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Construction and Comprehensive Analysis for Dysregulated Long Non-Coding RNA (lncRNA)-Associated Competing Endogenous RNA (ceRNA) Network in Gastric Cancer

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Data Interpretation D
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Long non-coding RNA (lncRNA) is a kind of non-coding RNA with transcripts more than 200 bp in length. LncRNA can interact with the miRNA as a competing endogenous RNA (ceRNA) to regulate the expression of target genes, which play a significant role in the initiation and progression of tumors. In this study, we explored the functional roles and regulatory mechanisms of lncRNAs as ceRNAs in gastric cancer, and their potential implications for prognosis.

The lncRNAs, miRNAs, and mRNAs expression profiles of 375 gastric cancer tissues and 32 non-tumor gastric tissues were downloaded from The Cancer Genome Atlas (TCGA) database. Differential expression of RNAs was identified using the DESeq package. Survival analysis was estimated based on Kaplan-Meier curve analysis. KEGG pathway analysis was performed using KOBAS 3.0. The dysregulated lncRNA-associated ceRNA network was constructed in gastric cancer based on bioinformatics generated from miRcode and miRTarBase.

A total of 237 differentially expressed lncRNAs and 198 miRNAs between gastric cancer and matched normal tissues were screened in our study with thresholds of |log2FC| >2 and adjusted P value <0.01. Eleven discriminatively expressed lncRNAs may be correlated with tumorigenesis of gastric cancer. Seven out of 11 dysregulated lncRNA were found to be significantly associated with overall survival in gastric cancer (*P* value <0.05). The newly identified ceRNA network includes 11 gastric cancer-specific lncRNAs, 9 miRNAs, and 41 mRNAs.

Collectively, our study will contribute to improving the understanding of the lncRNA-associated ceRNA network regulatory mechanisms in the pathogenesis of gastric cancer and provide and identify novel lncRNAs as candidate prognostic biomarkers or potential therapeutic targets.

MeSH Keywords: MicroRNAs • RNA, Long Noncoding • Stomach Neoplasms

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Background

Gastric cancer is one of the most common malignancies in the world. According to the data of cancer incidence and mortality worldwide, in 2012 there are 952 000 new cases of gastric cancer in the world each year and about 723 000 people die from it each year. It is one of the 3 leading causes of cancer death worldwide [1]. Due to its high prevalence, poor prognosis, and limited treatment options, gastric cancer is still an important clinical challenge worldwide. By analyzing a large gastric cancer patient clinical database, a retrospective study found that factors such as age, sex, tumor stage, and surgical method were associated with overall survival [2]. Therefore, identification of individualized treatment strategies, including potential biomarkers and therapeutic targets to combat gastric cancer, are urgently needed. The present study explores how the gastric cancer-specific lncRNAs act as ceRNAs to regulate target genes and participate in pathogenesis and prognosis of gastric cancer.

Encyclopedia of DNA elements (ENCODE) the latest research results show that more than 90% of the human genome sequence can be transcribed, but only 1~2% of the sequence is used to encode proteins [3–5].At present, lncRNA genes have been cloned and identified more than 50,000 in the human genome, but so far only a small part of the biological function of lncRNA got the experimental verification. Studies found that these lncRNAs have important potential application prospect in the diagnosis, treatment and prognosis in malignant tumor [6–8]. However, lncRNAs how to regulate the expression of genes has remained unclear. At present, much effort is being made to reveal that lncRNAs how to performance diverse biological functions in the malignant tumor.

In 2011, Salmena et al. proposed a competing endogenous RNA (ceRNA) hypothesis, which described a complex post-transcriptional regulatory network in which lncRNAs, mRNAs, and other RNAs act as natural miRNA sponges to suppress miRNA function by sharing 1 or more miRNA response elements (MREs) [9], which is supported by much evidence [10–13]. LncRNA as ceRNA regulates gene encoding protein level and participate in the regulation of cell biology by competing with miRNAs. miRNAs plays an important role in the ceRNA network through combining with target mRNA, inhibiting the action of mRNA expression [14]. It has been well-documented that the interaction between miRNAs and target genes is associated with tumor pathogenesis [15,16]. Studies have shown that each miRNA can control up to hundreds of expressions of transcription, while each RNA transcription with different miRNAs response elements (MREs) may be targeted by multiple miRNAs [17].

