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Circulating long noncoding RNA, Zfpm2-As1, and XIST based on medical data analysis are potential plasma biomarkers for gastric cancer diagnosis

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Abstract.
BACKGROUND: Long noncoding RNAs (lncRNAs) participate in diseases, especially tumorigenesis, including gastric cancer (GC). Although lncRNAs in GC tissues have been extensively studied in previous research, the possible significance of circulating lncRNAs in diagnosing GC is still unknown.
OBJECTIVE: The present work investigated lncRNAs ZFPM2-AS1 and XIST with high expression in GC tissues proved as potential plasma biomarkers from 20 early GC cases, 100 GC cases, and 90 normal subjects.
METHODS: The possible correlation between ZFPM2-AS1 and XIST expression levels was analyzed with general characteristics and clinicopathological features. The performance in diagnosis was assessed according to receiver operating characteristic (ROC) analysis.
RESULTS: According to the results, XIST and ZFPM2-AS1 expression remarkably increased within GC plasma relative to normal subjects ($P < 0.01$); besides, lncRNA XIST expression after surgery had a tendency of downregulation compared with preoperative levels ($P < 0.05$). Moreover, the area under ROC curve (AUC) values were 0.62 for ZFPM2-AS1 and 0.68 for XIST, while the pooled AUC value of CA-724 and two lncRNAs was 0.751.
CONCLUSION: Circulating lncRNAs ZFPM2-AS1 and XIST can serve as the candidate plasma biomarkers used to diagnose GC.

Keywords: Long noncoding RNA, gastric cancer, ZFPM2-AS1, XIST, diagnosis, plasma, biomarker

1. Introduction

Gastric cancer (GC) has high mortality and morbidity rates, and it is a huge burden worldwide [1]. Due

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to the wide spread of Helicobacter Pylori, China alone accounts for 42% of the world's GC patients [2], whereas $< 20\%$ of early GC is diagnosed in China compared with 30%–50% in Japan and South Korea [3, 4]. Strategies for treating GC may be greatly impacted by long noncoding RNAs (lncRNAs) ZFPM2-AS1 and XIST. They may eventually result in individualized therapies to improve prognostic evaluations, risk assessment, and early identification. Validation, uniformity, and cooperation are essential for successful translation. Gastritis, peptic ulcers, bleeding hemorrhage, GC, as well as MALT lymphoma are all associated with H. pylori infection. Ghrelin changes, cardiovascular health problems, and functional dyspepsia are also caused by it. Although advanced means of treatment have emerged gradually in recent years, the poor prognosis is still unfavorable owing to the limitations of early diagnosis. "Liquid biopsy" is considered a suitable candidate for noninvasive diagnosis compared with the invasive pathological examination [5]. A liquid biopsy is a noninvasive diagnostic method that finds biomarkers such as ctDNA, CTCs, and lncRNAs in biological fluids like blood. This method provides important information for early illness identification and individualized therapy. However, traditional GC biomarkers such as CEA, CA19-9, CA72-4, CA12-5, and CA15-3 have a low specificity or sensitivity [6]. Therefore, it is imperative to develop new diagnostic markers for GC in a noninvasive manner.

The development of GC is significantly influenced by lncRNAs, which also regulate gene expression, epigenetic alterations, cell cycle progression, apoptosis, survival, cell migration, invasion, angiogenesis, and immunological control and may provide therapeutic targets. lncRNAs are the RNAs over 200 nucleotides (nt) long [7]. Indeed, because of the lack of a complete open reading frame (ORF), lncRNAs are initially considered the genomic transcription noise [8]. Because of their high sensitivity and non-invasiveness, circulating lncRNAs hold great diagnostic promise in diagnosing GC. Personalized treatment plans, early identification, and insights into the development of the disease can all be obtained via monitoring lncRNA expression. The accuracy of the diagnosis is increased when lncRNA analysis is combined with additional biomarkers.

