RESEARCH ARTICLE

Global expression profiling of metabolic pathway-related lncRNAs in human gastric cancer and the identification of RP11-555H23.1 as a new diagnostic biomarker

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Background: Long noncoding RNAs (lncRNAs) have roles in regulating metabolism; however, the global expression profile of metabolic pathway-associated lncRNAs in gastric cancer is unknown. The purpose of our study was to examine metabolic pathway-related lncRNAs in gastric cancer and their possible diagnostic values.

Methods: Differential expression patterns of metabolic pathway-related lncRNAs between gastric cancer and paired nontumor tissues were detected using metabolic pathway-associated lncRNA microarrays. The expression of RP11-555H23.1, one representative metabolic pathway-associated lncRNA, was validated using quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR). The associations between RP11-55H23.1 expression and the clinicopathological features of gastric cancer patients were analyzed. A receiver operating characteristic (ROC) curve was further established.

Results: A total of 114 differentially expressed metabolic pathway-associated lncRNAs (fold change >2, $P < 0.05$) between cancer and nontumor tissues were found (GEO No. GSE96856). Among them, TUG1, RP11-555H23.1, RP1-257120.13, UGP2, GCSHP3, and XLOC_000889 lncRNAs were downregulated more than sixfold in gastric cancer tissues. In contrast, RP11-605F14.2, TBC1D3P5, BC130595, LINC00475, RP11-19P22.6, BC080653, XLOC_004923, AFAP1-AS1, EPB49, and RP11-296I10.3 lncRNAs were upregulated more than sixfold in gastric cancer tissues. We further demonstrated that RP11-555H23.1 expression was significantly correlated with TNM stage ($P = 0.038$). The area under the ROC curve (AUC) was 0.65, and the specificity and sensitivity were 62% and 81%, respectively.

Conclusions: Metabolic pathway-associated lncRNAs play an important role in the occurrence of gastric cancer, and metabolic pathway-associated lncRNAs, such as RP11-555H23.1, may represent novel biomarkers of gastric cancer.

KEYWORDS

biomarker, gastric cancer, long noncoding RNA, metabolic pathway, RP11-555H23.1

1 | INTRODUCTION

Gastric cancer is one of the most common gastrointestinal cancers worldwide.^{1,2} Although early diagnostic rates have improved due to the widespread use of gastroscopy, the prognosis for advanced patients with gastric cancer remains very poor, with a 5-year survival rate of less than 25%.^{3–5} Furthermore, gastric carcinogenesis is a complicated process, resulting from the dysregulation of numerous tumor-related genes. Therefore, more ideal biomarkers for the diagnosis of gastric cancer are urgently needed. Improved understanding of the molecular mechanisms underlying gastric carcinogenesis will enhance diagnosis and treatment of gastric cancer.

Metabolism is defined as the chemical reactions in living organisms by which energy is provided for life-sustaining activities and new materials are assimilated. Altered metabolism, a hallmark of cancers, contributes to cancers from initiation, growth, development, and maintenance to malignant transformation.⁶ Identifying these metabolic alterations and regulatory mechanisms in cancers may reveal a new avenue for therapeutic intervention.

Cancer cells prefer to utilize glucose to produce energy under aerobic or anaerobic conditions, with an associated rise in lactate production. The phenomenon of aerobic glycolysis is termed the Warburg effect.⁶ To date, the mammalian glucose transporter (GLUT) family, which is associated with glycolysis, is classified into three sub-families: class 1 (GLUT-1, -2, -3, -4, and GLUT-14), class 2 (GLUT-5, -7, -9, and -11), and class 3 (GLUT-6, -10, -12, and -13).^{7,8} Several studies have indicated that GLUT-1 and GLUT-14 are independent prognostic factors for gastric cancer.^{9–11} In addition, GLUT-4 is up-regulated by P38 MAPK signaling and enhances glycolysis in gastric cancer cells.¹² Metastasis associated with colon cancer-1 (MACC1), a long noncoding RNA (lncRNA), enhances the Warburg effect by increasing the activities and upregulating the expression of several glycolytic enzymes in gastric cancer cells.¹³ Moreover, amino acid metabolism is changed in gastric carcinoma. For example, cysteine, serine, isoleucine, tyrosine, and others are increased in gastric cancer.¹⁴ Furthermore, blood valine levels were significantly altered in patients with gastric cancer.¹⁵

