Identification of plasma RGS18 and PPBP mRNAs as potential biomarkers for gastric cancer using transcriptome arrays

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Abstract. Coding and noncoding RNAs serve a crucial role in tumorigenesis. Circulating RNAs have been recognized as a novel category of biomarkers for a variety of physiological and pathological conditions. To identify plasma RNA biomarkers for gastric cancer (GC), a genome-wide transcriptome analysis using GeneChip® Human Transcriptome Array, which contains probe sets covering exons of ~67500 coding and noncoding transcripts of annotated genes, was performed to screen for the RNAs that exhibited differential expression in the plasma samples of patients with GC and controls. The expression levels of 6 candidate RNAs, including regulator of G-protein signaling 18 (RGS18), integral membrane protein 2B, pro-platelet basic protein (PPBP), nucleosome assembly protein1-like 1, n324674 and ENST00000442382 were assessed in the plasma samples of 81 patients with GC and 77 healthy participants using reverse transcription-quantitative polymerase chain reaction. Furthermore, the expression levels of RGS18 and PPBP mRNAs were indicated to be significantly differentially expressed ($P<0.0001$) in an independent panel of plasma samples of 36 patients with GC compared with 34 healthy participants. The potential association of RGS18 and PPBP mRNA expression levels with clinicopathological features was subsequently analyzed. Receiver operating characteristic analysis indicated that the combination of these 2 mRNAs with an area under curve <0.812 was an improved indicator for gastric cancer compared with respective individual levels. The results of the present study indicate that RGS18 and PPBP mRNA expression was significantly downregulated in the plasma of patients with GC, and the combination of these 2 mRNAs may be a useful diagnostic or prognostic marker for GC.

Introduction

Gastric cancer (GC) is the third leading cause of cancer-associated mortality, particularly in East Asia, Eastern Europe and South America (1-3) and the 5-year overall survival rate remains <25% (4). Therefore, identification of molecular biomarkers, which contribute to the early stratification of patients with poor prognosis, would aid in selecting the most effective and appropriate therapeutic strategy (3-5). However, numerous GC-associated biomarkers, involving carcinoembryonic antigen (CEA), carbohydrate antigen 19-9, carbohydrate antigen 72-4 (CA72-4) and carbohydrate antigens 125, frequently used for diagnosis, prognosis prediction and monitoring of postoperative recurrence (6,7) are not sensitive or specific for the detection of GC, particularly in early diagnosis. Therefore, identification of novel biomarkers for GC diagnosis have been a major focus of recent investigations (8-10).

A previous study indicated that the release of nucleic acids into the blood was associated with apoptosis and necrosis of cancer cells, including thyroid cancer, nasopharyngeal carcinoma and lung cancer, in the tumor microenvironment. In addition, the study reported that secretion has also been indicated to be a potential source of cell-free nucleic acids (11). Therefore, the detection of cell-free (cf) RNAs in plasma or serum may serve as a 'liquid biopsy', which would be useful for numerous diagnostic applications and would reduce the requirement for tumor tissue biopsies (11,12). A variety of extracellular RNAs have been detected and used as diagnostic and prognostic indicators for early diagnosis and disease outcomes (11-13). In contrast to miRNA, complete mRNA and long non-coding RNA (lncRNA) molecules have been reported to be limited in the plasma (14). Plasma mRNAs and lncRNAs

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exist mainly in the form of RNA fragments. Therefore, it is difficult to screen potential plasma RNA markers using traditional lncRNA or mRNA microarray with a single probe mapped to one gene. GeneChip® Human Transcriptome Array 2.0 (HTA2.0) contains >6.0 million distinct probes, covering coding and non-coding transcripts, and was designed with ~10 probes per exon, therefore ensuring that complete and accurate expression data was obtained in the present study, even for fragmented RNA molecules.