In recent years, a growing number of studies have confirmed that the lncRNA – miRNAs – mRNAs regulation network plays an important role in tumor pathogenesis and progression, including breast cancer, gastric cancer, liver cancer, lung cancer, and kidney cancer, and other malignant tumors [11,12,18–21]. LncRNAs that harbor similar sequences to their targeted miRNAs can sequester miRNAs away from mRNAs. Poliseno et al. confirmed that lncRNA PTENP1 up-regulates expression of gene PTEN through acting as a molecular sponge adsorption miR – 19 and miR-20a in prostate cancer and inhibits tumor cell growth [11]. In addition, lncRNA FER1L4 can influence the expression of the genes PTEN and RB1 by competitively combining with miR-106a-5p and participating in gastric cancer pathogenesis [22].

Therefore, lncRNA as ceRNAs have diverse biological functions that deserve further exploration. In addition, the analysis of gastric cancer-associated lncRNA-mediated ceRNA network in a whole genome is lacking, especially studies with large sample sizes. In this study, according to the analysis of RNA expression profiles between the 375 tumor tissues and 32 non-tumor tissues of gastric cancer, we successfully established the gastric cancer-associated ceRNA network based on bioinformatics prediction and correlation analysis, which included 11 lncRNAs, 9 miRNAs, and 41 mRNAs.

Material and Methods

Study population

A total of 375 gastric cancer cases were enrolled for comprehensive integrated analysis. The data were download from The Cancer Genome Atlas (TCGA) database. In addition, we used the Data Transfer Tool (provided by GDC Apps) to download the level 3 mRNASeq gene expression data, miRNAseq data of samples, and clinical information of those patients (*https://tcga-data.nci.nih.gov/*). The sequenced data was derived from Illumina HiSeq RNASeq and Illumina HiSeq_miRNASeq platforms. Our research meets the publication guidelines provided by TCGA (*http://cancergenome.nih.gov/publications/publicationguidelines*).

Differentially expressed analysis

Gastric cancer mRNAseq and miRNASeq data derived from 407 samples, including 375 gastric cancer samples (cohort Tumor) and 32 normal samples (cohort Normal), were downloaded from TCGA. In addition, we merged tumor sample and normal sample data and deleted expressed data, which closed to zero. Compared to the normal group with gastric cancer, we used the "DESeq" package [23] in R software to identify the differentially expressed mRNAs (DEmRNAs) with thresholds of |log2foldChange(FC)| >2.0 and adjusted P value <0.01 and differentially expressed miRNAs with | log2FC| >1.5 and

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adjusted P value <0.01. We used the Encyclopedia of DNA Elements (ENCODE) to define and annotate the differentially expressed lncRNAs (DElncRNAs). In our study, we discovered the DElncRNAs from differentially expressed RNAs with the cut-off criteria of |log2FC| >2.0 and adjusted P value <0.01.

Constructing the ceRNA network

To clarify the roles of lncRNA and miRNA with mediated ceRNA network, we built the co-expression network of differentially expressed genes, lncRNAs and miRNAs, visualized using Cytoscape v3.5.0 software. miRNA-targeted mRNAs were retrieved from miRTarBase (*http://mirtarbase.mbc.nctu.edu.tw/*). The targeted mRNAs of miRNAs were verified by experimental study using reporter assay, qRT-PCR, Western blot, microarray, and next-generation sequencing experiments in miRTarBase. To further improve the ceRNA network reliability, we retained mRNAs included in different expression of RNAs between tumor tissues and normal tissues. In addition, lncRNA-miRNA interactions were constructed based on miRcode (*http://www.mircode.org/*).

Survival analysis

To identify the prognostic DERNAs signature, combining the clinical data of those patients with gastric cancer in TCGA, we plotted the survival curves of those samples with differentially expressed lncRNAs, miRNAs and mRNAs by using the "survival" package in R. This univariate survival analysis was estimated based on Kaplan-Meier curve analysis. *P* values less than 0.05 were considered as significant.