lncRNAs are transcripts longer than 200 nucleotides with nonprotein coding functions.^{16,17} They are divided into antisense, sense, bidirectional, intronic, and intergenic categories.¹⁸ lncRNAs play important roles in cancer incidence and development.^{19–24} Furthermore, lncRNAs regulate metabolism in cancer cells. For example, lincRNA p21 modulates glycolysis,²⁵ and urothelial cancer-associated 1 (UCA1) regulates glycolysis by upregulating hexokinase 2 (HK2), a key enzyme in the glycolytic pathway.²⁶ In hepatoma cells, the lncRNA highly upregulated in liver cancer (HULC) regulates lipid metabolism.²⁷

To understand the metabolic regulatory roles of lncRNAs in gastric cancer, we analyzed aberrantly expressed metabolic pathway-associated lncRNAs and examined their potential diagnostic values in gastric cancer.

2 | MATERIALS AND METHODS

2.1 | Sample collecting

Gastric cancer tissue samples and paired nontumor tissues samples were acquired from surgical specimens of gastric cancer patients between August 2011 and February 2014 at Yinzhou People's Hospital, China. Patients had no radiotherapy, chemotherapy, targeted therapy, or other therapies prior to surgery. Tissues were directly conserved in RNA fixer (Bioteke, Beijing, China) after removal and preserved at -80°C until isolation of total RNA. Histological grade was determined following the National Comprehensive Cancer Network (NCCN) clinical practice guidelines of oncology (V.1.2011). Patients were staged according to the International Union Against Cancer tumor-node-metastasis (TNM) staging system (7th edition).²³ Paired adjacent nontumor tissues, obtained 5 cm of the edge of the tumor, were confirmed to possess no tumor cells by two experienced pathologists. All aspects of this study were approved by the Human Research Ethics Committee of Ningbo University (IRB No. 20120303).

2.2 | RNA isolation

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's protocol. Quality and quantification of RNA were evaluated using a DS-11+ Spectrophotometer (DeNovix, Wilmington, DE). $\text{OD}_{260/280}$ values approximately 1.8 were regarded as a criterion for acceptable levels. Total RNA was stored at -80°C until use.

2.3 | Analysis of metabolic pathway-associated lncRNAs

Three pairs of gastric cancer tissues and their nontumor tissue counterparts were obtained from patients (62-year-old male, 55-year-old male, and 77-year-old female) by gastroendoscopy. Fluorescent cRNA was reverse transcribed from amplified total RNA and hybridized onto the LncPath™ Human Metabolism Array ($6 \times 7 \text{ K}$, Arraystar, Rockville, MD), which simultaneously detects the expression of lncRNAs from RefSeq, Ensembl, UCSC, and related literature. The LncPath™ human metabolic pathway lncRNA microarray simultaneously profiles expression of 965 lncRNAs and 458 of their protein-coding gene targets related to metabolic signaling pathways. lncRNAs whose genes are located at or near protein-coding genes critical in metabolic pathways and lncRNAs were carefully collected from authoritative databases using rigorous selection processes (<https://www.arraystar.com/lncpath-human-metabolism-pathway-microarray/>). After having washed the slides, arrays were scanned using the Axon GenePix 4000B microarray scanner and imported into GenePix Pro 6.0 software (Axon). Normalization and data processing were completed using the R software package. Differential expression of lncRNAs is shown in volcano plot. lncRNAs whose fold changes ≥ 2 and P values ≤ 0.05 were selected.