Furthermore, RNAs have a long lifetime and high stability, making them the optimal candidate for diagnosis in a noninvasive manner [9]. In recent years, lncRNAs have been reported to play a crucial role in tumorigenesis [10]. The lncRNA XIST was identified to be related to GC, pancreatic cancer, and thyroid cancer (TC) [11]. The expression of lncRNA XIST is downregulated during surgery due to several variables impacting cellular proliferation and differentiation, including tumor excision, host immunological response, inflammatory responses, epigenetic modifications, microenvironment alterations, and postoperative therapies. Chen et al. found that the lncRNA XIST modulated GC development through sponging miR-101 to modulate the EZH2 level [12].

Moreover, XIST regulates TC growth and proliferation via the MET-PI3K-AKT pathway [13]. The lncRNA ZFPM2-AS1 is reported to regulate GC progression by ZFPM2-AS1/MIF/p53 axis [14]. Its involvement in the advancement of GC is suggested by the increased expression of XIST in these individuals. It could encourage angiogenesis, impact stromal interactions, and facilitate invasion, migration, and the epithelial-mesenchymal transition. As a possible target for therapy, XIST may function as a biomarker for the risk of metastasis. Consistent sampling techniques, patient characteristics, stable biological conditions, precise surgical intervention, and data normalization account for the minimal variation in ZFPM2-AS1 expression in GC patients. This underscores the significance of study design and validation in independent cohorts. With the type of lncRNA possibly impacting outcomes, the meta-analysis of 42 research on circulating long noncoding RNA (lncRNA) in stomach cancer (SC) showed good accuracy in identifying the illness, suggesting its potential for therapeutic use [15]. This study investigates the function of long noncoding RNA ZNFX1-AS1 (ZFAS1) as a screening biomarker and its clinical relevance in GC. The findings indicate that individuals with preoperative GC had elevated ZFAS1 plasma levels, which

are linked to lymph node metastases and a poor prognosis [16]. Thus, previous studies illuminate the significance of developing lncRNAs as the new diagnostic marker for GC in a noninvasive manner.

The present work analyzed lncRNA XIST and ZFPM2-AS1 plasma levels among GC cases, early GC patients, and age-matched normal subjects. Afterward, ZFPM2-AS1 and XIST levels were compared in preoperative and postoperative patients. This work analyzed the candidate associations of plasma expression of 2 lncRNAs with clinicopathological characteristics among GC cases, including gender, age, differentiation, tumor size, TNM classification, distant metastasis (DM), and lymph node invasion (LNM). Moreover, we also respectively tested the applicability of plasma ZFPM2-AS1 and plasma XIST as a potential GC diagnosis biomarker and analyzed their diagnosis performance compared with traditional biomarkers, including CEA, CA19-9, CA 12-5, CA 15-3, and CA 72-4. As a result, circulating lncRNA XIST and ZFPM2-AS1 may serve as the candidate diagnostic markers for GC.

The study examines the possible role of circulating lncRNAs ZFPM2-AS1 and XIST in diagnosing GC. When comparing GC plasma to regular patients, the researchers discovered that XIST and ZFPM2-AS1 expression were higher. XIST expression dropped following surgery in comparison to preoperative levels. The findings suggest circulating lncRNAs may be used as prospective plasma biomarkers for diagnosing GC.

The rest of the paper is structured as follows: Section 2 describes the methods, which include the study design and objectives. Section 3 shows the results of the proposed methodology; Section 4 summarizes the discussion part.

