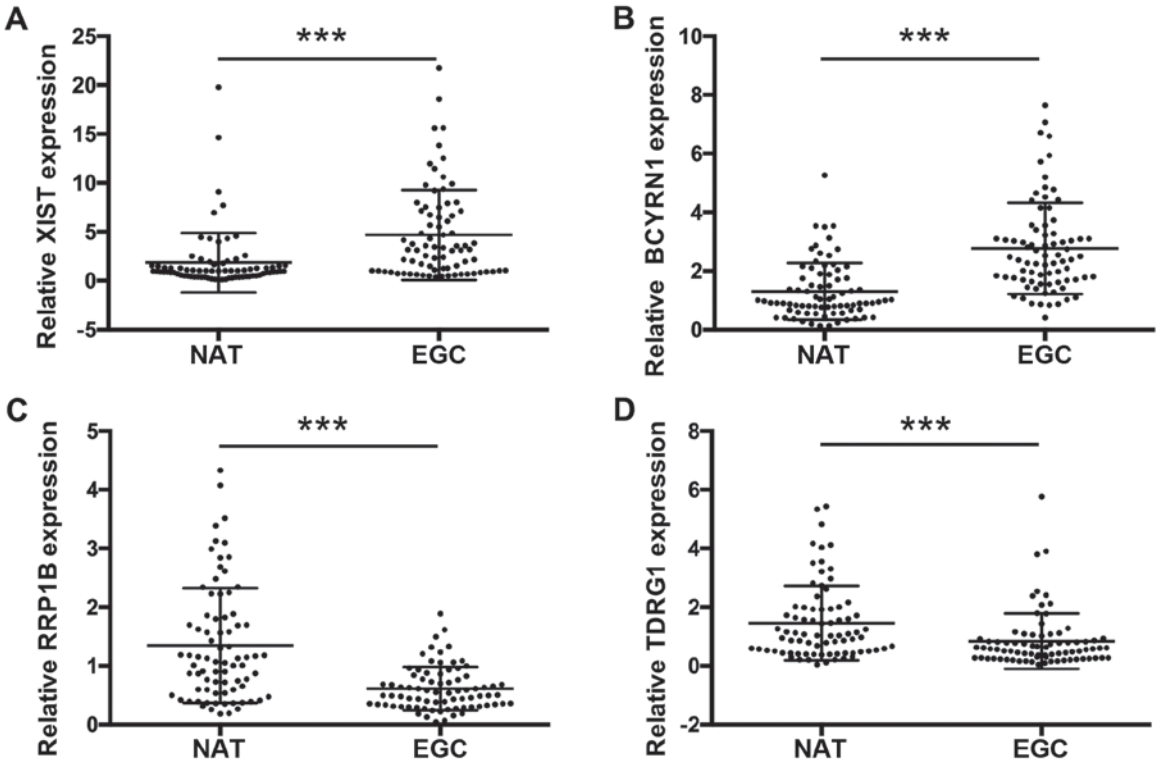

Figure 2. XIST, BCYRN1, RRP1B and TDRG1 expression levels in EGC tissues and NATs. Reverse transcription-quantitative polymerase chain reaction was used to detect the expression levels of (A) XIST, (B) BCYRN1, (C) RRP1B and (D) TDRG1 in EGC tissues compared with paired NATs from 78 patients. Relative expression levels were normalized to GAPDH using the $2^{-\Delta\Delta Cq}$ method. ***P<0.001. BCYRN1, brain cytoplasmic RNA 1; EGC, early gastric cancer; NAT, normal adjacent tissue; RRP1B, ribosomal RNA processing 1B; TDRG1, testes development related 1; XIST, X inactive-specific transcript.

described above, XIST, BCYRN1, RRP1B and TDRG1 were differentially expressed in EGC tissues (n=10) compared with NATs (n=10), while Yiya and KCNQ1OT1 expressions exhibited no significant alterations in EGC tissues compared with NATs. Therefore, we further collected the EGC tissues and paired NATs from 76 patients. The expression levels of XIST, BCYRN1, RRP1B and TDRG1 were measured by RT-qPCR. The results demonstrated that the expression levels of XIST (Fig. 2A) and BCYRN1 (Fig. 2B) were significantly increased in EGC tissues compared with NATs (n=76; ***P<0.001).

Table I. Associations between XIST expression level and clinicopathological characteristics in 76 patients.