TABLE 1 Downregulated lncRNAs in gastric cancer tissues compared with those in paired nontumor tissues (fold changes ≥ 2 and P values ≤ 0.05)

Name	Fold change	Chromosome	Source	P value
TUG1	33.93	22	RefSeq	0.001
RP11-555H23.1	20.36	X	gencode	0.006
RP1-257I20.13	13.03	22	gencode	0.007
UGP2	9.88	2	gencode	0.017
GCSHP3	7.83	2	RefSeq	0.035
XLOC_000889	6.40	1	lincRNA	0.035
HULC	5.89	6	RefSeq	0.00
AC099522.2	5.62	5	gencode	0.00
HAUS7	5.54	X	UCSC_knowngene	0.00
RP4-620F22.3	5.14	1	gencode	0.04
RP11-720L2.3	5.13	18	gencode	0.00
RP11-954J6.3	5.08	X	gencode	0.00
RP11-655M14.14	4.87	11	gencode	0.03
AX747742	4.65	2	UCSC_knowngene	0.01
RP5-903G2.2	4.14	11	gencode	0.00
PISRT1	4.01	3	RefSeq	0.02
AC097523.1	3.89	2	gencode	0.00
LOC100507053	3.82	4	RefSeq	0.01
GAPDHP25	3.59	2	gencode	0.03
DQ600483	3.51	22	UCSC_knowngene	0.00
RP11-442N24__B.1	3.45	1	gencode	0.01
RP5-875H18.4	3.41	17	gencode	0.00
RP1-172N19.3	3.36	X	gencode	0.00
DENND2C	3.15	1	gencode	0.00
RPL15P3	3.12	6	gencode	0.00
AC016697.6	2.99	2	gencode	0.00
PSORS1C3	2.97	6	RefSeq	0.03
RP3-400B16.3	2.74	6	gencode	0.00
RP4-680D5.2	2.69	1	gencode	0.00
RP11-316E14.2	2.69	14	gencode	0.00
ZFAS1	2.67	20	RefSeq	0.01
RP11-299D14.2	2.58	8	gencode	0.00
PASK	2.55	2	gencode	0.01
AFG3L1P	2.45	16	UCSC_knowngene	0.01
NAP1L4P3	2.43	13	gencode	0.00
RP11-320M2.1	2.41	2	gencode	0.04
SLC25A5P8	2.30	9	gencode	0.00
CTD-2315E11.1	2.27	15	gencode	0.05
RP11-481E4.1	2.25	16	gencode	0.00
RP11-82H13.2	2.25	1	gencode	0.01
ESPL1	2.24	12	gencode	0.00
AC021849.1	2.21	2	gencode	0.01
RP11-637I8.2	2.21	16	gencode	0.02
CHCHD2P6	2.21	1	gencode	0.00

(Continues)

TABLE 1 (Continued)

Name	Fold change	Chromosome	Source	P value
RP11-325P15.2	2.18	1	gencode	0.03
CTD-2061E19.1	2.17	5	gencode	0.00
RP11-363E6.1	2.16	8	gencode	0.02
RP11-568J23.1	2.14	16	gencode	0.01
RP11-626I20.2	2.11	12	gencode	0.04
TOPORS-AS1	2.04	9	gencode	0.01
XLOC_004244	2.04	5	LincRNAs identified by Cabili et al	0.02

2.4 | qRT-PCR detection of RP11-555H23.1

To confirm the results of the lncRNA microarray, cDNA was reverse transcribed from 2 µg of total RNA from another 104 pairs of gastric carcinoma tissue and adjacent noncancer tissue using the GoScript reverse transcription (RT) system (Promega, Madison, WI). cDNA, primers, and GoTaq qPCR master mix (Promega) were mixed and real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed using a Mx3005P real-time PCR system (Stratagene, La Jolla, CA).²⁴ Sequences of the PCR primers for RP11-555H23.1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, a reference) are as follows: 5'-TGACTACCGTCTTGACTTACACT-3' (forward) and 5'-TCGCAACTGGGAGGAATAGC-3' (reverse) for RP11-555H23.1; 5'-ACCCACTCCTCCACCTTTGAC-3' (forward) and 5'-TGTTGCTGTAGCCAAATTCGTT-3' (reverse) for GAPDH.²⁴ The relative expression level of RP11-555H23.1 was calculated with the comparative threshold cycle method. Relative fold change was calculated using the following formula: $2^{-\Delta\Delta C_q} = 2^{-(\Delta C_q(\text{tumor sample}) - \Delta C_q(\text{normal control}))}$, where $\Delta C_q = C_q(\text{RP11-555H23.1}) - C_q(\text{GAPDH})$. All experiments were independently repeated three times. The results are shown as the mean $\pm$ SD.

