

Plasma long noncoding RNAs PANDAR, FOXD2-AS1, and SMARCC2 as potential novel diagnostic biomarkers for gastric cancer

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Background: Gastric cancer is still a common cancer worldwide. Investigation of potential plasma biomarkers for gastric cancer diagnosis is essential for prevention strategies and early intervention for gastric cancer-control planning.

Objectives: This study was aimed to explore the lncRNAs' promoter of CDKN1A antisense DNA-damage-activated RNA (PANDAR), FOXD2-AS1, and SMARCC2 as potential novel diagnostic biomarkers for gastric cancer.

Method: 109 gastric cancer patients and 106 healthy controls were involved in this study. Plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 were detected by real-time PCR. Student's *t*-test, Mann-Whitney U test, and Chi-square test were used to verify the differences of clinical variables between two groups. Receiver operating characteristic curve (ROC) was used to evaluate the diagnostic value of every biomarker. Multivariable analysis of risk factors for gastric cancer was performed using logistic regression analysis.

Results: There were significant differences in age, gender, carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 153 between gastric cancer and healthy controls ($P < 0.05$). Compared with healthy subjects, the levels of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 were all significantly higher in gastric cancer patients ($P < 0.05$). These lncRNAs were significantly associated with clinicopathological parameters of gastric cancer, like pathological differentiation, TNM stage, and/or lymph nodes metastasis, and/or invasion depth ($P < 0.05$). The AUC for lncRNA PANDAR was 0.767, for FOXD2-AS1 was 0.700, for SMARCC2 was 0.748, and the AUC of the combinative diagnostic value of these three lncRNAs was 0.839. Adjusted by other variables, these lncRNAs' expressions were significantly associated with gastric cancer.

Conclusions: Plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 might be appropriate diagnostic biomarkers for gastric cancer.

Keywords: gastric cancer diagnosis, long noncoding RNA, PANDAR, FOXD2-AS1, SMARCC2

Introduction

Gastric cancer remains a common cancer worldwide with a considerable health burden.¹ The morbidity and mortality of gastric cancer are still high. It is reported to be responsible for more than 1 million new cases and about 783,000 deaths in 2018 for gastric cancer, which is the fifth most frequently diagnosed cancer and the third leading cause of cancer death.² Although the technologies for gastric cancer diagnosis have advanced recently, gastric cancer patients' prognosis is still poor. Approximately 70% gastric cancer patients died from this disease globally after

5 years follow-up.³ Pathological examination for biopsy tissues is the golden standard for gastric cancer diagnosis.⁴ However, endoscopy is invasive and uncomfortable, which is performed less frequently than blood test. If blood biomarkers are able to diagnose gastric cancer, the detection rate of gastric cancer should be increased effectively. The positive rates of current serum cancer biomarkers for gastric cancer diagnosis are relatively low, like carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), carbohydrate antigen (CA) 199, and CA125.^{5,6} It is necessary to investigate other novel biomarkers with high sensitivity and specificity.

Noncoding RNAs (ncRNAs) compose up to 75% of the human genome, which play important roles in regulating multiple biological processes.⁷ Long noncoding RNAs (lncRNAs) are a kind of noncoding RNAs longer than 200 nucleotides and lack protein-coding ability. It has been reported that lncRNA may regulate the tumor formation process.⁸ Some studies indicate that plasma lncRNAs can serve as potential biomarkers for gastric cancer detection.⁹ It is still far from clinical application because of the lack of large-range validation and consensus among researchers.

lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 have been reported to be able to promote gastric carcinogenesis.^{10–12} The previous studies investigated the lncRNA expression profiles and researched the mechanisms of the three lncRNAs in gastric cancer, indicating the three lncRNAs should play important part in gastric carcinogenesis. However, it has not been clear whether lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 in patients' plasma are potential biomarkers for gastric cancer diagnosis. In this study, we investigated the diagnostic value of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 in gastric cancer patients comparing with healthy controls.