Clinicopathological characteristic	n	XIST		
		High expression (%)	Low expression (%)	P-value
Age (year)				
>60	47	42 (51.1)	23 (48.9)	0.097
≤60	29	20 (69.0)	9 (31.0)	
Sex				
Male	50	29 (58.0)	21 (42.0)	0.585
Female	26	15 (57.7)	11 (42.3)	
pN status				
N0	44	29 (65.9)	15 (34.1)	0.077
N1-N2	32	15 (46.9)	17 (53.1)	
pM status				
M0	71	41 (57.7)	30 (42.3)	0.649
M1	5	3 (60.0)	2 (40.0)	
Clinical stage				
I and II	46	28 (60.9)	18 (39.1)	0.339
III and IV	30	16 (53.3)	14 (46.7)	

The P-values of XIST expressions (high expression vs. low expression) were 0.097, 0.585, 0.077, 0.649 and 0.339 compared with age (>60 vs. <60 vs.), sex (Male vs. Female), pN status (N0 vs. N1-N2), pM status (M0 vs. M1), and clinical stage (I and II vs. III and IV). pM, pathological metastasis; pN, pathological node; XIST, X inactive-specific transcript. The differences of XIST expression in tissues among the patients was analyzed using a χ^2 test concerning clinical parameters.

In addition, the expression levels of RRP1B (Fig. 2C) and TDRG1 (Fig. 2D) were significantly decreased in EGC tissues compared with NATs (n=76; ***P<0.001). Therefore, XIST, BCYRN1, RRP1B and TDRG1may serve as potential candidates as biomarkers for EGC.

In addition, no significant correlations were identified between the expression levels of XIST or BCYRN1 and clinicopathological characteristics (Tables I and II, respectively). Conversely, the expression level of RRP1B was significantly correlated with pathological metastasis (pM) and clinical stage (Table III), and the expression level of TDRG1 was significantly correlated with pathological node (pN) (Table IV).

XIST, BCYRN1, RRP1B and TDRG1 may be used as noninvasive biomarkers for EGC. The ROC curve is a comprehensive index that reflects the sensitivity and specificity of continuous variables. In the present study, the occurrence of EGC was predicted by ROC curve analysis using XIST, BCYRN1, RRP1B and TDRG1 expressions in 76 EGC samples and paired NATs (controls). The AUC for XIST was 0.733 (sensitivity=0.846; specificity=0.590; ***P<0.001; Fig. 3A). The AUC for BCYRN1 was 0.821 (sensitivity=0.679; specificity=0.859; ***P<0.001; Fig. 3B). The AUC for RRP1B was 0.753 (sensitivity=0.859; specificity=0.564; ***P<0.001; Fig. 3C). The AUC for TDRG1 was 0.681 (sensitivity=0.731; specificity=0.603; ***P<0.001; Fig. 3D). These data suggested that XIST, BCYRN1, RRP1B and TDRG1 may be able to serve as biomarkers of EGC.

Positive correlation of XIST, BCYRN1, RRP1B and TDRG1 expression between EGC tissue and plasma. Similar to EGC tissues, the expression levels of XIST and BCYRN1 were increased and the expression levels of RRP1B and TDRG1 were decreased in the plasma from patients with EGC. The correlations for XIST, BCYRN1, RRP1B and TDRG1 expression levels between EGC tissue and plasma were further analyzed and the results indicated that there was a positive correlation for XIST expression between EGC tissues and plasma (R^2=0.2650; ***P<0.001; Fig. 4A). A positive correlation was also made between EGC tissues and plasma for BCYRN1 expression (R^2=0.2686; ***P<0.001; Fig. 4B), RRP1B expression (R^2=0.2920; ***P<0.001; Fig. 4C), and TDRG1 expression (R^2=0.4120; ***P<0.001; Fig. 4D). These results demonstrated that XIST, BCYRN1, RRP1B and TDRG1 were aberrantly expressed both in EGC tissues and plasma, which may be related to EGC disease progression.

Discussion

Currently, the study of biomarkers study has focused on noncoding RNAs, particularly lncRNAs, most of which are transcribed by RNA polymerase (Pol) II and Pol I, but some are transcribed by RNA Pol III (24). A number of previous studies have indicated that lncRNAs serve important roles in regulating gene expression (20,25-27) and participate in cell cycle and differentiation (10), apoptosis (11,12) and chromatin remodeling (28-30). Other studies have demonstrated that lncRNAs were involved in the development of various

Table II. Associations between BCYRN expression level and clinicopathological characteristics in 76 patients.