2.5 | Sequencing of qRT-PCR products

To determine the validity of qRT-PCR results, we first used a UNIQU-10 PCR product purification kit (Sangon Biotech, Shanghai, China) to purify PCR products and then cloned them into the pUCm-T vector (Sangon Biotech).²³ Finally, sequencing was performed by Tsingke Biotech Co., Ltd., Hangzhou, China.

2.6 | Serological biomarker analysis

An Elecsys 2010 machine (Roche Diagnostics, Basel, Switzerland) was used to detect serum carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) levels. Their cutoff values were set at 35 U/mL and 5 ng/mL, respectively.

2.7 | Statistical analysis

Statistical Program for Social Sciences (SPSS) 18.0 software (SPSS, Chicago, IL) was used for all data analysis. Differences in

the RP11-555H23.1 expression levels between cancer tissues and paired nontumor tissues were evaluated using paired sample *t* test. Relationships between the RP11-555H23.1 levels and clinicopathological features of the gastric cancer patients were further analyzed by a two-tailed Student's *t* test, rank-sum test, or one-way analysis of variance (ANOVA). To assess diagnostic value, a receiver operating characteristic (ROC) curve was created. The statistical significance was set at $P < 0.05$.

3 | RESULTS

3.1 | Global metabolic pathway-associated lncRNA expression patterns in gastric cancer

Volcano plots are useful tools for visualizing differential expression of metabolic pathway-associated lncRNAs between gastric cancer tissues and paired nontumor tissues (Figure S1). Microarray detection data showed that, of 114 metabolic pathway-associated lncRNAs, 51 downregulated lncRNAs (Table 1) and 63 upregulated lncRNAs (Table 2) were significantly differentially expressed between the gastric cancer and adjacent nontumor tissues (fold change > 2.0 , $P < 0.05$; GEO accession number 96856: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE96856>). The lowest expressed lncRNAs in the gastric cancer group were TUG1, RP11-555H23.1, RP1-257I20.13, UGP2, GCSHP3, and XLOC_000889 (Table 1). The highest expressed lncRNAs in the gastric cancer group were RP11-605F14.2, TBC1D3P5, BC130595, LINC00475, RP11-19P22.6, BC080653, XLOC_004923, AFAP1-AS1, EPB49, and RP11-296I10.3 (Table 2).

3.2 | Expression of RP11-555H23.1 is reduced in gastric cancer samples

In our previous experiments, we found that expression of TOPORS antisense RNA 1 (TOPORS-AS1) in expanded sample numbers of gastric cancer tissues was consistent with our microarray results (Table 1).²² TOPORS-AS1 was one of the downregulated metabolic pathway-associated lncRNAs in gastric cancer. For upregulated metabolic pathway-associated lncRNAs (Table 2), we found that DiGeorge syndrome critical region gene 9 (DGCR9) exhibited increased expression, not only in gastric cancer tissues but also in

TABLE 2 Upregulated lncRNAs in gastric cancer tissues compared with those in paired nontumor tissues (fold changes ≥ 2 and P values ≤ 0.05)