2. Methods

2.1. Study design and subjects

The histologically confirmed plasma samples were collected from 100 GC cases at the First Affiliated Hospital of China Medical University between 2018 and 2019. In the same period, plasma samples were collected from 90 genders- and age-matched randomly selected normal subjects at the same hospital. Endoscopic Submucosal Dissection (ESD) is a minimally invasive method of removing gastrointestinal tumors that are in their early stages. It preserves healthy tissue and enables precise marking, incision, and submucosal dissection. The technique is customized for each patient based on their unique characteristics and lesion characteristics. Controls are chosen to reduce bias and assure excellent health based on various factors, including age, gender, inclusion/exclusion criteria, medical history, physical examination, laboratory testing, questionnaires, and ethnicity/geographic matching. Specimens were excluded if patients had coexisting tumors or had undergone gastrectomy for benign tumors. Among these subjects, 20 matching preoperative and postoperative plasma specimens were collected, and the postoperative plasma samples were obtained 5–10 days after operation. Twenty preoperative specimens of early GC with histopathological confirmation screening from patients undergoing endoscopic submucosal dissection (ESD) in the same period were recruited as well. A noninvasive diagnostic technique called liquid biopsy provides cost-effectiveness, real-time monitoring, and early illness diagnosis. It's being utilized more and more in oncology because it offers a thorough understanding of tumor heterogeneity, facilitates personalized therapy, and makes it simple to repeat for minimal residual disease monitoring.

Subsequently, we analyzed the relative plasma expression of lncRNAs XIST and ZFPM2-AS1 from 100 GC cases, 90 healthy controls, 20 preoperative and postoperatively, and 20 specimens of early GC. GC biomarkers Zfpm2-As1 and Xist are assessed for their diagnostic accuracy, sensitivity, and specificity. Zfpm2-As1's expression patterns and any correlation with particular cancer subtypes may be

evaluated. Evaluations of Xist may concentrate on its performance metrics and diagnostic usefulness, such as expression levels, association with clinical outcomes, and involvement in disease development. In addition, five conventional biomarkers, such as CEA, CA19-9, CA 12-5, CA 15-3, and CA 72-4, were also measured. Additionally, the clinicopathological features (gender, age, differentiation, tumor size, TNM stage, lymph node invasion, and distant metastasis) of these cases were collected. The purpose is to analyze the diagnosis value of plasma ZFPM2-AS1 and XIST compared with the traditional biomarkers and their possible associations with clinicopathological characteristics among GC cases.

2.2. RNA extraction and real-time PCR analysis

This study isolated plasma total RNA according to specific instructions (Biotake, Beijing, China). Lower statistical power, biases, and mistakes might make it more challenging to interpret diagnostic values in lncRNA preoperative validation studies with smaller sample numbers. Especially in Cancer, larger and more diverse patient cohorts are preferable for determining diagnostic markers' clinical usefulness and strength. The reverse transcription of lncRNAs XIST and ZFPM2-AS1 was performed using a PrimeScript^{TM}RT reagent Kit (Takara Bio, Inc., Kyushu, Japan). It is necessary to conduct additional experimental research because of the increased expression of long noncoding RNA (lncRNA) XIST in patients with positive distant metastasis. These factors include aggressive cancer subtypes, tumor biology, epigenetic regulation, an inflammatory tumor microenvironment, genetic changes, and tumor heterogeneity. A susceptible and effective reverse transcription instrument for gene expression research is the PrimeScriptTM RT Reagent Kit. Its optimization for quantitative PCR enables precise measurement of gene expression and full-length cDNA synthesis. It is more thermostable, doesn't contaminate genomic DNA, and maintains coding areas. The reaction conditions were as follows: 15 min at 37°C, 5 sec at 85°C, and was then held at 4°C. TB Green^{TM}Premix Ex Taq^{TM}II (Takara Bio, Inc., Kyushu, Japan) was utilized for qRT-PCR, with GAPDH being the endogenous reference. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is widely employed in gene expression research as a reference gene for qRT-PCR because of its historical usage, glycolysis, and steady expression function.