Materials and methods

Patients and plasma sample collections

We enrolled 109 GC patients and 106 normal healthy subjects between December, 2017 and October, 2018 at The First Hospital of China Medical University. All enrolled patients with GC were diagnosed by histopathological examination after radical resection or endoscopic biopsy. Clinicopathological data were obtained from the medical records. Healthy controls consisted of patients with benign diseases such as hernias, stones, or varicose veins who had no evidence of any stomach disease or other malignancy. All clinical parameters were estimated

according to the 8th AJCC/TNM staging system. The present study was approved by the Research Ethics Committee of The First Hospital of China Medical University. We confirmed that all participants provided written informed consent, and that this study was conducted in accordance with the Declaration of Helsinki.

Blood sampling was standardized. All blood samples were collected from each enrolled individual and held in a purple-top EDTA tube before subjects had received any surgery, chemotherapy, or radiation therapy. Plasma samples were separated within 4 hrs after collection following a two-step centrifugation protocol (3,000 g for 10 mins at 4°C, 12,000 g for 10 mins at 4°C) to thoroughly remove cellular nucleic acids, transfer to RNase/DNase-free tubes and immediately frozen and store at –80°C until total RNA extraction.

RNA extraction from plasma

Total RNA was extracted from plasma using the blood total RNA isolation kit (BioTeke). 300 µL plasma was thoroughly mixed with an equal volume of denaturing solution and incubated on ice for 5 mins. 600 µL acid-phenol:chloroform was added and thoroughly mixed via vortex, then centrifuged at 12,000× g for 10 mins at room temperature. The upper aqueous phase was transferred to a fresh RNase-free tube. Washing steps were performed according to the manufacturer's protocol, and the RNA was finally eluted with 30 µL RNase-free water pre-heated to 65°C. Plasma samples for evaluating the internal control were randomly selected and processed under identical conditions.

Reverse transcription (RT) and real-time PCR

The cDNA was synthesized using a PrimeScript RT reagent kit (TaKaRa) according to the manufacturer's protocol. The relative expression levels of lncRNAs in all samples were determined using SYBR Premix Ex TaqTM (TaKaRa) on a Light Cycler 480 Real-time PCR system (Roche). The reactions were incubated at 95°C for 30 seconds and then underwent 45 cycles of 95°C for 5 seconds, 59°C for 10 seconds and 72°C for 30 seconds. Samples were analyzed in triplicate, and the products were confirmed by melting curve analysis following each reaction. GAPDH was used as an internal control. The sequences of the primers for real-time PCR are shown in Table S1. All qRT-PCR reactions were performed in triplicate.

Statistical analysis

Statistical analyses were performed by SPSS 22.0 computer software, GraphPad Prism 5, and MedCalc statistical software. Δ CT, which is the difference between CT values of the target and the endogenous reference GAPDH (Δ CT = $CT_{\text{IncRNA}} - CT_{\text{GAPDH}}$), was estimated to reflect the expression of lncRNA. Descriptive statistics are given as mean \pm s.d., median (minimum, maximum), and percentage. A parametric comparison was performed using Student's *t*-test, and a non-parametric comparison was conducted using the Mann–Whitney U test. Chi-square test was used to verify the differences of nominative variables between the two groups. Multivariable analysis of risk factors for gastric cancer was performed using logistic regression analysis. Variables including patients' gender, age, CEA, AFP, CA125, CA153, and CA199 were adjusted in the multivariable logistic regression analysis. Receiver operating characteristic curve (ROC) and area under curve (AUC) were used to evaluate the diagnostic value of every biomarker. A combined ROC was calculated based on the logistic regression model. *P*-values less than 0.05 were considered to be statistically significant.

Results

General characteristics of gastric cancer patients compared with normal participants

In the healthy control group, there were a total of 106 individuals, and 55 individuals were female, 51 male. Among them, 80 individuals were younger than 65 years

old. In gastric cancer group, there were a total of 109 individuals, and 27 individuals were female, 82 male. Among them, 66 individuals were younger than 65 years old. There were significant differences in both age and gender between these two groups. The levels of serum biomarkers including CEA, AFP, CA125, CA153, and CA199 were shown in Table 1. There were significant differences in CEA and CA153 between the two groups (Table 1). These variables were adjusted in the following logistic regression analysis.

Expression of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 and its relationship with clinicopathological features in gastric cancer patients

A total of 106 healthy subjects and 109 gastric cancer patients were enrolled in this study. Levels of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 in these samples were measured using real-time PCR. Compared with healthy subjects, the levels of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 were all significantly higher in gastric cancer patients ($P < 0.05$). The results were shown in Figure 1.