Clinicopathological characteristic	n	BCYRN1		
		High expression (%)	Low expression (%)	P-value
Age (year)				
>60	47	41 (87.2)	6 (12.8)	0.413
≤60	29	24 (82.8)	5 (17.2)	
Sex				
Male	50	44 (88.0)	6 (12.0)	0.300
Female	26	21 (80.8)	5 (19.2)	
pN status				
N0	44	37 (84.1)	7 (15.9)	0.471
N1-N2	32	28 (87.5)	4 (12.5)	
pM status				
M0	71	60 (84.5)	11 (15.5)	0.447
M1	5	5 (100.0)	0 (0.0)	
Clinical stage				
I and II	46	40 (87.0)	6 (13.0)	0.452
III and IV	30	25 (83.3)	5 (16.7)	

None of the P-values in the Table indicates <0.05. The P-values of BCYRN1 expressions (high expression vs. low expression) were 0.413, 0.300, 0.471, 0.447 and 0.452 compared with age (>60 vs. <60 vs.), sex (Male vs. Female), pN status (N0 vs. N1-N2), pM status (M0 vs. M1), and clinical stage (I and II vs. III and IV). BCYRN1, brain cytoplasmic RNA 1; pM, pathological metastasis; pN, pathological node. The differences of BCYRN1 expression in tissues among the patients was analyzed using a χ^2 test concerning clinical parameters.

Table III. Associations between RRP1B expression level and clinicopathological characteristics in 76 patients.

Clinicopathological characteristic	n	RRP1B		
		High expression (%)	Low expression (%)	P-value
Age (year)				
>60	47	7 (14.9)	40 (85.1)	0.587
≤60	29	4 (13.8)	25 (86.2)	
Sex				
Male	50	7 (14.0)	43 (86.0)	0.561
Female	26	4 (15.4)	22 (84.6)	
pN status				
N0	44	7 (15.9)	37 (84.1)	0.471
N1-N2	32	4 (12.5)	28 (87.5)	
pM status				
M0	71	8 (11.3)	63 (88.7)	0.020[a]
M1	5	3 (60.0)	2 (40.0)	
Clinical stage				
I and II	46	8 (6.5)	43 (93.5)	0.018[a]
III and IV	30	3 (26.7)	22 (73.3)	

None of the P-values in the Table indicates <0.05. P<0.05 was considered to indicate a statistically significant difference. The P-values of RRP1B expressions (high expression vs. low expression) were 0.587, 0.561, 0.471, 0.020 and 0.018 compared with age (>60 vs. <60 vs.), sex (Male vs. Female), pN status (N0 vs. N1-N2), pM status (M0 vs. M1), and clinical stage (I and II vs. III and IV). [a]P<0.05. pM, pathological metastasis; pN, pathological node; RRP1B, ribosomal RNA processing 1B. The differences of RRP1B expression in tissues among the patients was analyzed using a χ^2 test concerning clinical parameters.

Table IV. Associations between TDRG1 expression level and clinicopathological characteristics in 76 patients.

Clinicopathological characteristic	n	TDRG1		
		High expression (%)	Low expression (%)	P-value
Age (year)				
>60	47	16 (34.0)	31 (66.0)	0.162
≤60	29	6 (20.7)	23 (79.3)	
Sex				
Male	50	17 (34.0)	33 (66.0)	0.140
Female	26	5 (19.2)	21 (80.8)	
pN status				
N0	44	19 (43.2)	25 (26.8)	0.001[a]
N1-N2	32	3 (9.4)	29 (90.6)	
pM status				
M0	71	21 (29.6)	50 (70.4)	0.548
M1	5	1 (20.0)	4 (80.0)	
Clinical stage				
I and II	46	11 (23.9)	35 (76.1)	0.174
III and IV	30	11 (36.7)	19 (63.3)	

None of the P-values in the Table indicates <0.05. P<0.05 was considered to indicate a statistically significant difference. The P-values of TDRG1 expressions (high expression vs. low expression) were 0.162, 0.140, 0.001, 0.548 and 0.174 compared with age (>60 vs. <60 vs.), sex (Male vs. Female), pN status (N0 vs. N1-N2), pM status (M0 vs. M1), and clinical stage (I and II vs. III and IV). [a]P<0.05. pM, pathological metastasis; pN, pathological node; TDRG1, testes development related 1. The differences of TDRG1 expression in tissues among the patients was analyzed using a χ^2 test concerning clinical parameters.

cancers (31). For example, long intergenic noncoding RNA for kinase activation (LINK-A) was reported to activate normoxic HIF1α signaling in certain breast cancers (32); antisense noncoding RNA in the INK4 locus (ANRIL) may be a potential prognostic biomarker in GC and has been demonstrated to regulate microRNA (miR)-99a/miR-449a (33); and lncRNA-n336928 has been correlated with bladder cancer tumor stage and overall survival (34). Therefore, lncRNAs may be important regulatory factors for gene expressions, yet their functions in cancer remain unclear and requires a deeper understanding of the regulatory networks that may be involved.