On the other hand, accurate normalization in qRT-PCR studies depends on stability validation. It is the standard reference for precise plasma long noncoding RNA (lncRNA) detection due to several considerations, including its extensive expression, stability, consistency, validation, historical use, and examination using normalization utilities. The PCR procedure was as follows: 30 s under 95°C, 5 s under 95°C and 30 s under 60°C for 45 cycles; 5 s under 95°C, 30 s under 60°C, and 15 s under 95°C. For molecular biology investigations, selecting the proper PCR protocol – including cycle lengths and temperature – is essential as it influences the effectiveness of DNA amplification, guarantees specific results, and prevents PCR artifacts. Thermal Cycler Dice Real real-time system was utilized for RT-PCR, and the 2^{-CT} approach was used to quantify the fold change (FC). Table 1 lists all primers utilized in qRT-PCR. The Thermal Cycler Dice Real Time System is a real-time RT-PCR monitoring equipment that measures the amount of DNA in the reaction using fluorescence detection. It is helpful for high-throughput analysis, pathogen identification, and gene expression analysis, allowing several samples to be processed simultaneously.

2.3. Serum traditional biomarker assay

ECLIA is a very reliable and efficient clinical diagnostic tool for illness evaluation and monitoring because of its excellent sensitivity, accuracy, and adaptability in assessing blood biomarker levels. We use electrochemiluminescence immunoassay (Combas e601, Roche) to measure the levels of CEA (reference, $\leqslant$ 4.3 ng/ml), CA19-9 ($\leqslant$ 27 U/ml), CA 12-5 (35 ng/ml), CA 15-3 (25 ng/ml) and CA 72-4 (6.9 ng/ml).

Table 1
All primers for the quantitave real-time PCR

Gene	Sense sequences (5'–3')	Antisense sequences (5'–3')
lncRNA PANDAR	CTCCATCATGCCAAGTTCTGC	GAAGGCAGGCAAGACTCGAA
lncRNA SMARCC2	ACAGCAGAATGAACTCCGCT	GTCTGAGTGCTGCAGGTAGG
lncRNA D63785	ACTGACGTATTTCTGGACCCAC	TTGCTGCTGACACGCCG
lncRNA ZFPM2-AS1	CAATGGGACTAAGCCAGGCA	GGGCTCCACCAACAACCATA
lncRNA HNF1A-AS1	TCAAGAAATGGTGGCTAT	GCTCTGAGACTGGCTGAA
lncRNA SNHG8	CCCGAGAACCGTCAGTTTGA	ACACCCGTTTCCCCAACTAC
lncRNA TP73-AS1	CCGGTTTTCCAGTTCTTGCAC	GCCTCACAGGGAAACTTCATGC
lncRNA XIST	TTGGGGAACCACCTACACTTGAG	CCATTTTGCTATGCGTTATCTGA
lncRNA RAB6C-AS1	CATTCAGAAGTGGAGAGTGTAGG	GAGGATTGCGAGTCATCAGC
lncRNA GapLinc	GCTTCTCTGCGTTCACACAC	ATCACTGTAAACGTGCCTGAGT
lncRNA HOTTIP	CCTAAAGCCACGCTTCTTTG	TGCAGGCTGGAGATCCTACT
GAPDH	GGGAGCCAAAAGGGTCAT	GAGTCCTTCCACGATACCAA

3. Results

3.1. Plasma expression of lncRNAs XIST and ZFPM2-AS1 among GC cases, early GC cases, and normal subjects

The plasma levels of lncRNAs XIST and ZFPM2-AS1 among GC cases significantly increased relative to those in normal subjects ($P < 0.01$).

3.2. Plasma expression levels of lncRNA XIST and ZFPM2-AS1 from 20 matched GC cases before and after surgery

Plasma expression of XIST decreased after surgery relative to that before ($P < 0.05$). However, the difference in relative ZFPM2-AS1 expression before and after surgery was insignificant.

3.3. Group differences between plasma lncRNA ZFPM2-AS1 and XIST levels and the clinicopathological characteristics of GC patients

According to Table 2, advanced GC (stage IV) had markedly increased ZFPM2-AS1 expression in comparison with that in early GC cases (I) ($P < 0.05$); the P-value is 0.037. For the lncRNA XIST, patients with positive distant metastasis had a significantly higher relative expression than negative distant metastasis ($P = 0.024$).

