

Competing endogenous RNA network analysis reveals potential long non-coding RNAs as predictive biomarkers of gastric cancer

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Abstract. Gastric cancer (GC) is one of the most frequently occurring life-threatening malignancies worldwide. Due to its high mortality rate, the discovery of putative biomarkers that may be sensitive and specific to GC is of seminal importance. Long non-coding RNAs (lncRNAs) are non-translatable RNAs whose transcript length exceeds 200 base pairs. The dysregulation of lncRNA expression plays a key role in tumorigenesis and development. In the present study, the expression profiles of lncRNAs, microRNAs and mRNAs of 361 GC tissues (and 32 normal gastric tissues) were downloaded from The Cancer Genome Atlas database. Furthermore, differentially expressed RNAs were analyzed by the DEseq package. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses confirmed some significant dysregulated signaling pathways and target RNAs. As a result, an lncRNA-associated competing endogenous RNA (ceRNA) network was constructed. Kaplan-Meier analysis of the differentially expressed RNAs associated with GC pathogenesis confirmed that the lncRNAs PVT1, HAND2-AS1 and ZNF667-AS1 were potentially associated with the prognosis of GC ($P < 0.05$). The present study suggests the mechanism of ceRNA networks in GC, and further demonstrates that aberrant lncRNA expression may be used as an effective diagnostic tool (or target) for the prognosis of GC.

Introduction

Gastric cancer (GC) is the fourth most commonly occurring cancer in the world, and the second cause of cancer-associated deaths worldwide. Approximately 990,000 new cases of GC occur every year, with ~738,000 associated deaths

worldwide (1). The high mortality rate of GC is mainly due to limited diagnosis at the earlier phases of the disease, which consequently means that gastric carcinoma is detected when it is established at a more advanced stage (2). Hence, the identification of GC-specific tumor biomarkers is warranted for the diagnosis and therapy of patients with GC.

A non-coding RNA (ncRNA) is a type of RNA that is not translated into a protein (3). Due to its structural characteristics, an ncRNA can regulate the expression of an mRNA to promote or inhibit the production of its encoded protein and indirectly exert its biological effects. The ncRNA family includes long ncRNAs (lncRNAs), microRNAs (miR/miRNAs), small interfering RNAs, small nucleolar RNAs and ribosomal RNAs (4). lncRNAs correspond to a class of ncRNAs, which lack open reading frames and are typically longer than 200 base pairs (5). A number of studies have demonstrated that lncRNAs are involved in the pathogenesis of many malignancies and, therefore, could be used as putative biomarkers for the prognosis of these various conditions. The function of lncRNAs can be either cancer-promoting or tumor-suppressing, depending on the level of regulation towards protein-coding genes and/or the chromosome structure (6,7). lncRNAs play roles in various cellular processes, mainly regulating cell growth, differentiation and apoptosis (8,9). miRNA is another type of RNA, ~22 nucleotides in length, that can also control the expression of protein-coding genes (10). This type of RNA can modulate gene expression at the post-transcriptional level, by attaching to miRNA response elements along the target transcript (11,12). In 2011, the competing endogenous RNA (ceRNA) theory emerged, introducing novel regulatory mechanisms driven by distinct non-coding RNAs (12). The main idea behind this theory involves the competitive cross-regulation of lncRNAs, mRNAs and miRNAs (13).

Recent studies have revealed that several lncRNAs are associated with the occurrence, metastasis, prognosis and drug resistance of GC (14-16). Notwithstanding this, the roles of lncRNA and miRNA in early and advanced GC are not yet fully understood. Therefore, further studies on the roles of lncRNA in GC could be conducive to the diagnosis and therapy of patients with GC.

In conclusion, the purpose of the present study was to identify novel and specific lncRNAs associated with GC, and to provide insight into the features of lncRNAs as biomarkers for the diagnosis and therapy of patients with GC, thus further improving the prognosis.

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Materials and methods

Data acquisition from The Cancer Genome Atlas (TCGA). The expression data of mRNA, lncRNA and miRNA in patients with GC were downloaded from TCGA database (<https://cancergenome.nih.gov>). Specifically, the expression profiles of 393 patients with GC were retrieved from the TCGA-Stomach Adenocarcinoma (STAD) dataset (17) via the University of California Santa Cruz Xena database (<https://xenabrowser.net/datapages/>). The inclusion criteria were as follows: i) Tissues samples with completed data for analysis and ii) histologic diagnosis is STAD without other malignancies. The exclusion criteria were set as follows: i) Tissues samples without completed data for analysis; ii) histologic diagnosis is not STAD; iii) patients with additional malignancies other than GC and iv) patients who received preoperative chemoradiation. For GC samples containing detailed RNA expression data, the corresponding clinical details were also accessed, including survival time, survival status, sex, staging and other clinical features. A total of 361 cases of GC (233 males and 128 females; mean age, 65.66 years; age range, 30-90 years) and 32 cases of normal (22 males and 10 females; mean age, 68.78 years; age range, 50-88 years) gastric tissue were included in the present study. The detailed clinical features of all patients with GC are summarized in Table I.

In silico RNA screening. The differentially expressed mRNAs, lncRNAs and miRNAs (DEmRNAs, DElncRNAs and DEmiRNAs, respectively), in both GC and non-tumor tissues, were independently selected using the edgeR package (R language; version 3.28.0; <http://bioconductor.org/packages/2.4/bioc/html/edgeR.html>) with a threshold of $|\log_2(\text{fold-change}(\log_2FC))| > 2$ and false discovery rate (FDR) < 0.01 (18). Hierarchical clustering analysis was completed using the pheatmap package in R (version 1.0.8), in order to estimate the divergence among the differentially expressed RNAs (19).

Differential mRNA enrichment analysis. DEmRNAs were added into the Database for Annotation, Visualization and Integrated Discovery (<https://david.ncifcrf.gov/>), which takes advantage of GO (<http://geneontology.org/>) to determine the gene function described in the respective gene profiles. The Kyoto Encyclopedia of Genes and Genomes (KEGG; <https://www.kegg.jp/>) was also utilized as a platform to investigate the likely functions of the aforementioned genes and their pathways.