Therefore, in the present study, the Human Transcriptome Array 2.0 was used to screen candidate RNAs that were differentially expressed between plasma samples from healthy participants and patients with GC. The differentially expressed RNA molecules were validated in a training set and assessed in the plasma samples of 81 patients with GC and 77 healthy participants using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The optimal candidate RNA biomarkers were isolated in a test set of 36 patients with GC and 34 healthy participants and the association between expression levels and patient clinicopathological data was determined to assess the diagnostic value of these biomarkers in patients with GC. The results of the present study demonstrated that certain RNAs, including G-protein signaling 18 (RGS18) and pro-platelet basic protein (PPBP), may be significantly downregulated in the plasmas of patients with GC. RGS18 has been reported to have a combined and cell-specific role in regulating the function of cancer cells, along with the progression of various types of cancer (15). PPBP has been reported to stimulate various cellular processes, including regulating the growth and metastasis of cholangiocarcinoma (16). The findings of the present study indicate that a combination of RGS18 and PPBP mRNAs may be used as a diagnostic or prognostic marker of GC.

Materials and methods

Tissue and blood samples. Peripheral blood samples were collected from 81 patients with GC and 77 healthy participants (Table I) at the Chinese People's Liberation Army General Hospital (Beijing, China) and 36 patients with GC and 34 healthy participants (Table II) at the China-Japan Union Hospital of Jilin University (Changchun, China). GC tissues and paired para-tumorous tissues were collected from 26 patients (Table III), who underwent surgery for GC at the Chinese PLA General Hospital (Beijing, China). None of the patients were administered radiotherapy or chemotherapy treatment prior to the operation. All tissue specimens and blood samples were immediately frozen in liquid nitrogen and stored at -80˚C. All samples were staged in accordance with the Tumor-Node-Metastasis (TNM) classification and the criteria of the Union for International Cancer Control (UICC) and the tumor grade was assessed in accordance to the UICC criteria (17,18). Written informed consent was obtained from all patients. The Ethics Committee of the Chinese PLA General Hospital (Beijing, China) and the China-Japan Union Hospital of Jilin University (Changchun, China) approved the use of samples for the present study.

Microarray analysis. Cell-free RNA was extracted from the mixed plasma samples of patients with GC and healthy participants (n=20, individual blood samples) using the miRNeasy Serum/Plasma kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol, and

Table I. Basic characteristics of enrolled individuals in the training set.

	Control group (n=77)	GC group (n=81)
Clinical parameter	Number of cases	Number of cases
Age (years)		
≤60	37 (48.1%)	42 (51.9%)
>60	40 (51.9%)	39 (48.1%)
Mean age	61	60
Age range	36-84	36-84
Sex		
Male	49 (63.6%)	64 (79.0%)
Female	28 (36.4%)	17 (21.0%)
Differentiated[a]		
PDAC[b]		56
MDAC		20
WDAC		5
Invasion depth		
T1		10
T2		19
T3		7
T4		37
Uncategorized		8
Regional lymph nodes		
N0		25
N1		17
N2		11
N3		11
Uncategorized		17
Distant metastasis		
Yes		10
No		63
Uncategorized		8
TNM staging[c]		
I		19
II-IV		52
Size (cm^3)		
≥4		42
<4		24
Uncategorized		15

[a]World Health Organization classification of tumors of the digestive system (17). [b]Mucinous carcinoma and poorly differentiated adenocarcinoma were classified into a group due to the analogously poor prognosis. [c]American Joint Committee on Cancer staging system (18). GC, gastric cancer; TNM, Tumor-Node-Metastasis; PDAC, poorly-differentiated adenocarcinoma; MDAC, moderately-differentiated adenocarcinoma; WDAC, well-differentiated adenocarcinoma.

Table II. Basic characteristics of enrolled individuals in testing set.

	Control group (n=34)	GC group (n=36)
Clinical parameter	Number of cases	Number of cases
Age (years)		
≤60	19 (55.9%)	11 (30.6%)
>60	15 (44.1%)	25 (69.4%)
Mean age	59	58
Age range	36-78	36-83
Sex		
Male	16 (47.1%)	20 (55.6%)
Female	18 (52.9)	16 (44.4%)
Differentiated[a]		
PDAC[b]		6
MDAC		12
WDAC		1
Invasion depth		
T1-T2		2
T3-T4		7
Regional lymph nodes		
N0		3
N1-N3		6
Distant metastasis		
Yes		1
No		8
TNM staging[c]		
I		2
II-IV		7
Size (cm^3)		
≥4		16
<4		4

[a]World Health Organization classification of tumors of the digestive system (17). [b]Mucinous carcinoma and poorly differentiated adenocarcinoma were classified into a group due to the analogously poor prognosis. [c]American Joint Committee on Cancer staging system (18). GC, gastric cancer; TNM, Tumor-Node-Metastasis; PDAC, poorly-differentiated adenocarcinoma; MDAC, moderately-differentiated adenocarcinoma; WDAC, well-differentiated adenocarcinoma.