Then we analyzed the association between clinicopathological parameters of gastric cancer and the levels of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 (Table 2). The results showed that for plasma lncRNA PANDAR, the levels were significantly higher in female than in male, higher in poor pathological differentiation than in well and moderate differentiation, higher in TNM

Table 1 General characteristics of gastric cancer patients compared with health controls

		Health controls	Gastric cancer	P-value
		N (%) / Median (min,max)	N (%) / Median (min,max)	
Gender				
	Female	55 (51.9%)	27 (24.8%)	<0.001*
	Male	51 (48.1%)	82 (75.2%)	
Age				
	<65	80 (75.5%)	66 (60.6%)	0.02*
	≥65	26 (24.5%)	43 (39.4%)	
CEA		2.45 (0.51,8.19)	3.08 (0.63,147.10)	<0.001*
AFP		2.84 (0.66,10.03)	2.74 (0.88,22.46)	0.42
CA125		9.57 (3.25,37.95)	11.85 (4.39,114.20)	0.11
CA153		9.05 (3.41,27.75)	7.76 (2.50,92.65)	0.03*
CA199		10.51 (0.00,47.01)	9.93 (1.18,770.50)	0.40

Notes: For variable including gender and age, Chi-square was used. For CEA, AFP, CA125, CA153, and CA199, which did not fit normal distribution, nonparametric statistical test was used. * $P < 0.05$.

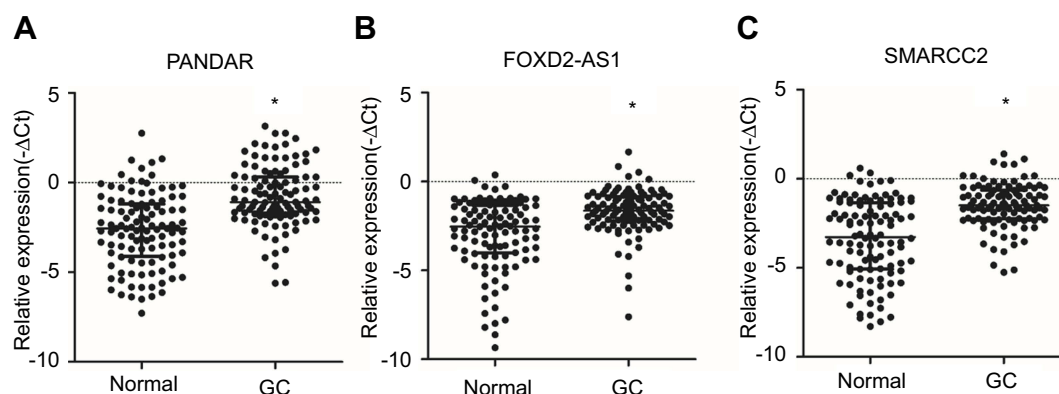


Figure 1 Expression levels of plasma lncRNAs PANDAR (A), FOXD2-AS1 (B), and SMARCC2 (C) in gastric cancer (GC) and healthy subjects (Normal) evaluated by real-time PCR. *When compared with healthy subjects, a $P < 0.05$ was considered statistically significant.

Table 2 Correlation between plasma lncRNAs and clinicopathological parameters of gastric cancer (n=109)