Construction of lncRNAs and mRNA co-expression networks. lncRNA-mRNA networks were designed as the basis for lncRNA-mediated control of mRNA abundance. For this, DElncRNAs and DEmRNAs were selected by comparing GC with normal control tissues. To determine the respective RNA contents, a standard selection criteria was set at $P < 0.05$ and fold change > 2 . The lncRNA and mRNA co-expression networks were constructed by correlating DElncRNAs and their respective target mRNAs.

Construction of ceRNA network. TargetScan (http://www.targetscan.org/vert_72/), miRTarBase (<http://mirtarbase.mbc>

Table I. Clinicopathological characteristics of patients with gastric cancer.

Characteristic	Patients (n=361)	
	n	%
Age category, years		
≤65	161	44.6
>65	200	55.4
Sex		
Male	233	64.5
Female	128	35.5
Pathological stage		
I	63	17.5
II	112	31.0
III	152	42.1
IV	34	9.4
Pathological T stage		
Tis	8	2.2
T1	18	5.0
T2	75	20.8
T3	164	45.4
T4	96	26.6
Pathological N stage		
N0	123	34.1
N1	96	26.6
N2	66	18.3
N3	76	21.1
Pathological M stage		
M0	322	89.2
M1	23	6.4
Mx	16	4.4
Survival status		
Alive	219	60.7
Dead	142	39.3

Tis, carcinoma *in situ*; Mx, distant metastasis is unknown.

nctu.edu.tw/php/index.php) and miRDB (<http://mirdb.org/>) databases were used to predict target mRNAs (combined with DEmiRNAs). The prediction analysis of lncRNA binding to DEmiRNAs was performed using miRcode software (version 11; <http://www.mircode.org>). Datasets were intersected in order to strengthen the correlations between mRNAs and DEmRNAs. The lncRNA-miRNA-mRNA ceRNA network was formed on the basis of the DEmiRNA-DElncRNA and DEmiRNA-DEmRNA interactions, and further visualized using Cytoscape software (version 3.7.2; <https://cytoscape.org>). A flow diagram of the lncRNA-miRNA-mRNA ceRNA network analysis is shown in Fig. 1.

Survival analysis. Using the median expression of the differentially expressed RNAs as the cutoff value, patients with GC were divided into high and low expression groups. The

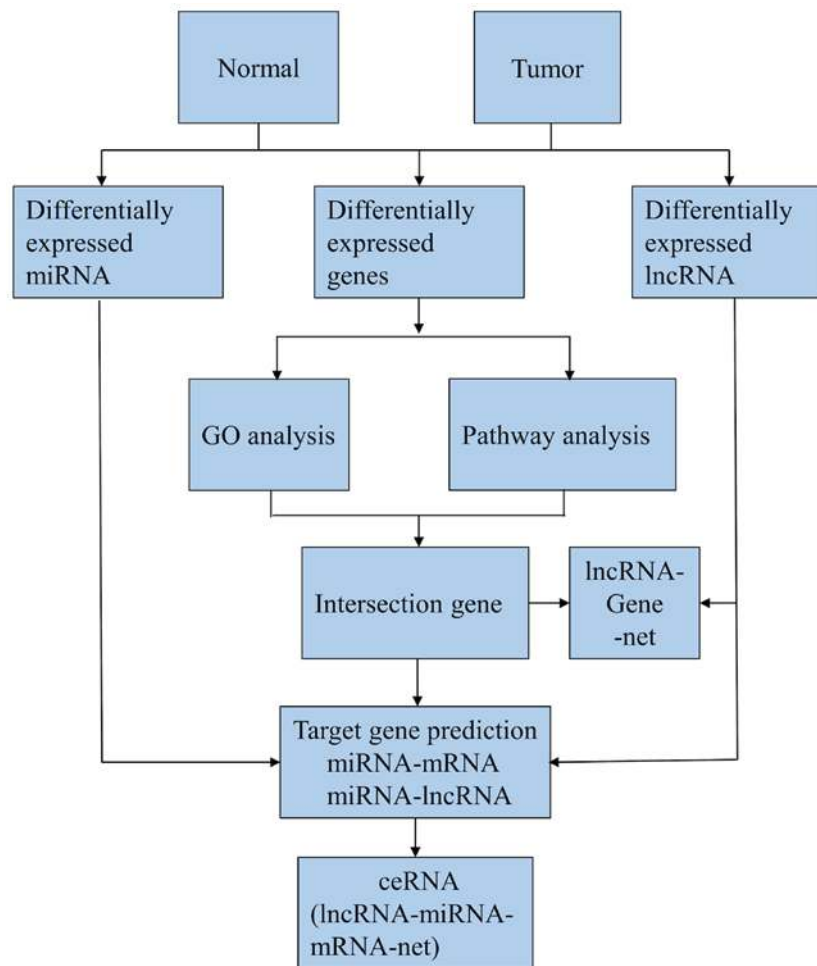


Figure 1. Flow chart of bioinformatics analysis.

difference in survival time between the two groups was evaluated by Kaplan-Meier survival curve and log-rank test analysis via the SPSS Statistics software, version 21 (IBM Corp.). As a result, lncRNAs were found accordingly. $P < 0.05$ was used as a threshold for statistical significance.

Results

Differential expression of mRNAs, lncRNAs and miRNAs in GC. Based on the cutoff threshold, a total of 1,370 DEmRNAs (627 upregulated and 743 downregulated RNAs) was selected from both GC and normal tissues. A total of 393 DELncRNAs (197 upregulated and 196 downregulated RNAs) and 46 DE miRNAs (24 upregulated and 22 downregulated RNAs) were differentially expressed in GC compared with normal tissues. Unsupervised hierarchical clustering analysis was achieved for all selected DEmRNAs, DELncRNAs and DE miRNAs. A heatmap was constructed to display the distinctive pattern of RNAs expressed in GC compared with non-tumor samples (Fig. 2A-C). Thereafter, all differentially expressed RNAs were used for further analyses.

GO and KEGG pathway analysis. GO and KEGG pathway analysis uncovered 25 upregulated and downregulated GO and KEGG terms. The most significant GO terms were associated with 'cell division', 'proliferation' and 'mitotic cell cycle' (Fig. 3A and B).

KEGG pathway enrichment analysis was also performed using the selected DEmRNAs. The leading 25 upregulated and downregulated KEGG pathways were found to be permanently enriched by DEmRNAs in the ceRNA network, among which the 'PI3K-Akt signaling pathway', 'cell cycle', 'pathways in cancer' and the 'p53 signaling pathway' were most closely associated with the pathogenesis of cancer (Fig. 3C and D).