treated with RNase-Free DNase set (Qiagen GmbH), according to the manufacturer's protocol. The sample preparation and microarray hybridization were performed by Shanghai Bohao Biotechnology Co., Ltd. (Shanghai, China). Total RNA was amplified, labelled and purified using an Affymetrix WT PLUS reagent kit (Affymetrix; Thermo Fisher Scientific Inc., Waltham, MA, USA) to obtain labelled cDNA. Samples were hybridized to a GeneChip® Human Transcriptome Array 2.0 (Affymetrix; Thermo Fisher Scientific Inc.), according to the manufacturer's protocol. Arrays were scanned using the GeneChip® Scanner 3000 (Affymetrix; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Command Console software 4.10 (Affymetrix; Thermo Fisher Scientific Inc.) was used with default settings to control the scanner and summarize probe cell intensity data. Raw data were then normalized using Expression Console software 4.10 (Affymetrix; Thermo Fisher Scientific, Inc.).

Total RNA preparation and RT-qPCR. Total RNA was extracted from tissue samples using TRIzol reagent (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), according to the manufacturer's protocol. Plasma cfRNA was extracted from 200 μl plasma using the RNeasy Serum/Plasma kit (Qiagen GmbH), according to the manufacturer's protocols. cDNA was synthesized and amplified using ImProm-II™ reverse transcriptase (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. The primer sequences are indicated in Table IV. qPCR was subsequently performed using the SYBR qPCR mix (Takara Biotechnology Co., Ltd., Tokyo, Japan) with an Mx 3000p instrument (Agilent Technologies Inc., Santa Clara, CA, USA), according to the manufacturer's protocols. Amplification of the cDNA was confirmed using melting curve analysis. cDNA was reverse transcribed from 20 μl plasma cfRNA and the following thermocycling conditions were used for the qPCR: Initial denaturation at 95˚C for 5 min; 45 cycles of denaturation at 95˚C for 10 sec and annealing and extending at 60˚C for 20 sec. The relative expression level of each RNA was quantified using the $2^{-\Delta\Delta Cq}$ method (19) with the 18S rRNA gene as the endogenous control for data normalization, since its expression level does not significantly differ in the plasma samples of patients with GC and healthy participants (20,21).

Statistical analyses. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) and SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Student's t-tests were used to evaluate differences in the RNA expression levels of tissue and plasma samples from patients with GC and healthy controls. The specificity, sensitivity and area under the curve (AUC) of each RNA sample was determined using receiver operating characteristic (ROC) curve analysis. Using the binary outcome of patients with GC or healthy control samples as dependent variables, a logistic regression model was established using the stepwise model selection method. All statistical tests were 2-tailed and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Plasma RNA screening and data analysis. To investigate whether aberrantly expressed RNAs are present in the plasma of patients with GC, 2 pools of plasma specimens were generated from the GC group (n=16) and the control group (n=16). Circulating RNAs in the 2 plasma pools were analyzed using microarray [GeneChip® Human Transcriptome Array 2.0 (Affymetrix; Thermo Fisher Scientific Inc.)]. Numerous significantly differentially expressed RNAs between the 2 pools were detected, including 716 RNAs with a fold-change ≥2. Among these, 577 RNAs were upregulated and 139 RNAs were downregulated in patients with GC compared with the control group. There were 333 RNAs with a fold-change ≥3, including 272 RNAs that were upregulated and 61 RNAs that

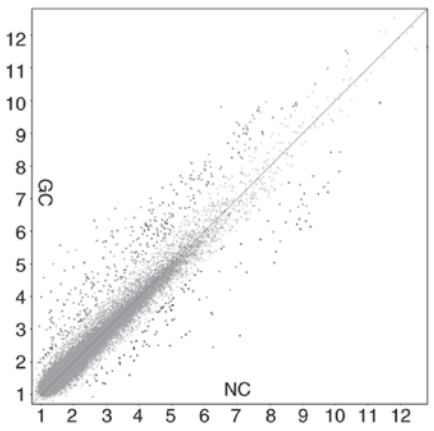
Table III. The clinical parameters of patients with GC and GC tumors.