Features	Number of cases	plasma PANDAR levels(-ΔCt)	P-value	plasma FOXD2-AS1 levels(-ΔCt)	P-value	plasma SMARCC2 levels (-ΔCt)	P-value
Age (years)							
<65	66	-0.51 ± 0.21	0.054	-1.60 ± 0.16	0.37	-1.37 ± 0.15	0.20
≥65	43	-1.13 ± 0.23		-1.82 ± 0.18		-1.68 ± 0.20	
Gender							
Male	82	-0.94 ± 0.18	0.04*	-1.56 ± 0.12	0.15	-1.56 ± 0.14	0.33
Female	27	-0.19 ± 0.28		-1.98 ± 0.32		-1.29 ± 0.21	
Tumor size (cm)							
<5	63	-0.96 ± 0.20	0.12	-1.88 ± 0.16	0.053	-1.67 ± 0.16	0.07
≥5	46	-0.46 ± 0.24		-1.41 ± 0.17		-1.25 ± 0.17	
Pathological differentiation							
Well+Moderate	37	-1.28 ± 0.24	0.02*	-2.14 ± 0.24	0.006*	-1.90 ± 0.22	0.01*
Poor	72	-0.48 ± 0.20		-1.45 ± 0.13		-1.28 ± 0.13	
Invasion depth							
T1+ T2+ T3	50	-1.15 ± 0.22	0.20	-1.93 ± 0.20	0.054	-1.76 ± 0.14	0.04*
T4	59	-0.42 ± 0.22		-1.47 ± 0.14		-1.27 ± 0.18	
Lymph nodes metastasis							
Negative	35	-1.11 ± 0.28	0.12	-2.15 ± 0.24	0.007*	-1.94 ± 0.19	0.008*
Positive	74	-0.58 ± 0.19		-1.46 ± 0.13		-1.28 ± 0.14	
TNM stage							
I and II	44	-1.17 ± 0.25	0.03*	-2.06 ± 0.21	0.009*	-1.92 ± 0.17	0.002*
III and IV	65	-0.47 ± 0.20		-1.43 ± 0.13		-1.20 ± 0.15	

Notes: A parametric comparison was performed using Student's t-test, and a non-parametric comparison was conducted using the Mann-Whitney U test. Chi-square test was used to verify the differences of nominative variables between the two groups. * $P < 0.05$.

III and IV stage than in I and II stage. For lncRNA FOXD2-AS1, the levels were significantly higher in poor pathological differentiation than in well and moderate differentiation, higher in patients with lymph nodes metastasis than without lymph nodes metastasis, higher in TNM III and IV stage than

in I and II stage. For lncRNA SMARCC2, the levels were significantly higher in poor pathological differentiation than in well and moderate differentiation, higher in T4 invasion depth than in T1+T2+T3 invasion depth, higher in TNM III and IV stage than in I and II stage.

Diagnostic value of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 for gastric cancer patients

ROC curves were used to evaluate the potential diagnostic capacity of each biomarkers for gastric cancer detection. Relative expression levels of the lncRNAs were obtained using real-time PCR as described previously. The area under the curve (AUC) for CEA was 0.661 (95% CI, 0.592–0.724, $P < 0.05$). The AUC for CA153 was 0.589 (95% CI, 0.520–0.657, $P < 0.05$). The AUC for AFP was 0.536 (95% CI, 0.467–0.604, $P > 0.05$). The AUC for CA125 was 0.558 (95% CI, 0.489–0.626, $P > 0.05$). The AUC for CA199 was 0.533 (95% CI, 0.464–0.602, $P > 0.05$). We calculated the combinative diagnostic value of CEA, CA153, AFP, CA125, and CA199, and the AUC was 0.709 (95% CI, 0.639–0.779, $P < 0.05$). The results are shown in Figure 2.

For the lncRNAs, the AUCs are generally larger than the above biomarkers. For lncRNA PANDAR, the AUC was 0.767 (95% CI, 0.703–0.822, $P < 0.05$). For lncRNA FOXD2-AS1, the AUC was 0.700 (95% CI, 0.629–0.772, $P < 0.05$). For lncRNA SMARCC2, the AUC was 0.748 (95% CI, 0.682–0.815, $P < 0.05$). We calculated the combinative diagnostic value of the three lncRNAs, and the AUC was 0.839 (95% CI, 0.785–0.894, $P < 0.05$). The results are shown in Figure 3. These results indicated that plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 might be appropriate diagnostic biomarkers for gastric cancer.

Multivariate analysis of risk factors for gastric cancer

Logistic regression analysis was used to analyze the risk factors for gastric cancer. The 10 variables including gender, age, current biomarkers (CEA, AFP, CA125, CA153, CA199), and the three lncRNAs investigated in this study (PANDAR, FOXD2-AS1, SMARCC2) were analyzed by multivariate logistic regression analysis. The results in Table 3 showed that gender (OR, 0.219; 95% CI, 0.090–0.532; $P < 0.01$), CEA (OR, 0.022; 95% CI, 1.032–1.496; $P < 0.05$), and lncRNA PANDAR (OR, 1.690; 95% CI, 1.313–2.176; $P < 0.001$), FOXD2-AS1 (OR, 1.358; 95% CI, 1.032–1.787; $P < 0.05$), SMARCC2 (OR, 1.681; 95% CI, 1.293–2.186; $P < 0.001$) were significantly associated with gastric cancer.