A previous study identified 33 differentially expressed lncRNAs using a human lncRNA microarray to screen GC tissues and paired NATs (21). Other studies have reported that H19 promotes proliferation of GC cells and high expression of H19 indicates a poor prognosis in patients with GC (35,36); prostate cancer associated 3 (PCA3) is highly expressed in prostate cancer (37); HOX transcript antisense RNA (HOTAIR) promotes tumor invasion and reverses EMT in GC (38,39). In addition, a decrease in the expression of growth arrest specific 5 (GAS5) was revealed to induce a poor prognosis and accelerate cell proliferation in GC (40), and metastasis associate lung adenocarcinoma transcript 1 (MALAT1) was reported to enhance GC cell proliferation through pre-mRNA-splicing factor SF2/alternative splicing factor (ASF) (41). HIF1A-AS1, PVT1, CBR3-AS1 and UCA1 have also been identified in GC (21), and the present study examined the expression levels of XIST, Yiya, BCYRN1, RRP1B, KCNQ1OT1 and TDRG1 in EGC.

In the present study, it was demonstrated that XIST and BCYRN1 were significantly upregulated, and RRP1B and TDRG1 were significantly downregulated, in EGC tissues compared with NATs. RRP1B was correlated with pM and clinical stage, and TDRG1 was correlated with pN. In addition, there were positive correlations for XIST, BCYRN1, RRP1B and TDRG1 expressions between EGC tissue and plasma. Therefore, it was suggested that XIST, BCYRN1, RRP1B and TDRG1 may be promising candidates for the diagnosis of EGC.

In conclusion, RT-qPCR analysis demonstrated that XIST, BCYRN1, RRP1B and TDRG1 were differentially expressed in EGC tissues compared with NATs, and ROC curve analysis indicated that these lncRNAs have potential diagnostic values for the detection of EGC. Furthermore, the results indicated that there were significant positive correlations of XIST, BCYRN1, RRP1B and TDRG1 expression levels between the EGC tissues and plasmas. Therefore, the present study suggested that XIST, BCYRN1, RRP1B and TDRG1 may potentially serve as diagnostic biomarkers for EGC.

Acknowledgements

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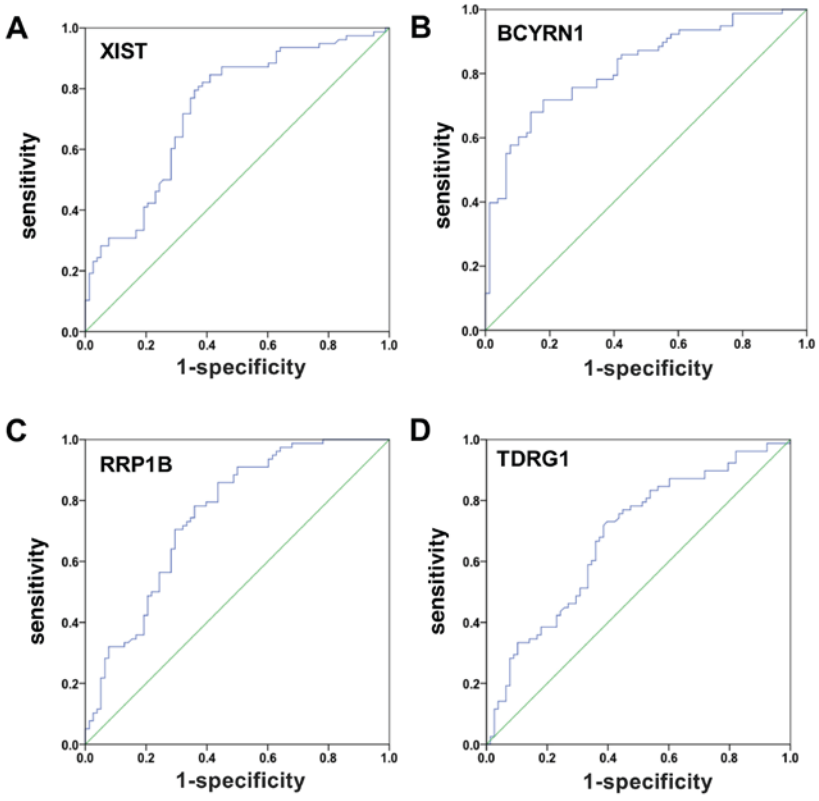