Sample	Sex	Age (years)	Differentiated[a]	TNM staging[b]	Depth of invasion	LN metastasis
1	Male	58	MDAC[c]	IIIB	T3	N2
2	Male	74	PDAC	II	T3	N0
3	Female	79	PDAC	III	T3	N2
4	Female	76	PDAC	IIIB	T3	N2
5	Male	73	MDAC	IV	T3	N3
6	Male	87	MDAC	IA	T1	N0
7	Female	71	MDAC	IV	T4	N2
8	Male	73	MDAC	II	T2	N1
9	Male	57	PDAC	IB	T2	N0
10	Female	34	PDAC	IA	T1	N0
11	Male	75	PDAC	IIIB	T3	N2
12	Male	54	MDAC	IB	T2	N0
13	Female	70	PDAC	IV	T3	N3
14	Male	62	MDAC	II	T2	N1
15	Female	67	MDAC	IV	T4	N2
16	Male	26	MDAC	IIIA	T3	N1
17	Female	45	MDAC	IIIB	T3	N2
18	Male	59	PDAC	IV	T3	N3[a]
19	Male	50	MDAC	II	T2	N1
20	Male	52	MDAC	IV	T4[a]	N3[b]
21	Female	42	MDAC	II	T3	N0
22	Female	67	MDAC	IIIB	T3	N2
23	Female	41	PDAC	IV	T4[a]	N3[b]
24	Male	53	MDAC	IIIB	T3	N2
25	Male	46	PDAC	IV	T4[a]	N3[b]
26	Female	34	PDAC	IB	T2	N0

Mean age, 59 years; age range, 26-87 years. [a]World Health Organization classification of tumors of the digestive system (17). [b]The American Joint Committee on Cancer staging system (18). [c]Mucinous carcinoma and poorly differentiated adenocarcinoma were classified into a group due to the analogously poor prognosis. TNM, Tumor-Node-Metastasis; LN, lymph node; PDAC, poorly-differentiated adenocarcinoma; MDAC, moderately-differentiated adenocarcinoma; WDAC, well-differentiated adenocarcinoma.

were downregulated in patients with GC compared with the control group (Fig. 1). Based on the expression levels of probe set regions and integrated transcripts, 19 RNA molecules were selected for verification in the 2 plasma pools using RT-qPCR. It was indicated that the expression levels of 2 non-coding RNAs (n324674 and ENST00000442382) were significantly elevated, while a total of 4 mRNAs, regulator of G-protein signaling 18 (RGS18), integral membrane protein 2B (ITM2B), pro-platelet basic protein (PPBP) and nucleosome assembly protein1-like 1 (NAP1L1), were reduced in the plasma of patients with GC (Figs. 2 and 4). Therefore, the expression levels of these 6 RNAs were further verified in the large-scale training sample of patients with GC.

Large-scale validation in plasma samples. In the large-scale training sample of patients with GC (n=81) and healthy participants (n=77), the expression level of n324674 (P =0.0069) was indicated to be increased in the plasma of patients with GC compared with that of healthy participants. However, the expression level of RGS18 (P<0.0001), ITM2B (P=0.0078), PPBP (P<0.0001) and NAP1L1 (P=0.0237) were



Fold-change	Genes	Upregulated	Downregulated
2	716	577	139
3	333	272	61

Figure 1. Scatter plot analysis of the transcriptome array. NC, negative control; GC, gastric cancer.

Table IV. Sequences of primers used in the present study.

Gene	Forward (5'-3')	Reverse (5'-3')
RGS18	TGGACTAGAGGCTTTTAC	ATTTGTTGAGGTCCCTTG
ITM2B	TATTCAGAAACGTGAAGC	CTTGACTGTTCAAGAAC
PPBP	TGAGACAGAATGAAACAC	AGGTGATGAATCTGCTG
NAP1L1	TGGCCAGCATCTGAGAAC	CACGATGAACCTATTCTG
n324674	TGGATCACCTGAGGTCAG	GGGTTCAAGCAATTCTCC
ENST00000442382	TTCAGCATGGCTGGGAAG	CTCTGTCCTGCTGCCTTG
18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

RGS18, regulator of G protein signaling 18; ITM2B, integral membrane protein 2B; PPBP, pro-platelet basic protein; NAP1L1, nucleosome assembly protein 1 like 1.