Discussion/conclusion

Many patients may be diagnosed with cancer every year in the following years. The number will increase from 14 million a year worldwide in 2012 to 21.6 million a year by 2030. Prompt diagnosis is necessary to improve the patients' survival. Monitoring the effectiveness of national and regional health systems in treating and caring for patients with cancer becomes ever more crucial.¹³ Despite a steady decline in gastric cancer incidence and mortality rates observed in developed countries over the past 50 years, gastric cancer is still a considerable global health burden.^{1,2,14} The incidence rate of gastric cancer is highest in Eastern Asia, including China.^{2,14} China

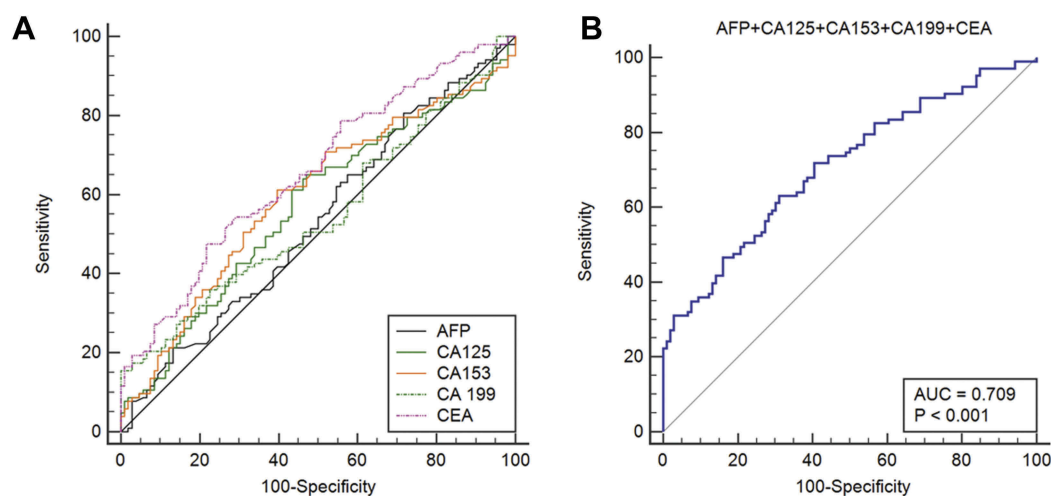


Figure 2 The ROC curves of current biomarkers including CEA, CA153, AFP, CA125, and CA199. (A) The ROC curves of each current biomarker. The area under the curve (AUC) for CEA was 0.661. The AUC for CA153 was 0.589. The AUC for AFP was 0.536. The AUC for CA125 was 0.558. The AUC for CA199 was 0.533. (B) The ROC curve of combined CEA, CA153, AFP, CA125, and CA199. The AUC was 0.709, $P < 0.001$.