Results

Identification of DEmRNAs and DEmiRNAs

RNAs expression profiles of gastric cancer patients and corresponding clinical information were downloaded using the Data Transfer Tool of the TCGA database. We identified the significant DEmRNAs and DEmiRNAs in gastric cancer samples compared with the normal samples. A total of 2024 differentially expressed mRNAs and 198 miRNAs were identified by the "DESeq" package in R. Then, the heat map with complete linkage clustering of DEmRNAs and DEmiRNAs was built using the "gplots" package in R. (Supplementary Figures 1, 2). As a result, there were 1042 (51.48%) up-regulated and 982 (48.52%) down-regulated DEGs. Moreover, a total of 158 (79.79%) up-regulated and 40 (20.21%) down-regulated DEmiRNAs were identified. The DERNAs were enriched in the KEGG pathway by KOBAS 3.0 (*http://kobas.cbi.pku.edu.cn/*), in order to preliminarily investigate the tumorigenesis of gastric cancer. We found that the DERNAs were mainly enriched in "Transcriptional misregulation in cancer, Metabolic pathways, and Chemical carcinogenesis", which are closely correlated with tumorigenesis (Table 1).

Differentially expressed lncRNAs (DElncRNAs) in gastric cancer

A total of 237 DElncRNAs were identified in our study, with thresholds of |log2FC| >2 and adjusted P value <0.01. To enhance the data reliability, those not annotated in ENCODE were removed. Finally, 11 DElncRNAs (9 up-regulated and 2 down-regulated) were identified in gastric cancer samples compared to the normal samples (Table 2). Subsequently, to explore the relationship between DElncRNAs and the prognosis of patients with gastric cancer, the overall survival for 11 DElncRNAs in gastric cancer patients was investigated using Kaplan-Meier curve analysis. We found that 7 of 11 DElncRNAs were considered as key DElncRNAs responsible for the prognosis of gastric cancer. As a result, 7 DElncRNAs were significantly associated with overall survival, lncRNA RP11-120K18.2 were positively correlated with overall survival, while the remaining 6 DElncRNAs were negatively associated with overall survival (log-rank P <0.05) (Figure 1).

Differentially expressed miRNAs (DEmiRNAs) in gastric cancer

In our study, 198 DEmiRNAs were identified in gastric cancer samples compared with normal samples with thresholds of |log2FC| >1.5 and adjusted P value <0.01. Nine DEmiRNAs (5 up-regulated and 4 down-regulated) were selected from 198 gastric cancer-associated DEmiRNAs in TCGA data (Table 3). As with the DElncRNAs, the overall survival for 9 DEmiRNAs in gastric cancer patients was also investigated using Kaplan-Meier curve analysis. Four out of 9 significant DEmiRNAs were significantly associated with overall survival (log-rank P <0.05), and 2 DEmiRNAs, mir-137 and mir-145, were demonstrated to be associated with high levels of DEmiRNAs and with poor prognosis. On the contrary, high levels of the remaining 2 DEmiRNAs, mir-96 and mir-183, were associated with prolonged patient survival time (Figure 2).

Construction of a ceRNA network in gastric cancer

To better understand how lncRNA mediates mRNA through combining miRNA in gastric cancer, a ceRNA network graph was constructed based on the above data and visualized using Cytoscape v3.5.0. (Figure 3). We found that 11 DElncRNAs interact with the 9 DEmiRNAs retrieved in the miRcode database (Table 4). We searched for targeted mRNAs based on the 9 miRNAs using the miRTarBase database. MiRNAs targeted mRNAs not included in DERNAs (|log2FC| >1.0 and adjusted P value <0.01) were discarded. Each miRNA-mRNA pair was

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Table 1. DERNAs were enriched KEGG pathways in gastric cancer.

Pathway ID	Description	P-value	Number of DERNAs
hsa01100	Metabolic pathways	5.20E-08	90
hsa04080	Neuroactive ligand-receptor interaction	7.86E-11	39
hsa04151	PI3K-Akt signaling pathway	1.35E-05	32
hsa04060	Cytokine-cytokine receptor interaction	7.86E-07	30
hsa04974	Protein digestion and absorption	1.30E-13	26
hsa00980	Metabolism of xenobiotics by cytochrome P450	1.57E-14	25
hsa05204	Chemical carcinogenesis	1.40E-13	25
hsa05202	Transcriptional misregulation in cancer	7.42E-07	24
hsa05200	Pathways in cancer	0.02720	24
hsa04024	cAMP signaling pathway	1.08E-05	23

Table 2. Eleven gastric cancer specific lncRNAs in ceRNA network construction.