Name	Fold change	Chromosome	Source	P value
RP11-605F14.2	40.01	3	gencode	0.001
TBC1D3P5	29.53	17	RefSeq	0.006
BC130595	16.78	2	UCSC_knowngene	0.002
LINC00475	14.53	9	RefSeq	0.023
RP11-19P22.6	9.01	17	gencode	0.006
BC080653	8.38	9	UCSC_knowngene	0.015
XLOC_004923	8.34	5	lincRNA	0.004
AFAP1-AS1	7.87	4	RefSeq	0.044
EPB49	7.42	8	gencode	0.004
RP11-296I10.3	6.11	16	gencode	0.046
SMAD5-AS1	5.70	5	RefSeq	0.048
IGLL3P	5.51	22	RefSeq	0.025
RP11-1L12.3	4.68	11	gencode	0.020
TCL6	4.36	14	RefSeq	0.023
KRASP1	4.32	6	gencode	0.04
AK055628	4.26	10	GenBank	0.04
LOC151171	4.17	2	RefSeq	0.01
ANKRD36BP2	4.14	2	RefSeq	0.05
SNAR-A2	3.95	19	RefSeq	0.01
HCP5	3.68	6	RefSeq	0.03
XLOC_008852	3.66	10	LincRNAs identified by Cabili et al	0.01
XLOC_007858	3.62	9	LincRNAs identified by Cabili et al	0.00
AC004938.5	3.46	7	gencode	0.00
MIAT	3.34	22	RefSeq	0.01
AK024556	3.33	5	GenBank	0.01
LOC100289019	3.29	9	RefSeq	0.00
CTD-2194A8.2	3.28	16	gencode	0.01
RP11-377K22.2	3.23	1	gencode	0.00
AP000350.8	3.15	22	gencode	0.05
XLOC_012430	3.14	17	LincRNAs identified by Cabili et al	0.04
AC006022.4	3.13	7	gencode	0.03
AK056073	3.10	19	UCSC_knowngene	0.00
RP11-119D9.4	3.05	11	gencode	0.03
APOL2	2.99	22	gencode	0.01
XLOC_003808	2.96	4	LincRNAs identified by Cabili et al	0.01
XLOC_006599	2.81	7	LincRNAs identified by Cabili et al	0.00
RP11-774O3.1	2.80	4	gencode	0.00
C14orf63	2.68	14	gencode	0.02
XLOC_009198	2.67	11	LincRNAs identified by Cabili et al	0.01
RP11-410D17.2	2.65	16	gencode	0.02

(Continues)

TABLE 2 (Continued)

Name	Fold change	Chromosome	Source	P value
LOC729739	2.61	15	RefSeq	0.00
RP11-256G5.1	2.61	6	encode	0.01
RP11-106J23.1	2.58	16	encode	0.03
DGCR9	2.54	22	RefSeq	0.00
CASP3P1	2.53	1	encode	0.03
CYP4A22-AS1	2.53	1	encode	0.03
XLOC_002067	2.49	2	LincRNAs identified by Cabili et al	0.01
PPIHP1	2.47	11	encode	0.03
RP11-307C19.1	2.45	15	encode	0.00
TRIM31	2.37	6	encode	0.02
RP11-473P24.2	2.34	3	encode	0.00
FTLP2	2.28	X	encode	0.02
RPLP2	2.26	11	encode	0.00
MAFG-AS1	2.21	17	UCSC_knowngene	0.03
XLOC_003422	2.20	4	LincRNAs identified by Cabili et al	0.02
KCNQ1DN	2.19	11	RefSeq	0.02
RP1-66C13.3	2.16	17	encode	0.04
RP11-441O15.3	2.09	10	encode	0.02
C1orf191	2.05	1	RefSeq	0.03
PNPLA7	2.04	9	encode	0.04
ARAP1	2.04	11	encode	0.00
C1QTNF9B-AS1	2.03	13	Ensembl	0.01
TERC	2.01	3	RefSeq	0.01

gastric cancer cell lines.²⁴ In this study, RP11-555H23.1 was chosen to confirm the microarray results. RP11-555H23.1, a newly discovered lncRNA, is transcribed from the antisense strand of chromosome X, and there are no reports regarding its expression in cancers. In the microarray results, the expression patterns of three patients between gastric cancer and nontumor tissues were the same. As illustrated in the microarray results (Table 1), RP11-555H23.1 is the second most downregulated lncRNA. To verify RP11-555H23.1 expression in gastric cancer tissues, we expanded the sample number and used qRT-PCR to assess its expression levels. We first found that RP11-555H23.1 was significantly reduced in gastric cancer tissues (Figure 1, $P < 0.001$). The qRT-PCR product was then sequenced, and the results showed that the RP11-555H23.1 sequence (Figure S2) was consistent with that observed in the database (https://asia.ensembl.org/Homo_sapiens/Transcript/Sequence_cDNA?db=core;g=ENSG00000238193;r=X:5305714-5307128;t=ENST00000434972).