Construction of lncRNA-mRNA co-expression network. Subsequently, an lncRNA-mRNA co-expression network was established using the GO terms and pathways compiled by respective target genes and DELncRNAs. The statistical basis of the co-expression network was Pearson correlation index $|r| > 0.9$ in a network where both lncRNA-mRNA and mRNA-mRNA connections were mutually regulated. The co-expression network in GC and normal gastric tissues was constructed according to the Pearson's correlation coefficient, which measured values of lncRNA and mRNA. Based on preceding conclusions (Figs. 2A and 3), the lncRNA-mRNA co-expression network was created among differentially expressed lncRNAs and mRNAs in both GC and normal gastric tissues. The results revealed network associations between 12 lncRNAs and 57 mRNAs (Fig. S1).

DELncRNA-DE miRNA interactions predicted by miRcode. The prediction analysis of lncRNA binding to DE miRNAs was

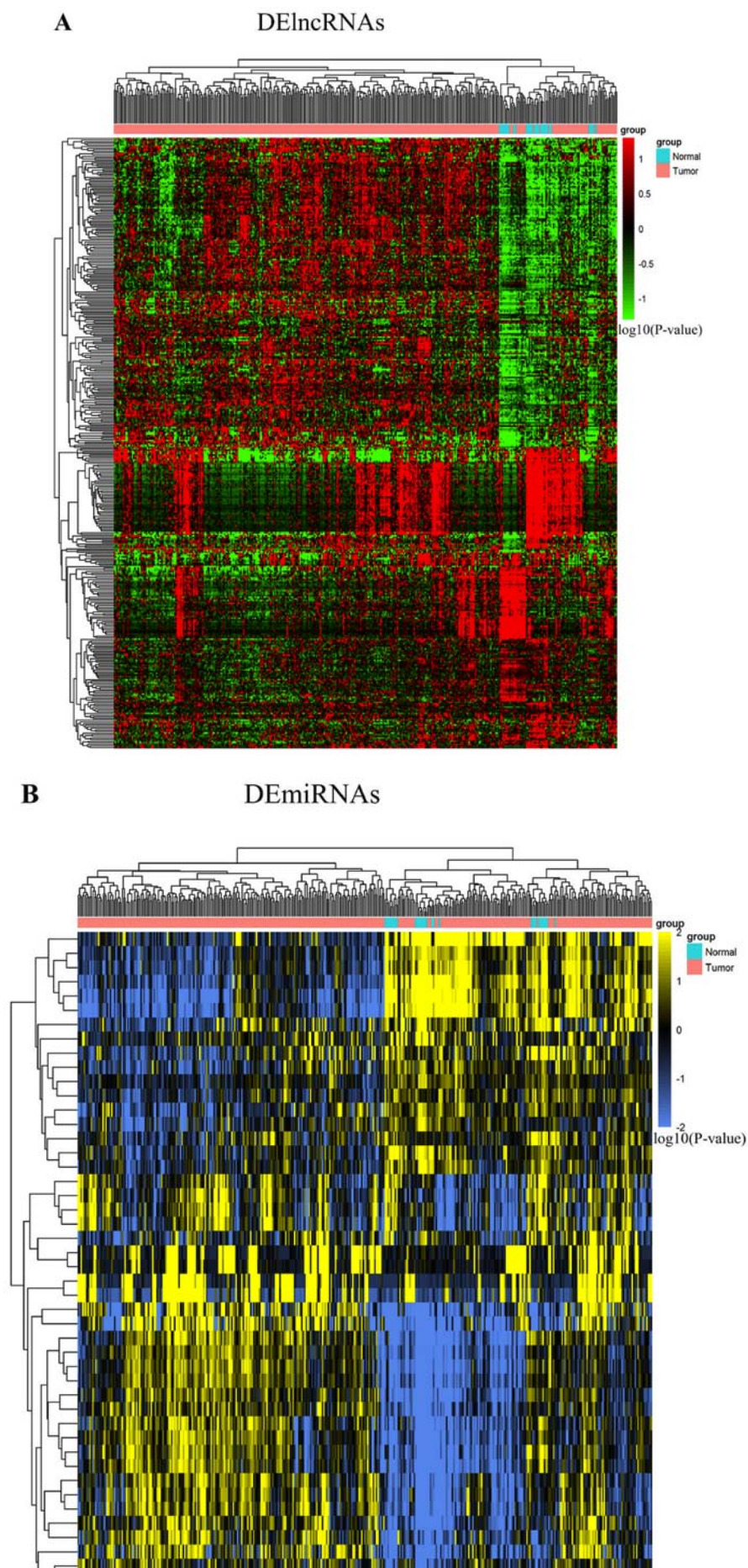


Figure 2. Differential expression of intersecting RNAs in GC. (A) DElncRNAs and (B) DEmiRNAs. A heatmap showing the differentially expressed RNAs.