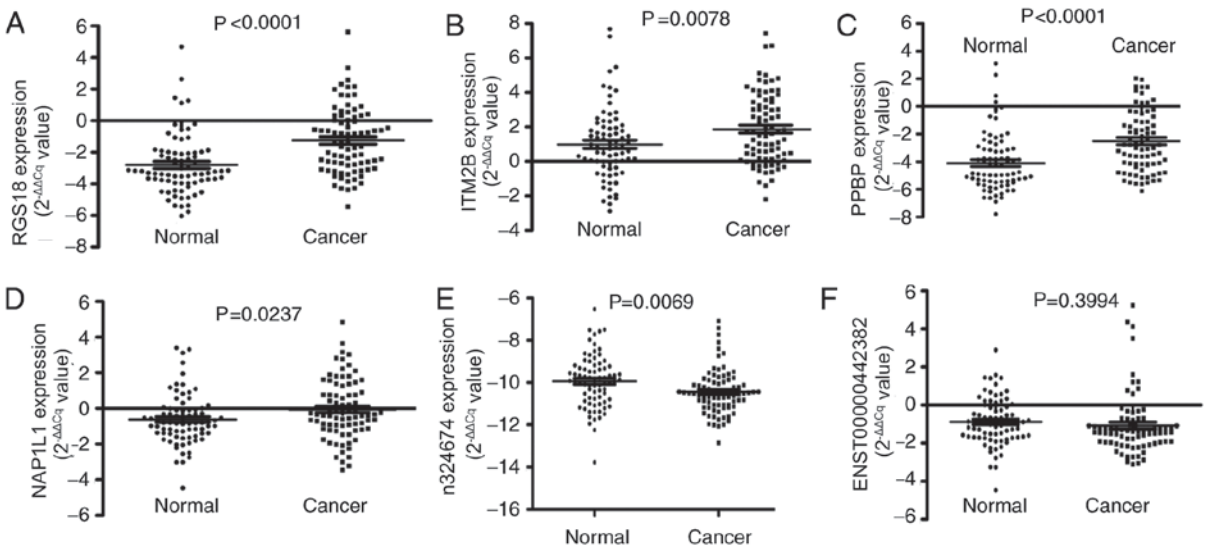
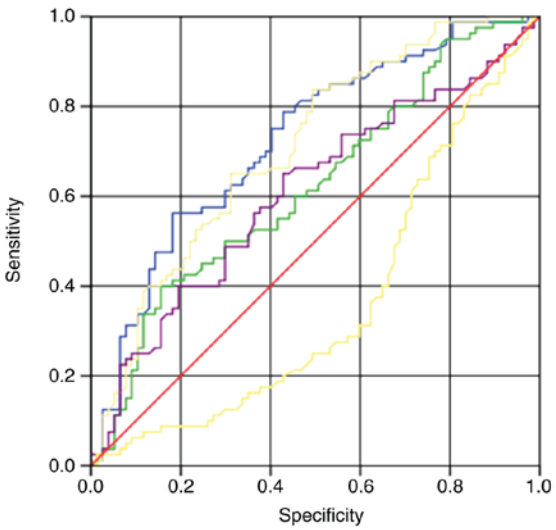
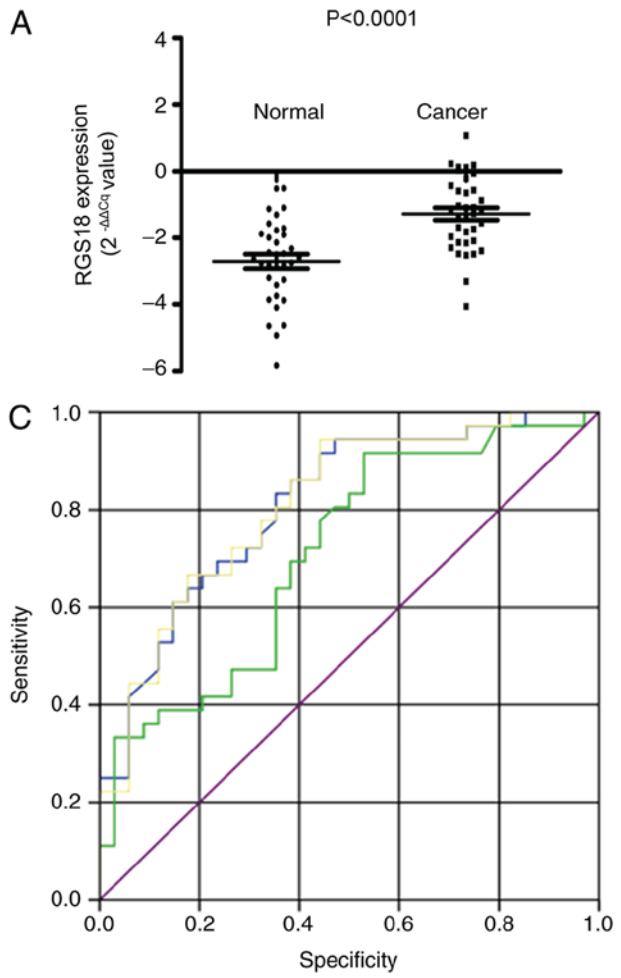