lncRNA	Gene ID	Expression change	log2 fold change (T/N)	*P*-value
RP11-389G6.3	ENSG00000261292	Down-regulation	–3.37	2.31E-18
ADAMTS9-AS2	ENSG00000241684	Down-regulation	–2.30	1.08E-23
RP11-499F3.2	ENSG00000259692	Up-regulation	2.07	0.00047
DLX6-AS1	ENSG00000231764	Up-regulation	2.97	1.46E-07
RP11-120K18.2	ENSG00000260757	Up-regulation	3.40	1.57E-15
AC027119.1	ENSG00000229642	Up-regulation	3.86	1.78E-06
RP11-474D1.3	ENSG00000214039	Up-regulation	4.25	2.24E-07
AC012531.25	ENSG00000260597	Up-regulation	4.33	4.07E-20
RP11-445F12.1	ENSG00000277268	Up-regulation	4.71	1.76E-08
CTD-2147F2.1	ENSG00000259485	Up-regulation	5.22	3.01E-09
AC007099.1	ENSG00000231172	Up-regulation	5.55	2.31E-10

T – tumor; N – normal.

Table 3. Nine gastric cancer specific miRNAs in ceRNA network construction.

Name	log2 fold change (T/N)	*P*-value	FDR
hsa-mir-183	1.94	5.44E-18	1.99E-16
hsa-mir-96	1.79	4.15E-16	1.33E-14
hsa-mir-217	1.69	9.92E-08	8.39E-07
hsa-mir-182	1.55	4.10E-14	9.62E-13
hsa-mir-19a	1.54	2.42E-18	9.51E-17
hsa-mir-137	–1.78	6.74E-07	4.84E-06
hsa-mir-139	–2.27	4.93E-54	7.75E-51
hsa-mir-145	–2.39	3.23E-41	1.02E-38
hsa-mir-204	–1.96	1.69E-14	4.16E-13

T – tumor; N – normal.

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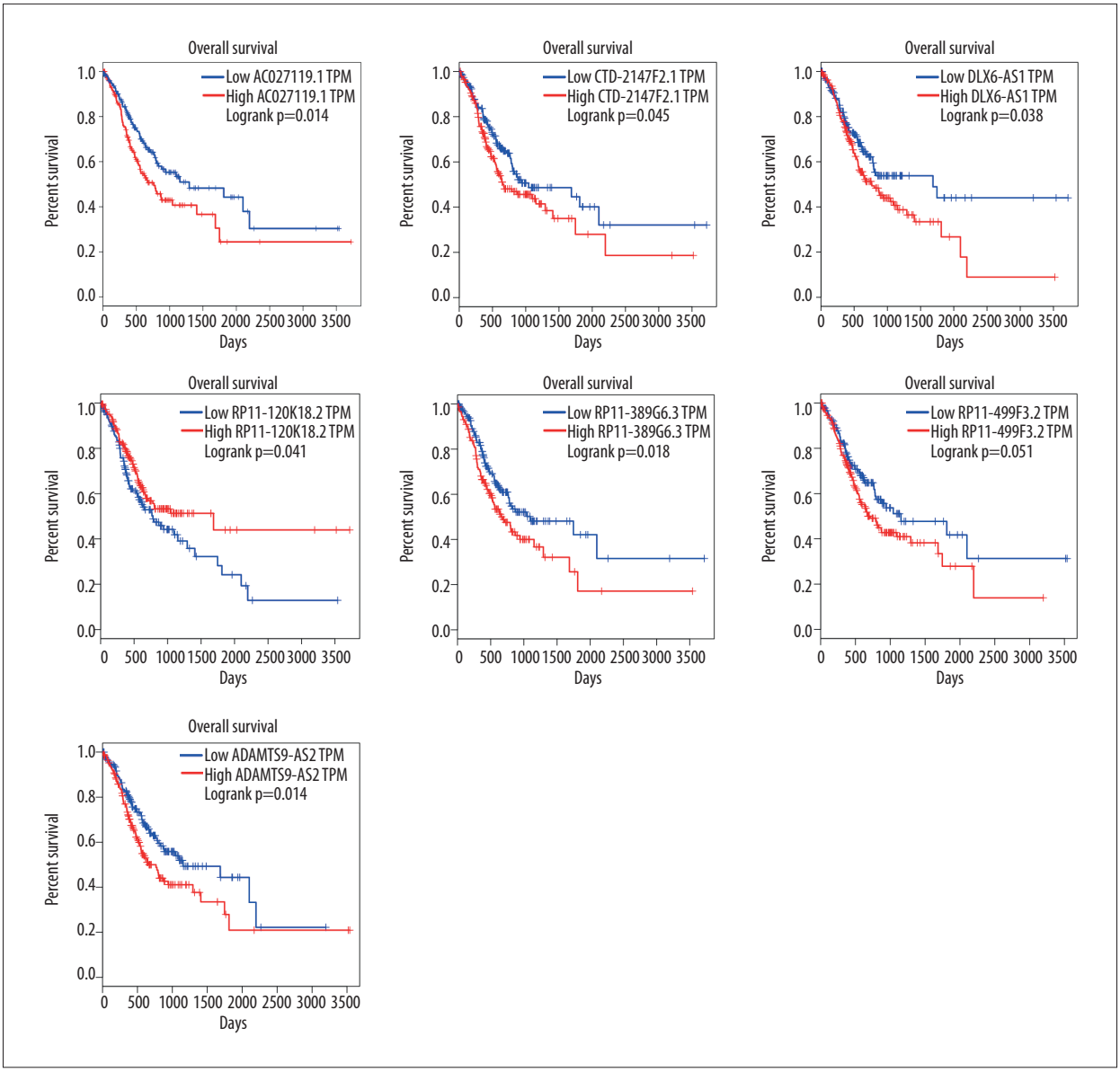