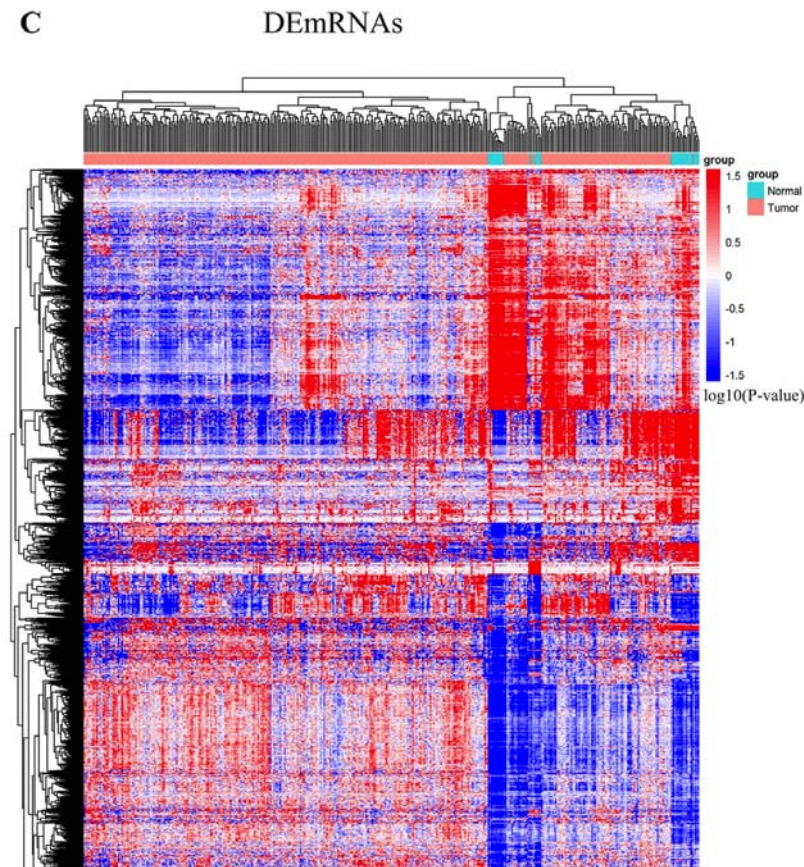


Figure 2. Continued. Differential expression of intersecting RNAs in GC. (C) DEmRNAs. A heatmap showing the differentially expressed RNAs. DE, differentially expressed; lncRNA, long non-coding RNA; miRNA, microRNA.

performed using miRcode. As such, predicted lncRNAs and DElncRNAs were intersected, and the intersecting lncRNAs were included into the ceRNA network. Correspondingly, 342 interactions between 64 DElncRNAs (including PVT1, RP11-284F21.7, RP11-284F21.10, HAND2-AS1 and ZNF667-AS1) and 35 DEMiRNAs (including hsa-miR-194-3p, hsa-miR-196b-5p, hsa-miR-218-5p and hsa-miR-145-3p) were annotated and further analyzed. The top 10 miRNA-mediated lncRNAs are shown in Table II.

Prediction of DEMiRNA-interacting DEmRNAs. Previously annotated DEMiRNAs were mapped into TargetScan, miRTarBase and miRDB databases to search for their target mRNAs. A total of 357 mRNAs were converged by these DEMiRNAs in all three databases. As a result, 118 mRNAs were determined as the DEMiRNA-targeted DEmRNAs. To underline the ceRNA characteristics of the network, DEMiRNAs that were capable of combining with both DEmRNAs and DElncRNAs were introduced into the ceRNA network. Ultimately, 37 DEMiRNAs, 118 DEmRNAs and 65 DElncRNAs were selected for ceRNA network construction. The top 27 miRNAs, which were predicted to modulate most of the mRNAs, are shown in Table III.

Profile of the ceRNA network in GC. Based on data from Tables II and III, and Fig. S1, an miRNA-lncRNA-mRNA ceRNA network was constructed. The miRNA-lncRNA-mRNA associations were integrated into the ceRNA network,

according to their mutual regulation roles. The ceRNA network was constructed with 220 modules (including 37 DEMiRNAs, 118 DEmRNAs and 65 DElncRNAs) and 717 interactions. The network was profiled using Cytoscape (Fig. 4A and B). This network demonstrated the possibility of indirect associations between DElncRNAs and DEmRNAs.

lncRNAs associated with the prognosis of patients with GC. In order to discover lncRNAs that are significantly correlated with the prognosis of patients with GC, Kaplan-Meier survival curves and log-rank test analyses were conducted for each of the DElncRNAs comprising the ceRNA network. The results demonstrated that three distinct DElncRNAs (PVT1, ZNF667-AS1 and HAND2-AS1) were associated with survival ($P < 0.05$; Fig. 5A-C). ZNF667-AS1 and HAND2-AS1 appeared to be pro-cancerous, since patients with higher levels of these RNAs presented with shorter overall survival time (Fig. 5A and C). By contrast, PVT1 appeared to have a protective role, since its high expression was associated with longer overall survival time in patients with GC (Fig. 5C).

Discussion

GC is one of the most common types of cancer worldwide, and it largely affects the quality of life and the health of Asian populations. In China, the incidence and mortality rates of GC have escalated to be the second highest among all cancer types (20). Although the diagnosis and treatment of GC have significantly

Table II. Top 10 DElncRNAs putatively targeted by most DEmiRNAs in the competing endogenous RNA network.

DElncRNAs	DEmiRNAs
MIR143HG	hsa-miR-141-5p, hsa-miR-146b-5p, hsa-miR-18a-5p, hsa-miR-196a-5p, hsa-miR-196b-5p, hsa-miR-335-3p, hsa-miR-552-5p, hsa-miR-767-5p
LINC00261	hsa-miR-105-5p, hsa-miR-135b-5p, hsa-miR-182-5p, hsa-miR-194-3p, hsa-miR-196a-5p, hsa-miR-335-3p, hsa-miR-552-3p
TP73-AS1	hsa-miR-105-5p, hsa-miR-141-3p, hsa-miR-141-5p, hsa-miR-194-3p, hsa-miR-552-3p
GS1-358P8.4	hsa-miR-141-3p, hsa-miR-146b-5p, hsa-miR-196a-5p, hsa-miR-196b-5p, hsa-miR-200a-3p
MBNL1-AS1	hsa-miR-141-3p, hsa-miR-196a-5p, hsa-miR-200a-3p, hsa-miR-767-5p
AFAP1-AS1	hsa-miR-129-5p, hsa-miR-145-3p, hsa-miR-149-5p, hsa-miR-30c-2-3p
NSUN5P1	hsa-miR-129-5p, hsa-miR-139-3p, hsa-miR-30c-2-3p, hsa-miR-5683
RP11-242D8.1	hsa-miR-145-3p, hsa-miR-149-5p, hsa-miR-30c-2-3p
MIR4435-2HG	hsa-miR-1-3p, hsa-miR-145-5p, hsa-miR-149-5p
TUG1	hsa-miR-144-3p, hsa-miR-29c-3p, hsa-miR-5683

DE, differentially expressed; lncRNA, long non-coding RNA; miRNA, microRNA.