Figure 2. Large-scale training plasma samples of patients with GC (n=81) and healthy participants (n=77) of candidate RNAs. (A) Scatter plots of plasma levels of regulator of G-protein signaling 18. (B) Scatter plots of plasma levels of integral membrane protein 2B. (C) Scatter plots of plasma levels of pro-platelet basic protein. (D) Scatter plots of plasma levels of nucleosome assembly protein1-like 1. (E) Scatter plots of plasma levels of n324674. (F) Scatter plots of plasma levels of ENST00000442382. Expression levels of RNAs ($2^{-\Delta\Delta Cq}$ scale y-axis) were normalized to that of the 18S rRNA gene. RGS18, regulator of G-protein signaling 18; ITM2B, integral membrane protein 2B; PPBP, pro-platelet basic protein; NAP1L1, nucleosome assembly protein1-like 1.



Training set	AUC	Sensitivity	Specificity	95% CI	P-value
-RGS18	0.735	0.584	0.818	0.657–0814	*P<0.0001*
-ITM2B	0.635	0.416	0.844	0.548–0.723	*P<0.01*
-PPBP	0.721	0.675	0.688	0.641–0.801	*P<0.0001*
-NAP1L1	0.629	0.675	0.429	0.541–0.718	*P<0.01*
-n324674	0.639	1.000	0.013	0.550–0.727	*P<0.01*

Figure 3. Receiver operating characteristic curve analysis of plasma RNAs: RGS18, ITM2B, PPBP, NAP1L1 and n324674. RGS18, regulator of G-protein signaling 18; ITM2B, integral membrane protein 2B; PPBP, pro-platelet basic protein; NAP1L1, nucleosome assembly protein1-like 1; CI, confidence interval, AUC, area under the curve.



Testing set	AUC	Sensitivity	Specificity	95% CI	P-value
-2-mRNA	0.812	0.944	0.559	0.712–0.912	P<0.0001
-RGS18	0.811	0.833	0.647	0.711–0.911	P<0.0001
-PPBP	0.710	0.917	0.471	0.589–0.862	P<0.01

Figure 4. Validation of RNAs in plasma samples of the testing set (n=70). (A) Scatter plot of plasma levels of RGS18 expression in patients with gastric cancer (n=36) and healthy participants (n=34). (B) Scatter plot of plasma levels of PPBP expression in patients with gastric cancer (n=36) and healthy participants (n=34). Expression levels of RNAs ($2^{-\Delta\Delta Cq}$ scale y-axis) are normalized to that of 18S rRNA. (C) Receiver operating characteristic curve analysis of plasma RNAs: RGS18 and PPBP. RGS18, regulator of G-protein signaling 18; PPBP, pro-platelet basic protein; CI, confidence interval; AUC, area under the curve.

reduced in the plasma of patients with GC (Fig. 2). To evaluate whether plasma levels of the 5 candidate RNAs could be used as diagnostic markers for GC, ROC curve analyses were performed. It was indicated that the plasma levels of RGS18, ITM2B, PPBP, NAP1L1 and n324674 discriminated patients with GC from healthy participants, with AUCs of the ROC curves of 0.735 [95% confidence interval (CI), 0.657-0.814], 0.635 (95% CI, 0.548-0.723), 0.721 (95% CI, 0.641-0.801), 0.629 (95% CI, 0.541-0.718) and 0.639 (95% CI, 0.550-0.727), respectively (Fig. 3).