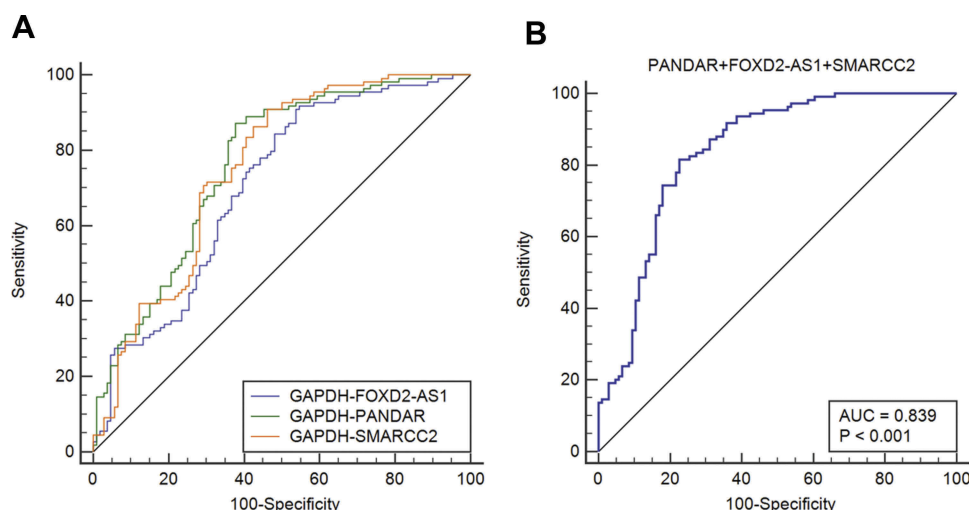


Figure 3 The ROC curves of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2. **(A)** The ROC curves of each plasma lncRNA. The AUC for PANDAR was 0.767. The AUC for FOXD2-AS1 was 0.700. The AUC for SMARCC2 was 0.748. **(B)** The ROC curve of combined lncRNAs PANDAR, FOXD2-AS1, and SMARCC2. The AUC was 0.839, $P < 0.001$.

Table 3 Multivariable analysis of risk factors for gastric cancer using logistic regression analysis

	Multivariable		95% CI	
	P-value	OR		
Gender Female vs male	0.001	0.219	0.090	0.532
Age >65 years vs ≤65 years	0.358	1.496	0.633	3.537
CEA	0.022	1.243	1.032	1.496
AFP	0.807	0.980	0.836	1.150
CA125	0.540	1.013	0.972	1.055
CA153	0.074	0.941	0.881	1.006
CA199	0.089	1.025	0.996	1.056
PANDAR	<0.001	1.690	1.313	2.176
FOX D2-AS1	0.029	1.358	1.032	1.787
SMARCC2	<0.001	1.681	1.293	2.186

contributes about 40% of new cases of gastric cancer every year around the world.¹⁵ Early detection and timely

intervention are considered to be a particularly effective way to fight against cancer. Investigation of the techniques for prompt diagnosis is extremely important for appropriate prevention strategies and prioritization of cancer-control planning.¹⁶

In recent years, gastric endoscopy has been rapidly advanced for gastric cancer diagnosis. Endoscopic diagnosis has been revolutionized by dye-based and image-enhanced endoscopic techniques.¹⁷ Endoscopic ultrasound-guided needle-based confocal laser endomicroscopy has even been used to improve the diagnostic accuracy.¹⁸ However, endoscopy is invasive, uncomfortable, and not suitable for everyone.¹⁹ Endoscopy followed by pathological examination are limited by operator's experience and medical instruments. Serum-based biomarkers are considered to be valuable in cancer diagnosis and monitoring. In our study, we analyzed current serum cancer biomarkers for gastric cancer diagnosis, including CEA, AFP, CA125, CA153, and CA199,²⁰ and their positivity rates showed low, which were consistent with previous studies.^{21–23} It is urgent to investigate novel biomarkers for diagnosis in gastric cancer patients.²¹

lncRNAs have been reported to be associated with kinds of diseases,^{24–26} including cancer phenotypes and carcinogenesis.^{27–31} Some lncRNAs can regulate the expression of nearby genes through RNA–protein interactions, and other lncRNAs can also act as local regulators.³² lncRNAs play critical roles in genome stability,³³ which is considered to be important in carcinogenesis.³⁴ It has been demonstrated that lncRNAs are stable in plasma even

in extreme conditions,³⁵ indicating it could be used as diagnostic biomarkers. The relative expression levels of lncRNAs can be measured using real-time PCR. PCR-based testing is widely used for detection of genes in clinics. PCR is often faster and less costly to perform, and can obtain extremely high sensitivities.³⁶ In this study, we measured the expression levels of three plasma lncRNAs using real-time PCR in order to investigate whether plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 could be potential diagnostic biomarkers for gastric cancer.