Figure 1. Kaplan-Meier survival curves for 7 lncRNAs associated with overall survival in gastric cancer. (Seven DElncRNAs are presented (P<0.05), including ADAMTS9-AS2, DLX6-AS1, RP11-389G6.3, RP11-499F3.2, AC027119.1, RP11-120K18.2, and CTD-2147F2.1. Horizontal axis: overall survival time: days, Vertical axis: survival function).

experimentally validated. Finally, 41 DEmRNAs were included in the ceRNA network. We found that most of them are tumor-related genes, such as HMGA2, HOXA9, CDK6, EZH2, E2F3, FSCN1, CSE1L, BCL2, CDKN1A, AKAP12, CHL1, KIT, KLF4, EGR1, MXD1, MEIS1, and SOX4, all retrieved from the Onco database (*http://www.bushmanlab.org/links/genelists*). Moreover, we noted that some of the mRNAs were also associated with overall survival in patients with gastric cancer (Figure 4). Overall survival for DEmRNAs was investigated using Kaplan-Meier curve analysis. To better understand the KEGG pathways involved in the ceRNA network, the mRNAs were performed using KEGG pathway analysis by KOBAS 3.0, and the top 10 KEGG pathways were significantly enriched in our study (Table 5). The signal pathways were cancer-associated, such as "MicroRNAs in cancer, Transcriptional misregulation in cancer, Pathways in cancer, Glioma, Pancreatic cancer and Melanoma".

Moreover, we found that the ADAMTS9-AS2 maybe play an important lncRNA role in the ceRNA network. ADAMTS9-AS2 interacted with 6 miRNAs (mir-96, mir-137, mir-145, mir-182, mir-204, and mir-19a) and indirectly interacted with 37 miRNA-targeted mRNAs in this network. We used different expression levels of DElncRNAs and DEmRNAs in regression analysis. Interesting, we found that the results uncovered a strong