RGS18 and PPBP, the most significantly differentially expressed RNAs, were subsequently selected for further validation in an independent testing set of patients with GC (n=36) and healthy participants (n=34) (Table II). The results confirmed that the expression levels of RGS18 (P<0.0001) and PPBP (P=0.0012) were reduced in the plasma samples from patients with GC compared with those from healthy participants (Fig. 4). In addition, the AUC value of the 2-mRNA panel was 0.812, higher than that of the individual mRNAs. The association between various clinicopathological features (age, sex, tumor size, histological differentiation, invasion depth, regional lymph nodes, distant metastasis and TNM stage) and expression levels of RGS18 and PPBP in GC plasma samples (n=117), including the training (n=81) and testing sets (n=36), was subsequently investigated. It was indicated that PPBP expression was reduced in female patients with GC compared with male patients with GC (P=0.0328; Fig. 5).

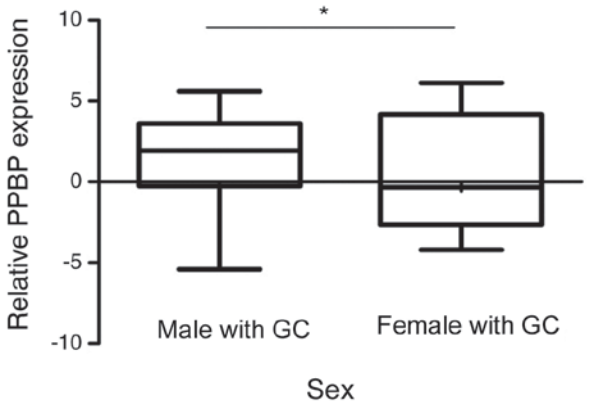


Figure 5. Relative PPBP expression in gastric cancer plasma samples and its clinical significance. PPBP expression was significantly decreased in female patients with GC compared with male patients with GC. *P<0.05. PPBP, pro-platelet basic protein

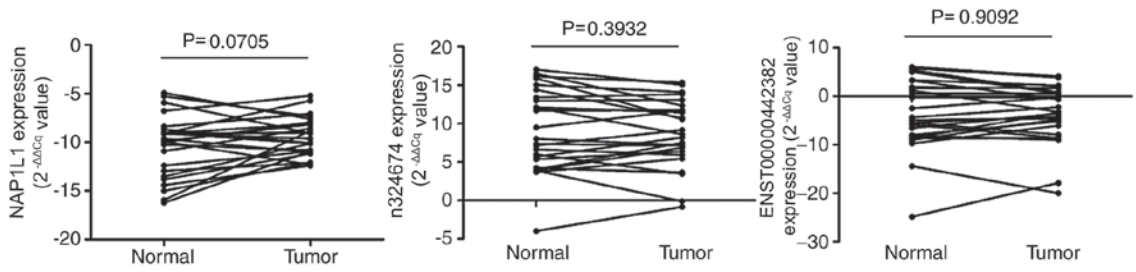


Figure 6. Relative expression levels of NAP1L1, n324674 and ENST00000442382 in 26 gastric cancer and paired normal gastric tissue samples. No significant differences between normal and tumor tissues were observed according to paired t-tests. NAP1L1, nucleosome assembly protein1-like.

Detection of RNA expression in GC tissues. The levels of the 6 candidate RNAs in 26 GC tissue samples and paired adjacent histologically normal tissues were assessed. RGS18, ITM2B and PPBP expression could not be detected using RT-qPCR in GC tissues, whereas the expression of NAP1L1, n324674 and ENST00000442382 was detected. However, their expression patterns in the tissues were different from those in plasma samples. NAP1L1 was downregulated in 9 tumor tissues compared with levels in normal tissues, while n324674 was upregulated in 11 tissues and ENST00000442382 was upregulated in 12 tissues (Fig. 6). According to these results, it was speculated that these plasma RNAs may not derive directly from gastric carcinoma tissues.