LncRNA PANDAR (promoter of CDKN1A antisense DNA damage activated RNA) has been reported to be over-expressed in cancer tissues and associated with poor prognosis in many types of human cancer, including colorectal cancer,³⁷ renal cell carcinoma,³⁸ and bladder cancer;³⁹ while in lung cancer the correlation of PANDAR is found to be opposite.⁴⁰ In gastric cancer, PANDAR could promote the malignant progression by regulating CDKN1A gene transcription in a p53-dependent manner.¹¹ LncRNA FOXD2-AS1 might play important roles in kinds of cancer like glioma,⁴¹ colorectal cancer.⁴² It has been found that upregulation of FOXD2-AS1 could promote carcinogenesis and predict poor prognosis in gastric cancer both in vitro and in vivo. Cell cycle and DNA-replication-related genes should be involved in FOXD2-AS1 function. EphB3 downregulation mediated by Zeste homolog 2 (EZH2) and lysine (K)-specific demethylase 1A (LSD1) might promote FOXD2-AS1 leading to gastric cancer.¹⁰ LncRNA SMARCC2 has been reported to be involved in gastric carcinogenesis. LncRNA SMARCC2 might inhibit miR-551b-3p expression. TMPRSS4 is reported to be the target gene of miR-551b-3p,¹² and could activate NF-kappa B/MMP-9 signaling and promote gastric cancer cells invasiveness.⁴³ However, no study of PANDAR, FOXD2-AS1, and SMARCC2 in gastric cancer plasma has been performed.

In this study, based on the feasibility of real-time PCR testing on plasma lncRNAs and the carcinogenic functions of PANDAR, FOXD2-AS1, and SMARCC2 in gastric carcinogenesis, the levels of three lncRNAs in gastric cancer patients' plasma were measured using real-time PCR. Our results showed that compared with healthy controls, the levels of plasma lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 were all significantly higher in gastric cancer patients, indicating that these lncRNAs might be valuable for gastric cancer diagnosis. To confirm the hypothesis, diagnostic value of every marker was evaluated using ROC. Our results showed that the AUC of every lncRNA

was significantly larger than current serum cancer biomarkers, including CEA, AFP, CA125, CA153, and CA199. The AUC of combinative diagnostic value of the three lncRNAs was up to 0.839, indicating that these lncRNAs are potential biomarkers for gastric cancer diagnosis. To further analyze the risk factors for gastric cancer, multi-variable analysis was performed. As well known, gender in our study also showed to be significantly associated with gastric cancer.¹ Male might be an independent risk factor for gastric cancer. We did not find the association of age and gastric cancer. Adjusted by gender and age, there were four variables which would be independent risk factors for gastric cancer, including CEA, PANDAR, FOXD2-AS1, and SMARCC2. Elevation of the four markers would independently increase gastric cancer risk. LncRNAs could serve as novel targets for gastric cancer diagnosis.^{35,44}

It is necessary to state that in this study, we used GAPDH as the reference gene. Some reference genes including GAPDH might be deregulated in cancer tissues.⁴⁵ While in recent researches, GAPDH is still used as a common reference gene.^{46,47} In the future, the re-recognition of reference genes might be performed with the development of technology and instrument.

In summary, our results showed that lncRNAs PANDAR, FOXD2-AS1, and SMARCC2 in plasma could be used as novel diagnostic biomarkers for gastric cancer detection. The combination of these three lncRNAs could provide a high enough diagnostic accuracy. In this study, we did not have the clinical follow-up survey yet, leading to the lack of prognostic value of the lncRNAs. Further investigation should be performed to complete the follow-up process to study the prognostic value of these biomarkers. As some of these lncRNAs play important effects in many kinds of cancer except for gastric cancer, it should be further studied to identify the specific diagnostic value of these lncRNAs for gastric cancer detection.

Statement of ethics

The study protocol has been approved by the Research Ethics Committee of The First Hospital of China Medical University on human research.

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Author contributions

Ziwei Yang performed the research examinations. Yanfei Sun and Rongfeng Liu collated the data. Yanyan Shi performed the statistical analyses and wrote the manuscript. Yanyan Shi and Shigang Ding designed the research. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Primers for lncRNAs

LncRNAs	Forward primer (5'-3')	Reverse primer (5'-3')	Product length (bp)	TM (°C)
PANDAR	TCCCAACAAACAAGGGGTGG	GTGGCCAAAGGATCTGACGA	92	60
FOX2-AS1	AAGCGATCAGCTCCCTTAGC	CAGACGCGTGGTGGTTATCT	184	60
SMARCC2	ACAGCAGAATGAACTCCGCT	GTCTGAGTGCTGCAGGTAGG	110	60

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