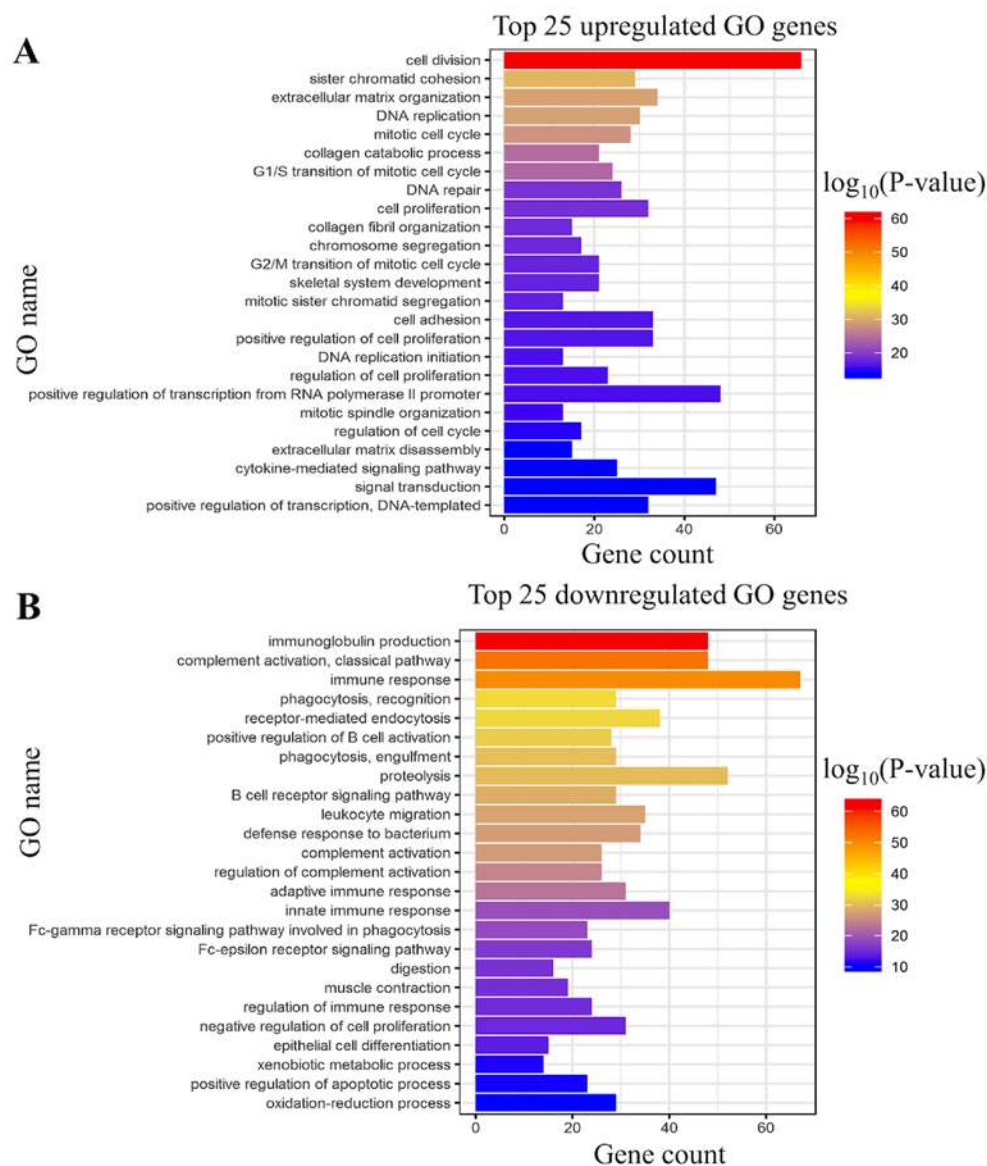


Figure 3. Top 25 enrichment GO terms and pathways of differentially expressed intersecting mRNAs. (A) Upregulated GO terms, and (B) downregulated GO terms.