Discussion

GC is the third leading cause of cancer-associated mortality worldwide (1-3). GC tumor development and progression is a multi-step processes, involving numerous genetic and epigenetic alterations. However, only a few oncogenes and tumor suppressor genes have been previously identified as being involved in gastric carcinogenesis (22-25). Previous research has reported the use of biomarkers for early detection and prediction of chemotherapeutic sensitivity and prognosis in patients with various types of cancer (11). Among the various approaches reported in previous research, blood-based testing has been indicated as the ideal method for identifying biomarkers in cancer, due to its simplicity and minimal invasiveness (26). However, conventional plasma biomarkers, including CEA and CA72-4, lack sufficient sensitivity and specificity (6,7). Similar to other molecules, cfRNA has been stably detected in plasma or serum samples (27-29). Previous studies have indicated that plasma mRNAs and lncRNAs may be protected by exosome encapsulation and complex formation with proteins, similar to plasma microRNAs (27,30,31). Genome-wide screening approaches have provided opportunities to develop novel diagnostic or prognostic markers and to identify novel therapeutic targets (32).

In the present study, the Human Transcriptome Array was used to identify RNAs that are differentially expressed in plasma samples from patients with GC and healthy participants. The results indicated that the expression levels of RGS18, ITM2B, PPBP and NAP1L1 were significantly decreased in GC plasma samples compared with healthy participant plasma samples, whereas those of n324674 were significantly increased, confirming the differential expression of the aforementioned RNAs in 81 patients with GC and 77 healthy participants. The association between the plasma levels of these 2 RNAs in 117 patients with GC (training and testing sets) and various clinicopathological factors was analyzed. The results indicated that the differential expression of PPBP between patients with GC and healthy participants was more significant in females, as females with GC exhibited decreased expression levels of PPBP compared with male patients with GC. In terms of the utility of these RNAs as biomarkers, it was indicated that plasma levels of RGS18 and PPBP discriminated patients with GC from healthy participants with a combined AUC of 0.812.

Among the 6 candidate RNAs, n324674 and ENST00000442382 were lncRNAs. Numerous lncRNAs have been reported to be associated with disease, particularly cancerous diseases (33). lncRNAs, including H19, imprinted maternally expressed transcript (non-protein coding) (H19), hepatocellular carcinoma upregulated lncRNA, colon cancer associated transcript 1 and run-related transcription factor 1, have been reported to serve functional roles in GC (23,34,35). H19 has also been reported to circulate in the plasmas of patients with GC (24). Therefore, n324674 may be a novel lncRNA associated with GC.

The remaining RNAs are mRNAs. PPBP has been reported to stimulate various cellular processes, including DNA synthesis, mitosis, glycolysis and intracellular cyclic adenosine monophosphate accumulation (16). NAP1L1 participates in DNA replication and may serve a role in modulating chromatin formation and regulating proliferation (36). The underlying mechanisms of PPBP and NAP1L1 in GC require further investigation. RGS proteins have been reported to interact with and negatively regulate G protein activation, and the RGS gene has been indicated to regulate platelet aggregation, haemostasis and thrombosis (37). The ITM2B gene has been reported to be a target of BCL6 repression in lymphomas and neurodegenerative diseases and ITM2B protein generated by alternative splicing has been reported to induce apoptosis in hematopoietic cell lines (38).

Previous studies have demonstrated that the levels of particular RNAs in body fluids were inconsistent with their corresponding levels in tissues (39). It has been reported that one reason for this is that cfRNAs may be transferred to body fluids by exosomes (40,41). Previous studies have reported that certain RNAs can be enriched in exosomes and selectively released from healthy and malignant cells (42). In the present study, RGS18, ITM2B and PPBP could not be detected in GC tissues by RT-qPCR and the expression patterns of NAP1L1,

n324674 and ENST00000442382 in GC tissues were not consistent with those in plasma samples. This unexpected result suggests that these RNAs in plasma may derive from other tissues and not GC tissues.

In conclusion, the results of the present study demonstrate that certain RNAs, including RGS18 and PPBP, were significantly downregulated in the plasma of patients with GC compared with healthy controls. These findings imply that a combination of these 2 mRNAs could be used as a diagnostic or prognostic marker for GC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

HF and XZ conceived the project and contributed to the study design. CS, HL and ZP performed the data collection. CS, HL and DK analyzed and interpreted the data. HL, CS and HF wrote the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients. The Ethics Committee of the Chinese PLA General Hospital (Beijing, China) and the China-Japan Union Hospital of Jilin University (Changchun, China) approved the use of samples for the present study.

Patient consent for publication

Written informed consent for the publication of any associated data was obtained from all the patient, or parent, guardian or next of kin (in case of deceased patients).

Competing interests

The authors declare that they have no competing interests.

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