3.3 | Possible diagnostic value of RP11-555H23.1 in gastric cancer

We next investigated whether RP11-555H23.1 expression is related to the clinicopathological features of gastric cancer. Using another

104 pairs of gastric carcinoma tissues and the adjacent noncancer tissues, we found that levels of RP11-555H23.1 are correlated with TNM stage ($P = 0.038$; Table 3). Patients at Stage IV are typically not candidates for surgery, and obtaining cancer tissues for study is difficult. Among patients in this study, 11 suffered from metastatic gastric cancer. Eight of them developed pyloric obstruction and could not eat, necessitating surgery. Three other patients did not exhibit distant metastases during preoperative examination. However, in the middle of the surgery, these patients were found to have distant metastases, one hepatic metastasis and two peritoneal implantation metastases. Since the number of patients with Stage IV disease was small (11 patients), we pooled Stage III and Stage IV patients (Table 3). Although there was no significant difference in the results of the pT, pN, and M analyses (Table 3), a significant difference for the TNM stage was observed, and TNM staging, which uses combinations of pT, pN, and M, indicates the extent of cancer spread.

Furthermore, an ROC curve was constructed by grouping gastric cancer and nontumor samples to assess the diagnostic value of RP11-555H23.1. The area under the ROC (AUR) curve was 0.65 (Figure 2), and further analysis showed that, at a cutoff of 12.9 (ΔC_q value), sensitivity and specificity were 81% and 62%, respectively.

FIGURE 1 Expression of RP11-555H23.1 in gastric cancer tissues and paired adjacent normal tissues. RP11-555H23.1 and GAPDH mRNA levels were measured by qRT-PCR. ΔC_q was used to show relative expression of RP11-555H23.1 using $\Delta C_q = C_q(\text{RP11-555H23.1}) - C_q(\text{GAPDH})$. Small ΔC_q indicates higher expression. $n = 104$, $P < 0.001$

4 | DISCUSSION

Cancer is a class of diseases involving oncogene activation and tumor suppressor gene inhibition. In recent years, deep sequencing studies including large consortia and advances in technology, such as RNA deep sequencing and tiling arrays, have enabled identification of abundant tumor-associated coding sequences and noncoding sequences.^{28,29} In particular, lncRNAs have received increasing attention. Current knowledge regarding the functions of lncRNAs has shown that they are involved in a diversity of cancer-associated processes.³⁰ For example, LINC00982 regulates cell proliferation in gastric cancer.³¹ Moreover, another lncRNA, FOXP4 antisense RNA 1

(FOXP4-AS1), is overexpressed in colorectal cancer tissues and significantly related to TNM stages and regulation of cell proliferation.³² In addition, PVT1 expression levels were markedly upregulated in gastric cancer tissues, correlated with TNM stage, and regulate gastric cancer cell growth.³³

Reprogramming energy metabolism is one of the hallmarks of cancers.⁶ Pyruvate kinase isozyme type M2 (PKM2), which plays a critical role in glucose metabolism, is closely correlated with TNM stage in colorectal cancer.³⁴ Meanwhile, TNM stage and PKM2 are two independent factors of overall survival in patients with colorectal cancer.³⁴ Furthermore, in clear-cell renal cell carcinoma (ccRCC), solute carrier family 1 member 5 (SLC1A5), a major glutamine transporter, was shown to be significantly associated with TNM stage.³⁵

TABLE 3 Relationship between RP11-555H23.1 expression levels (ΔC_q) in cancer tissues and the clinicopathological factors of patients with gastric cancer