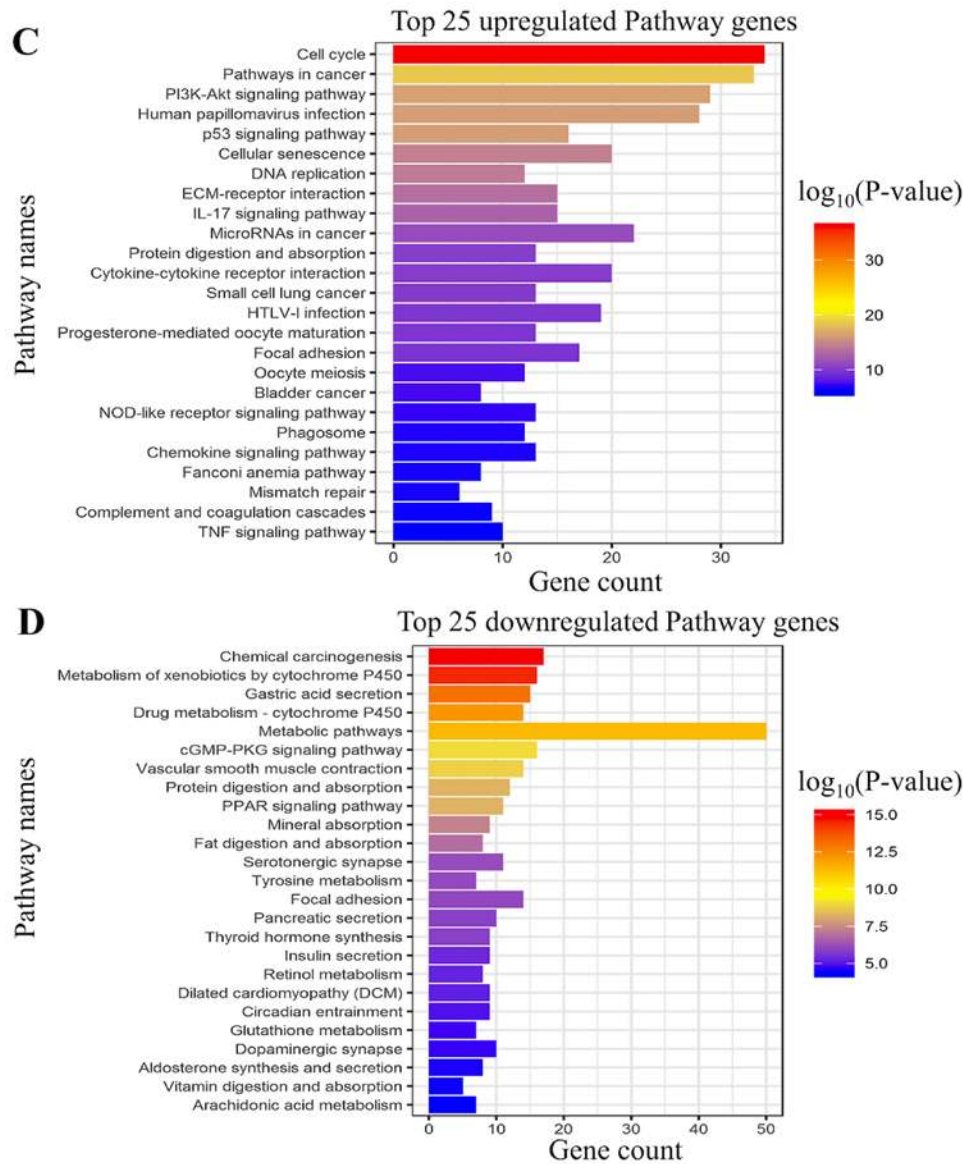


Figure 3. Continued. Top 25 enrichment GO terms and pathways of differentially expressed intersecting mRNAs. (C) upregulated pathways and (D) downregulated pathways. The bar plot shows the enrichment scores of the significant enrichment GO terms and pathways. GO, Gene Ontology.

improved, the 5-year overall survival rate of patients with advanced GC is still ~20%, mostly due to late diagnosis, rapid progression and metastatic characteristics (21,22). Therefore, the discovery and characterization of early GC biomarkers is imperative for a more efficient diagnosis and treatment of the disease.

The role of lncRNAs in cancer has become increasingly important. Moreover, lncRNAs appear to play a vital role in cancer proliferation, development and prognosis. For example, MEG3 inhibits GC metastasis by activating the p53 signaling pathway (23). The lncRNA AWPPH enhances the growth and proliferation of triple-negative breast cancer by upregulating FZD7 (24), whereas cancer progression and shortened survival in patients with colon cancer are associated with the overexpression of the lncRNA DANCR (25).

A number of studies have shown that lncRNAs can regulate diverse biological activities in tumor cells, including transcriptional, post-transcriptional and phenotype regulation (26-28). lncRNAs influence the stability

of target-encoding genes by modulating the activity of miRNAs (29). In the context of GC, a complicated regulatory network involving lncRNAs and miRNAs or mRNAs plays a vital role in the occurrence and development of the disease (30,31). Therefore, the concept of ceRNAs was proposed in order to explain the mutual regulatory mechanism between non-coding and coding RNAs during cancer progression, as well as its influence on the survival of patients with cancer (11). Compared with the coding RNAs, lncRNAs have a greater advantage as an effective biomarker for the diagnosis and prognosis of early GC (32).

A number of lncRNAs have been found to be associated with GC. It is also well known that *Helicobacter pylori* infection plays a role in the pathogenesis of GC. *In vitro* and *in vivo* studies have revealed that infection by *H. pylori* can, for example, drive the downregulation of lncRNA AF147447 in GC. lncRNA AF147447 can suppress the proliferation and invasion of GC by repressing MU2 and upregulating the expression of miR-34c (33). The lncRNA NORAD is highly

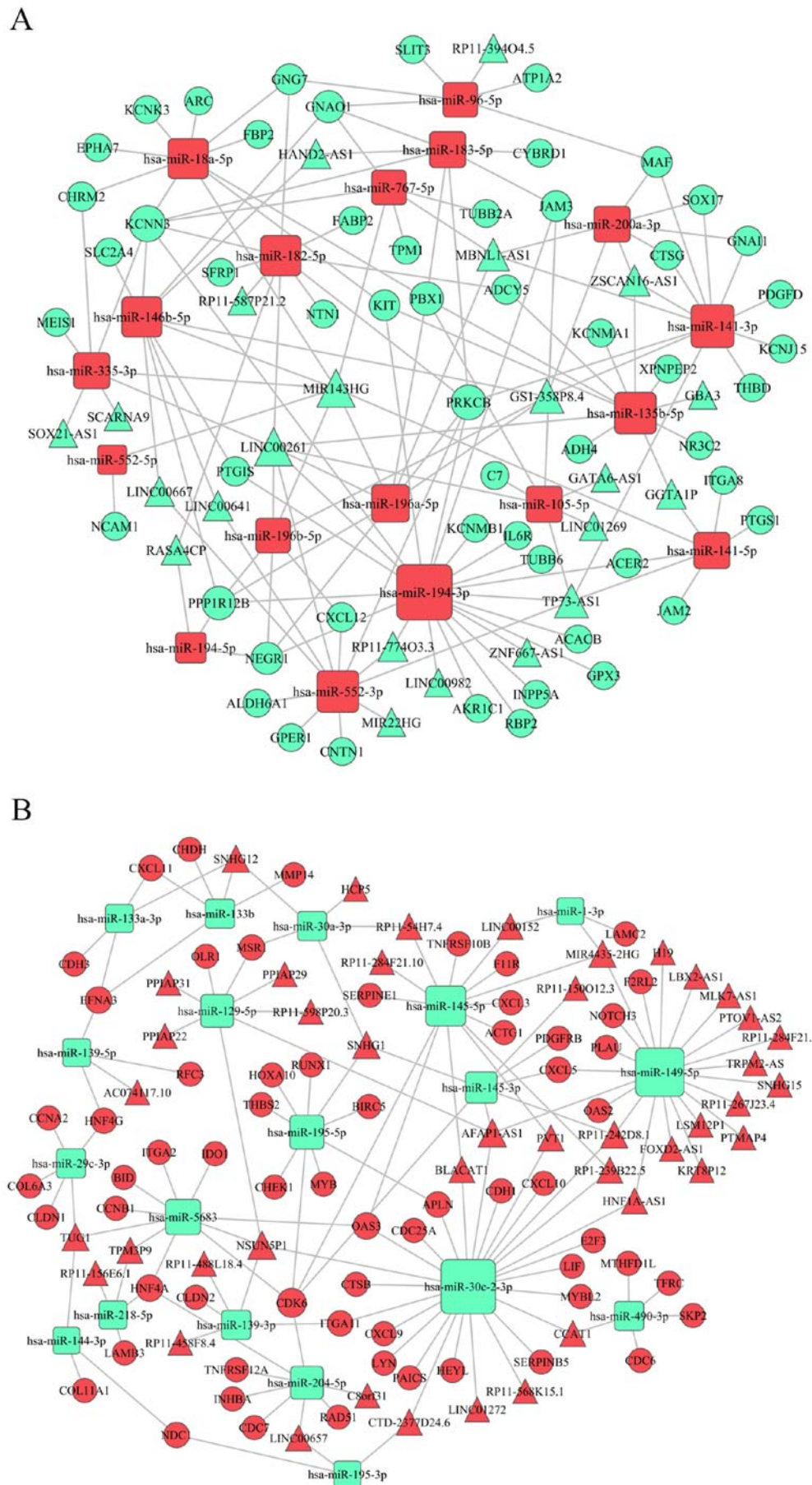


Figure 4. lncRNA-miRNA-mRNA competing endogenous RNA network. (A) Red squares represent upregulated miRNAs, green circles represent downregulated mRNAs and green triangles represent downregulated lncRNAs. (B) Green squares represent downregulated miRNAs, red circles represent upregulated mRNAs and red triangles represent upregulated lncRNAs. miRNA/miR, microRNA; lncRNA, long non-coding RNA.