3.4. Diagnostic value of lncRNAs ZFPM2-AS1 and XIST in the plasma for GC patients

Measurement of expression levels, ROC curve construction, and plasma sample collection from GC patients and healthy controls are all part of the ROC study for plasma XIST and ZFPM2-AS1. Their diagnostic power is measured by calculating the AUC and establishing the ideal cutoff threshold. As revealed by ROC analysis, the AUC values for plasma XIST and ZFPM2-AS1 were 0.68 and 0.62, separately, while those for conventional serum markers for GC were: CA-724 = 0.67, CEA = 0.64, CA-153 = 0.55, AFP = 0.58, CA-199 = 0.55, CA-125 = 0.53. The combination of ZFPM2-AS1 XIST and CA-724 had the highest AUC value of 0.751. ROC curve analysis, optimization algorithms, grid search, cross-validation, model selection strategies, machine learning approaches, and statistical tests for AUC comparison are some of the methodologies and statistical tools used to determine the ideal variables

Table 2
Relationships between lncRNA XIST/ZFPM2-AS1 levels and patient clinicopathological characteristics

Variables	Cases	ZFPM2-AS1 relative expression		XIST relative expression	
		Mean ± SD	P Value	Mean ± SD	P Value
Sex					
Male	74	10.230 ± 2.161	0.991	14.360 ± 2.629	0.739
Female	26	10.280 ± 3.956		12.780 ± 3.092	
Age (years)					
< **62**	36	6.528 ± 1.776	0.155	16.030 ± 4.439	0.478
⩾ **62**	64	12.220 ± 2.739		12.870 ± 2.211	
Tumor size*					
< 5 cm	38	5.636 ± 2.418	0.068	12.980 ± 3.287	0.736
⩾ 5 cm	23	15.410 ± 5.360		14.870 ± 4.552	
Differentiation*					
High/moderate	19	9.157 ± 4.089	0.835	11.290 ± 3.889	0.290
Poor	41	8.118 ± 2.664		18.350 ± 4.181	
TNM classification*					
Early cancer	20	7.299 ± 2.666	0.386	5.818 ± 1.977	0.238
I	13	3.649 ± 2.193		11.220 ± 4.700	
II	13	7.692 ± 3.398	0.341	18.310 ± 8.349	0.442
III	48	13.900 ± 3.398	0.172	17.130 ± 4.231	0.483
IV	13	36.020 ±13.040	0.037	25.620 ±12.970	0.278
Distant metastasis*					
Positive	14	12.800 ± 7.027	0.669	28.520 ± 8.621	0.024
Negative	77	10.230 ± 2.221		13.460 ± 2.364	
Lymphatic invasion*					
Positive	52	11.150 ± 2.767	0.330	12.610 ± 4.109	0.578
Negative	26	6.746 ± 2.728		15.520 ± 2.826	

Tumor size, Pathological differentiation, TNM classification, and Distant metastasis were assessed by the AJCC/UICC Cancer Staging Manual in Gastric Cancer: 2017 edition. *The analysis is performed in an environment of incomplete data.

for optimizing diagnostic accuracy. LncRNAs XIST and ZFPM2-AS1 performed well in diagnosing GC relative to conventional serum markers. Because of their specificity, early identification, sensitivity to disease changes, and functional significance in cancer pathogenesis, XIST, and ZFPM2-AS are very efficient diagnostic markers for GC. Their potential for personalized therapy and incorporation into diagnostic panels further enhance their diagnostic value.

4. Discussion

GC ranks 3rd place among the common causes resulting in cancer-associated mortality globally [17]. GC is usually diagnosed later; in this regard, it is essential to develop novel biomarkers for GC [18]. More and more studies have indicated the critical functions of lncRNAs in carcinogenesis and development of GC. The basis of lncRNAs serving as a noninvasive biomarker lies in the stability and longevity of lncRNAs in plasma [19]. Most existing articles are conducted to analyze the ability of some specific lncRNAs in diagnosing GC tumor tissues. Thus, gene difference expression in various unexplored lncRNAs of GC plasma samples remains further research.