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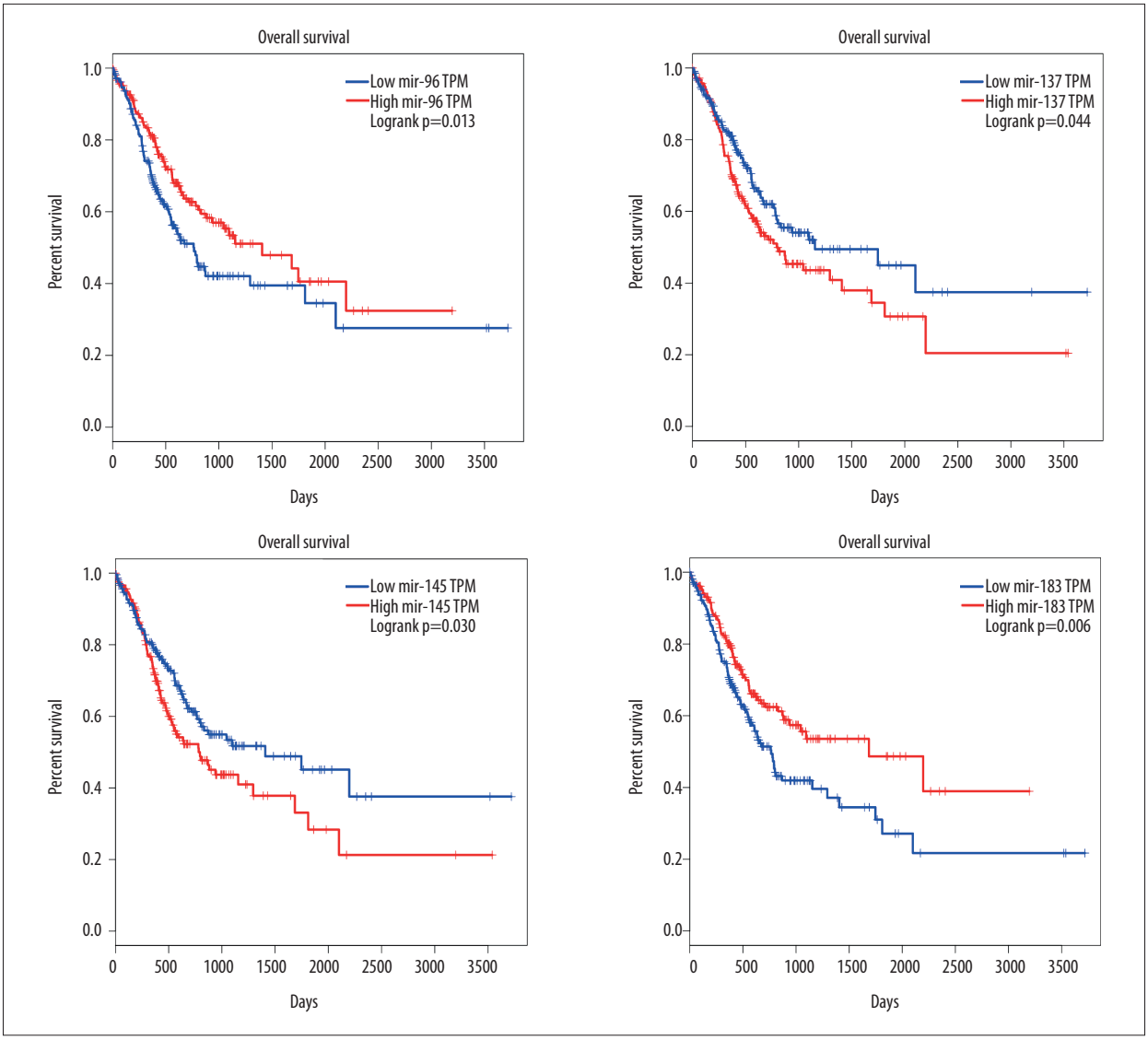

Figure 2. Kaplan-Meier survival curves for 4 miRNAs associated with overall survival in gastric cancer. (Seven DEmiRNAs were presented (P<0.05), including miR-96, miR-137, miR-145 and miR-183. Horizontal axis: overall survival time: days, Vertical axis: survival function).

positive correlation between the expression of DElncRNAs and DEmRNAs in the ceRNA network (Figure 5). It revealed that DElncRNAs may indirectly interact with mRNAs through miRNAs in gastric cancer. For instance, ADAMTS9-AS2 interacted with MEIS1, TCEAL7, ZEB1, and ILK, mediated through miR-96, miR-145, miR-182, and miR-204. Our findings suggest that DElncRNA ADAMTS9-AS2 may serve as key regulator and prognostic marker in gastric cancer.

Discussion

In recent years, many studies have shown that lncRNA has important biological functions by regulating gene expression at various levels, including epigenetic regulation, transcription regulation, and post-transcription regulation [24,25]. More and more studies have shown that lncRNA and miRNA play a significant role in the pathogenesis and progress of tumors. There is a complex regulatory network relationship between them. Different studies have revealed that the expression of aberrant lncRNAs presents an opportunity for different types of cancer diagnostics, prognostics, and therapeutics [26].