Table III. DEmiRNAs (n=27) with corresponding target DEMRNAs in the competing endogenous RNA network.

DEmiRNAs	DEmRNAs
hsa-miR-194-3p	ACACB,ACER2,AKR1C1,GPX3,IL6R,INPP5A,JAM3,KCNMB1,KCNN3,KIT,NEGR1,PPP1R12B,PRKCB,PTGIS,RBP2,TUBB6
hsa-miR-30c-2-3p	LYN,APLN,CDC25A,CDH1,CTSB,CXCL10,CXCL9,E2F3,HEYL,ITGA11,LIF,MYBL2,OAS3,PAICS,SERPINB5
hsa-miR-195-5p	APLN,BIRC5,CDK6,CHEK1,HOXA10,MYB,RUNX1,THBS2
hsa-miR-141-3p	CTSG,GNAI1,KCNJ15,MAF,PDGFD,PRKCB,SOX17,THBD
hsa-miR-145-5p	ACTG1,CDK6,CXCL3,F11R,OAS3,SERPINE1,TNFRSF10B
hsa-miR-135b-5p	ADCY5,ADH4,KCNMA1,KIT,NR3C2,PBX1,XPNPEP2
hsa-miR-18a-5p	ARC,CHRM2,EPHA7,FBP2,GNG7,KCNK3,KIT,PBX1
hsa-miR-182-5p	ADCY5,FABP2,GNG7,KCNN3,NTN1,PRKCB,SFRP1
hsa-miR-183-5p	CYBRD1,GNAO1,JAM3,KCNN3,PBX1,PRKCB
hsa-miR-5683	BID,CCNB1,CDK6,HNF4A,IDO1,ITGA2,OAS3
hsa-miR-552-3p	ALDH6A1,CNTN1,CXCL12,GPER1,PRKCB
hsa-miR-146b-5p	GNAO1,KCNN3,PPP1R12B,PTGIS,SLC2A4
hsa-miR-204-5p	CDC7,CDK6,INHBA,RAD51,TNFRSF12A
hsa-miR-149-5p	CXCL5,F2RL2,NOTCH3,OAS2,PLAU
hsa-miR-96-5p	ATP1A2,GNAO1,GNG7,MAF,SLIT3
hsa-miR-196a-5p	NEGR1,PBX1,PPP1R12B,PRKCB
hsa-miR-29c-3p	CCNA2,CLDN1,COL6A3,HNF4G
hsa-miR-767-5p	GNAO1,KCNN3,TPM1,TUBB2A
hsa-miR-133b	CHDH,CXCL11,EFNA3,MMP14
hsa-miR-490-3p	CDC6,MTHFD1L,SKP2,TFRC
hsa-miR-141-5p	ACER2,ITGA8,JAM2,PTGS1
hsa-miR-196b-5p	CXCL12,NEGR1,PPP1R12B
hsa-miR-200a-3p	CTSG,GNAI1,MAF,SOX17
hsa-miR-335-3p	CHRM2,KCNN3,MEIS1
hsa-miR-133a-3p	CDH3,CXCL11,EFNA3
hsa-miR-139-5p	EFNA3,HNF4G,RFC3
hsa-miR-144-3p	COL11A1,NDC1

DE, differentially expressed; miRNA, microRNA.

expressed in GC tissues and cells, and is strongly correlated with poor clinical outcomes in GC. Furthermore, NORAD may act as a ceRNA to promote the outgrowth of GC cells, by regulating the miR-608/FOXO6 signaling cascade (34). The lncRNA LINC00460 has also been suggested as an independent risk factor for the prognosis of GC, since it can promote the proliferation and migration of cancer cells by stimulating the Wnt/ β -catenin pathway (35). In addition, the lncRNA FLVCR1-AS1 sponges miR-155 to further promote the development of GC (36).

Following TCGA analysis of the DElncRNA content in GC and paracancerous tissue samples, MIR100HG was found to be highly expressed in GC and, thus, could be considered as a potential prognostic marker of GC (37). AKT protein kinase has been widely studied as a cancer-promoting factor. A recent study reported that lncRNA STXBP5-AS1 can inhibit the activation of AKT in GC, which leads to the inhibition of proliferation and migration of GC cells (38).

The present study attempted to identify a highly specific lncRNA in GC, and to further understand its impact during

the pathogenesis and progression of the disease. In order to achieve this aim, the expression profiles of lncRNAs, miRNAs and mRNAs were first assessed in 361 GC and 32 adjacent and normal tissues from TCGA database. A detailed bioinformatics analysis of GC compared with normal tissues uncovered some DElncRNAs (PVT1, RP11-284F21.7 and RP11-284F21.10) that were significantly upregulated, and at least two DElncRNAs (HAND2-AS1 and ZNF667-AS1) that were significantly downregulated. Moreover, a number of DEmiRNAs and DEMRNAs were upregulated and downregulated. In particular, enriched DEMRNAs were closely associated to processes that impact carcinogenesis, including 'cell division', 'proliferation' and the 'mitotic cell cycle'. Moreover, a number of DEMRNAs were associated with the 'PI3K-Akt signaling pathway' (39), 'pathways in cancer' and the 'p53 signaling pathway' (40) in GC, and abnormalities in these pathways are also associated with cancer. Subsequently, the association between the three RNAs was analyzed. Furthermore, a ceRNA network map was constructed, and Kaplan-Meier survival analyses were performed to establish a potential association between differential RNAs and