Figure 3. EGC was predicted by ROC curve analysis using XIST, BCYRN1, RRP1B and TDRG1 expression levels between EGC patients and controls. (A) ROC curve analysis using XIST.AUC=0.733; sensitivity=0.846; specificity=0.590. (B) ROC curve analysis using BCYRN1. AUC=0.821; sensitivity=0.679; specificity=0.859. (C) ROC curve analysis using RRP1B. AUC=0.753; sensitivity=0.859; specificity=0.564. (D) ROC curve analysis using TDRG1. AUC=0.681; sensitivity=0.731; specificity=0.603. An ROC curve plots the sensitivity on the y-axis against one minus the 1-specificity on the x-axis. A diagonal line at 45, known as the line of chance, would result from a test which allocated subjects randomly. AUC, area under the ROC curve; BCYRN1, brain cytoplasmic RNA 1; EGC, early gastric cancer; NAT, normal adjacent tissue; ROC, receiver-operator characteristic; RRP1B, ribosomal RNA processing 1B; TDRG1, testes development related 1; XIST, X inactive-specific transcript.

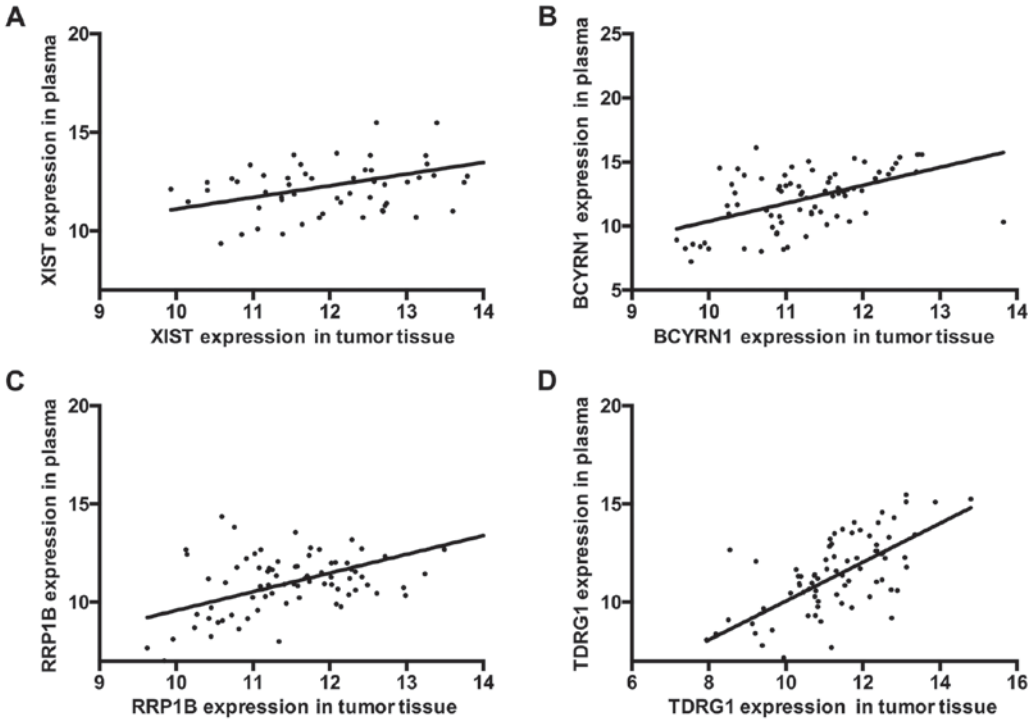

Figure 4. Correlations for XIST, BCYRN1, RRP1B and TDRG1 expression levels were determined between early gastric cancer tissue and plasma. (A) Correlation analysis of XIST. R^2=0.2650. (B) Correlation analysis of BCYRN1. R^2=0.2686. (C) Correlation analysis of RRP1B.R^2=0.2920. (D) Correlation analysis of TDRG1. R^2=0.4120. BCYRN1, brain cytoplasmic RNA 1; RRP1B, ribosomal RNA processing 1B; TDRG1, testes development related 1; XIST, X inactive-specific transcript.

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