The study used GAPDH as a standard reference to detect plasma lncRNA expression [19,20,21] accurately. We first identified ten highly expressed lncRNAs in GC tissues in previous studies: HOTTIP,

PANDAR, SMARCC2, D63785, GapLinc, SNHG8, TP73-AS1, HNF1A-AS1, ZFPM2-AS1 and XIST and measured the expression levels respectively in the pre-test [22,23,24,25,26,27,28,29,30,31,32,33,34, 35]. Subsequently, the top two lncRNAs, ZFPM2-AS1 and XIST, were chosen as candidate oncogenes involved in the following succession according to the results in the pre-test (Additional file: The results showed that the plasma levels of XIST and ZFPM2-AS1 were significantly up-regulated among GC cases relative to normal subjects ($P < 0.01$); besides, lncRNA XIST expression after surgery tended down-regulation close to that before ($P < 0.05$). After surgical treatment, XIST expression declines 5–10 days later due to the stress response, tissue alterations, hormone fluctuations, epigenetic modifications, inflammatory reactions, and postoperative drugs. Considering surgical settings, epigenetic research, and molecular profiling, a thorough examination is necessary to comprehend these parameters.

Moreover, relative ZFPM2-AS1 expression was markedly up-regulated in advanced GC (IV) relative to early GC patients (I) ($P < 0.05$). In contrast, XIST expression among patients developing DM significantly increased close to those with no DM ($P < 0.01$). Several possible uses for the elevated plasma expression of lncRNAs XIST and ZFPM2-AS1 in GC patients after surgery include postoperative recovery, prognosis, therapy response assessment, patient counseling, research, and diagnostic panels. For all we know, this is the first study that evaluates the possible relationship between plasma levels of XIST and ZFPM2-AS1 and GC clinicopathological factors. LncRNA ZFPM2-AS1 and XIST are released into the blood from apoptotic tumor cells, causing the increase of circulating ZFPM2-AS1 and XIST in peripheral blood [36,37,38]. Tumour stage, histological types, HER2 status, surgical margin involvement, mucus production, tumor size, patient demographics, concomitant diseases, and response to neoadjuvant therapy are all essential factors to consider while diagnosing and treating GC. As reported, ZFPM2-AS1 enhances GC genesis and suppresses the p53 pathway through the stabilization of MIF [14]. XIST can modulate GC development by sponging miR-101 to modulate the EZH2 level [12].

Furthermore, the diagnostic value of traditional serum biomarkers was accessed in the study. The ROC curve shows that the AUC was 0.62 and 0.68 for ZFPM2-AS1 and XIST, respectively, which demonstrated a higher diagnostic capability than CA19-9, CA12-5, and CA15-3 (AUC = 0.55, 0.53, 0.55 respectively), the combined area of ZFPM2-AS1, XIST and CA-724 was 0.751, which indicates that circulating lncRNAs ZFPM2-AS1 and XIST are the possible plasma markers used to diagnose GC. The combination of ZFPM2-AS1, XIST, and CA-724 as biomarkers for detecting GC has the most excellent AUC value, probably because of their synergistic effects, improved specificity, decreased variability, and greater statistical power. However, this combination has to be validated in separate cohorts. However, the analysis is performed in an environment of missing data such as the differentiation, TNM classification, tumor size, distant metastasis, and lymphatic invasion of GC patients so as to have an impact on the results to a certain extent. The robustness and generalizability of prediction models in clinical settings are evaluated by performance analysis in environments with incomplete data. It offers information for well-informed healthcare decision-making by recognizing biases, comprehending imputation techniques, and guaranteeing model relevance.