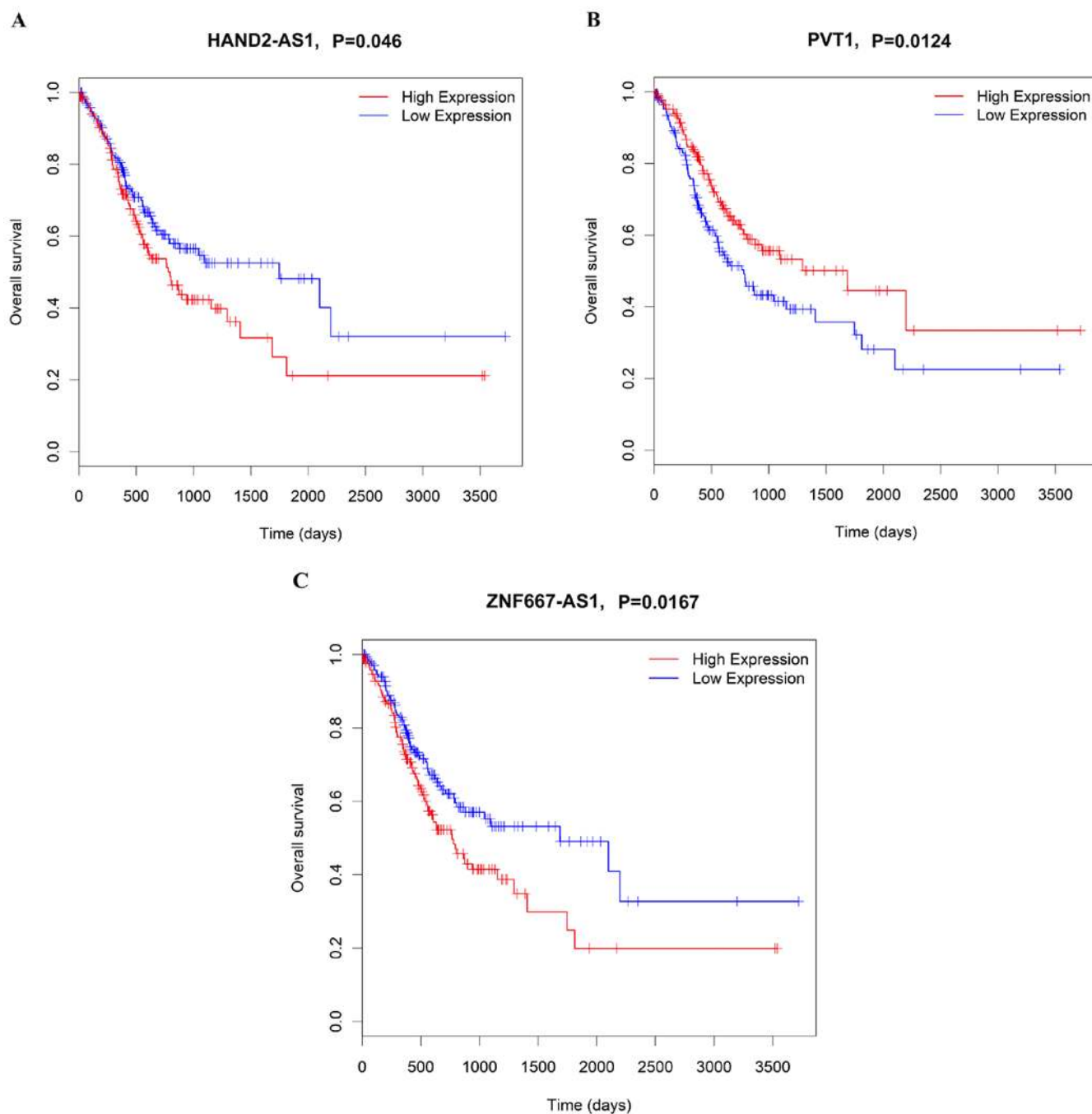


Figure 5. Kaplan-Meier survival curves of the three long non-coding RNAs. Kaplan-Meier survival curves of patients with low and high expression levels of (A) HAND2-AS1, (B) PVT1 and (C) ZNF667-AS1.

the prognosis of GC. The lncRNAs HAND2-AS1, PVT1 and ZNF667-AS1 were strongly associated with the prognosis of GC and could be used as biomarkers for predicting the prognosis of the disease.

The role of PVT1 in other cancer types has been previously reported. For instance, it was shown that PVT1 was highly expressed in patients with glioma at stages III-IV (versus patients at stages I-II), and the survival time of these patients with high expression of PVT1 was significantly shorter (41). PVT1 is also involved in the sensitivity of radiotherapy affecting lung cancer (42). The role of lncRNA HAND2-AS1, which was also reported in the present study, has been similarly identified in other cancer types. As an example, HAND2-AS1 appears to

inhibit the proliferation and migration of esophageal squamous cell tumors (43). HAND2-AS1 can also impede the growth and metastasis of colon cancer by upregulating KLF5 (44). In lung cancer, HAND2-AS1 can also act as a tumor suppressor (45). However, the roles of ZNF667-AS1 and HAND2-AS1 in GC has not been previously reported. To the best of our knowledge, the present study is the first to demonstrate that high expression of ZNF667-AS1 and HAND2-AS1 is negatively associated with the survival time of patients with GC.

The construction of an lncRNA-miRNA-mRNA ceRNA network revealed the associations between novel specific lncRNAs and other RNAs in GC. Moreover, a putative association between differentially expressed RNAs and the

prognosis of patients with GC was elucidated. The findings of the present study may have substantial clinical significance; however, some limitations should be taken into consideration. Firstly, the data were downloaded from TCGA databases; thus, experimental methods are required to verify the results. Secondly, the role of HAND2-AS1, PVT1 and ZNF667-AS1 in GC is still unknown; therefore, *in vivo* and *in vitro* experiments are required to answer this question.

In conclusion, the data from the present study will serve as a basis for the definition and validation of potential biomarkers for the diagnosis and targeted therapy for GC. Future functional investigations are required to explore the mechanisms underlying the roles of these lncRNAs in 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 upon reasonable request.

Authors' contributions

YF and SP conceived and designed the present study, SP drafted the manuscript and YF revised the initial manuscript. XY acquired the data. SP, XY, YZ, WM, TL, YY, JJ and QL analyzed and interpreted the data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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