Characteristics	No. of patients (%)	Mean $\pm$ SD	P value
Age (y)			
≥60	67 (64.4)	13.01 $\pm$ 3.42	0.843
<60	37 (35.6)	12.88 $\pm$ 2.60	
Gender			
Male	75 (72.1)	13.29 $\pm$ 3.05	0.092
Female	29 (27.9)	12.13 $\pm$ 3.27	
Diameter (cm)			
≥5	56 (53.8)	13.08 $\pm$ 3.22	0.706
<5	48 (46.2)	12.84 $\pm$ 3.08	
CA19-9			
Positive	64 (61.5)	13.13 $\pm$ 3.12	0.503
Negative	40 (38.5)	12.70 $\pm$ 3.20	
CEA			
Positive	98 (94.2)	12.97 $\pm$ 3.19	0.963
Negative	6 (5.8)	12.91 $\pm$ 2.59	
Differentiation			
Well	13 (12.5)	13.96 $\pm$ 2.20	0.225
Moderate	49 (47.1)	13.20 $\pm$ 3.33	
Poor	42 (40.4)	12.39 $\pm$ 3.12	
Lymphatic metastasis			
N0	37 (35.6)	13.56 $\pm$ 2.74	0.154
N1 & N2 & N3	67 (64.4)	12.64 $\pm$ 3.32	
Distal metastasis			
M0	93 (89.4)	13.00 $\pm$ 3.04	0.742
M1	11 (10.6)	12.67 $\pm$ 4.11	
Invasion			
Tis & T1-T3	40 (38.5)	13.25 $\pm$ 3.21	0.467
T4	64 (61.5)	12.79 $\pm$ 3.11	
TNM stage			
0 & I & II	42 (40.4)	13.74 $\pm$ 2.56	0.038
III & IV	62 (59.6)	12.44 $\pm$ 3.40

FIGURE 2 The ROC curve of RP11-555H23.1

In our study, the metabolic pathway-associated lncRNAs in gastric cancer were studied for the first time. A total of 114 differentially expressed lncRNAs between gastric cancer tissues and paired nontumorous tissues were found (Tables 1 and 2). Some of these lncRNAs have been found to play crucial roles in carcinogenesis. For example, RP11-19P22.6-001 and its target, nitric oxide synthase 2 (NOS2), are associated with gastric cancer, and their combined use might be a promising method to improve the diagnostic value of lncRNAs in cancers.³⁶ By following p53 activation, LINC00475 regulates levels of CDKN1A/p21, a cell cycle inhibitor and a major p53 target gene.³⁷ Interestingly, taurine upregulated gene 1 (TUG1), the most downregulated lncRNA in our study (Table 1), is upregulated in hepatocellular carcinoma (HCC) tissues and promotes cell growth and apoptosis through epigenetic silencing of Kruppel-like factor 2 (KLF2).³⁸

To confirm the microarray results, we expanded the sample size of gastric cancer tissue and examined expression levels of RP11-555H23.1, the second most downregulated lncRNA in metabolic pathway microarray detection (Table 1). The results showed consistency between these two assays (Figure 1). The ROC curve evidenced this lncRNA's diagnostic value (Figure 2), and its sensitivity and specificity were 81% and 62%, respectively. Furthermore, expression levels of RP11-555H23.1 were related to TNM stage (Table 3). Since lncRNA screening was performed through metabolic pathway microarray and since the patients with earlier gastric cancer expressed much lower levels of RP11-555H23.1 than did the advanced patients (Table 3), our results indicate that RP11-555H23.1 might alter metabolism in gastric cancer and play a role in gastric cancer initiation.

One of the weaknesses of this study is the results related to differences in the expression of RP11-555H23.1 between carcinoma tissues, which were heterogeneous with regard to cellular type and content, and adjacent "normal" tissues, which were also likely to exhibit cellular heterogeneity. As a result, an increased number of samples or more pure cells should be used in the future.

Little experimental evidence of an association with a known metabolic process was presented that would reflect the presence of gastric carcinoma. However, lncRNA species have been reported to be related to multidrug resistance and sensitivity,³⁹ as well as carcinogenesis.⁴⁰ In contrast to the goals of other studies, this study was focused on identifying diagnostic biomarkers.

In conclusion, lncRNAs play a role in gastric cancer through regulating metabolism, and the representative metabolic-associated lncRNA RP11-555H23.1 might represent a diagnostic biomarker in gastric cancer.

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CONFLICT OF INTERESTS

None.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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