To confirm the diagnosis value of ZFPM2-AS1 and XIST, we added 20 matching plasma samples preoperatively and postoperatively. XIST expression significantly decreased 5–10 days after surgical treatment ($P < 0.05$), indicating that the plasma lncRNA XIST may be an effective biomarker for diagnosis and GC dynamics monitoring. We carefully analyzed the specimens with increased relative expression levels after surgery, which led to the increasing release of lncRNA from tumors in the suturing procedures. Interpretation may become more challenging due to circulating lncRNA levels rising due to surgical procedures. Accurate postoperative monitoring requires an understanding of the kinetics of lncRNA release. Standardization of protocols, control groups, baseline comparisons, and timing is crucial.

However, the study had a limited sample size in the validation preoperatively and postoperatively and could have performed a better and more exact interpretation of the diagnosis value of candidate lncRNAs.

To sum up, plasma expression of XIST and ZFPM2-AS1 remarkably increased in GC patients before surgery compared with normal subjects ($P < 0.01$); moreover, ZFPM2-AS1 expression was related to TNM classification ($P < 0.05$), whereas XIST expression was associated with DM ($P < 0.05$). Both lncRNAs' expression declined at 5–10 days postoperatively relative to that preoperatively; typically, XIST expression dramatically decreased after surgery close to that before ($P < 0.05$). In addition, the ROC curve showed that AUC was 0.62 and 0.68 for ZFPM2-AS1 and XIST, respectively, and the combination of ZFPM2-AS1, XIST, and CA 72-4 was the highest at 0.751, indicating the circulating lncRNAs XIST and ZFPM2-AS1 were the candidate markers for GC. Helicobacter Pylori infection has been well-recognized as the risk factor for diagnosing GC, particularly in China [35]. In the present study, information on Helicobacter Pylori infection was unavailable from our subjects; as a result, we could not wholly assess the accuracy of those two lncRNAs in diagnosing GC. To reduce the danger of contamination, researchers often follow excellent laboratory procedures, which include using sterile methods, clean surroundings, routine cleaning, quality control checks, isolation protocols, environmental monitoring, inclusion of control samples, and rigorous record-keeping. The interindividual expression variability of ZFPM2-AS1 and XIST and the environmental contamination during the experiment are considered to be impacts for the illustration of the under-expressed samples, and further researches of lncRNA ZFPM2-AS1 and XIST are needed to confirm the diagnostic performance and the mechanism of the two lncRNAs in GC. Some of the study's drawbacks include small sample size, biological variability, possible heterogeneity, retrospective design, confounding factors, lack of validation, questionable clinical usefulness, publication bias, and limitations related to technology and methodology.

5. Conclusion

According to the study's findings, there is a great deal of promise for using circulating lncRNAs ZFPM2-AS1 and XIST as plasma biomarkers to diagnose GC. This study found elevated expression of XIST and ZFPM2-AS1 in GC plasma, including early GC cases, GC patients, and ordinary people. After surgery, there is also a propensity for XIST to be downregulated. Combined with the traditional tumor marker CA-724, the diagnostic performance, as determined by ROC analysis, demonstrates promising individual AUC values (0.68 for XIST and 0.62 for ZFPM2-AS1) and improved diagnostic accuracy (AUC 0.751). Overall, the research points to the possibility of using these circulating lncRNAs as reliable plasma biomarkers for diagnosing GC. In the future, it intends to track circulating levels of lncRNA, investigate functional roles, integrate with other biomarkers, evaluate clinical value, integrate diagnostic panels, and explore the potential of Zfpm2-As1 and Xist as diagnostic biomarkers. It also wants to validate findings in larger cohorts of patients with GC.

Data and material availability

All datasets analyzed/generated in this work are included in this article.

Conflict of interest

None of the authors have any conflicts of interest to declare.

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Author contributions

XG, ZY and PL planned the test program; ZY, JC and HL implemented experiments and conducted the data analysis; ZY and HL collected the samples; NX and LG obtained statutory and ethic approval; HL wrote the manuscript. All authors approved the final manuscript for publication.

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