A CeRNA hypothesis was proposed for the mechanism of tumorigenesis, providing an important new clue for direction of research for tumor diagnosis and treatment, providing a new guiding theory [9]. Compared with protein-coding genes, lncRNAs have significant advantages as diagnostic and prognostic

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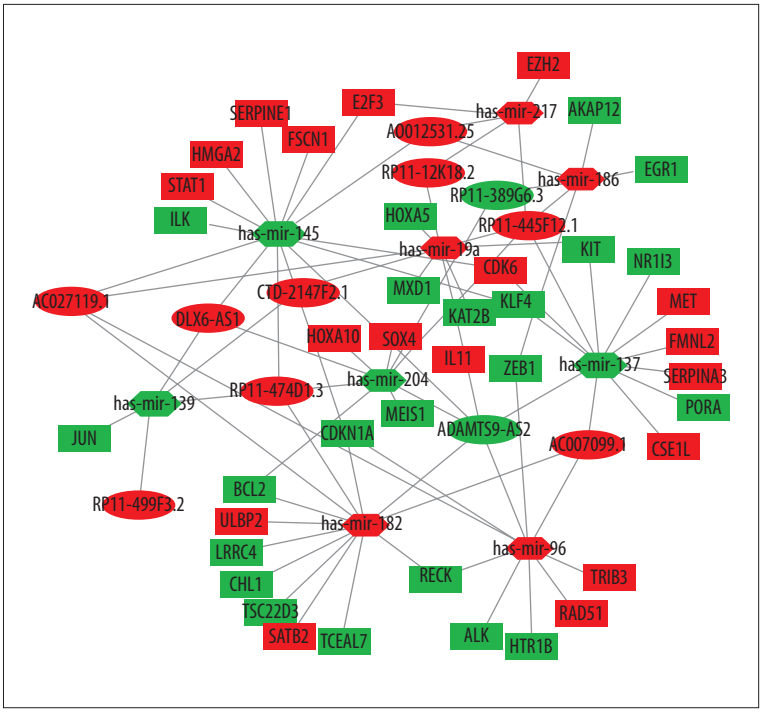

Figure 3. CeRNA network in gastric cancer. The red nodes represent increased level of expression, while the green nodes represent decreased level of expression. Round rectangles represent protein-coding genes; hexagon represent miRNAs; ellipse represent lncRNAs; gray edges indicate lncRNA-miRNA-mRNA interactions.

Table 4. Eleven DElncRNAs interact with the nine DEmiRNAs retrieving miRcode database.

lncRNA	miRNAs
ADAMTS9-AS2	miR-96, miR-137, miR-145, miR-182, miR-216b, miR-19a, miR-204
AC027119.1	miR-96, miR-145, miR-182, miR-19a
RP11-474D1.3	miR-139, miR-145, miR-182, miR-204
RP11-445F12.1	miR-137, miR-183, miR-217, miR-19a, miR-204
AC012531.25	miR-145, miR-183, miR-217
AC007099.1	miR-96, miR-137, miR-182
CTD-2147F2.1	miR-139,miR-19a
DLX6-AS1	miR-139, miR-145, miR-216b, miR-204
RP11-120K18.2	miR-217,miR-19a
RP11-389G6.3	miR-183,miR-216b,miR-204
RP11-499F3.2	miR-139

biomarkers [27]. Several studies have confirmed that the differential expression of lncRNAs is closely related to the pathogenesis and prognosis of tumors, and can be used as a tumor-associated predictors [28,29]. Ren et al. revealed that 5 gastric cancer-specific lncRNAs (CTD-2616J11.14, RP1-90G24.10, RP11-150O12.3, RP11-1149O23.2, and MLK7-AS1) were significantly associated with the overall survival of patients with gastric cancer. They confirmed that 5-lncRNA was an independent predictor of prognosis by multivariate Cox regression analysis [30]. Zhang et al. demonstrated that PTENP1 acts as a ceRNA and participates in carcinogenesis and progression of gastric cancer by sponge miR-106b and miR-93 from targeting PTEN [31]. In addition, Liu et al. found that lncRNA HOTAIR overexpression modulates the derepression of HER2 and promotes the proliferation, migration, and invasion of gastric cancer cells by competitively combining with miR-331-3p/miR-124 [32]. HOTAIR is considered as a novel target for HER2-positive patients with gastric cancer who have high metastatic potential and poor survival. Then, Lü et al. reported that downregulation of lncRNA BC032469 resulted in a significant inhibition of proliferation of gastric cancer cells by directly binding miR-1207-5p to modulate the derepression of hTERT [33]. Moreover, Peng

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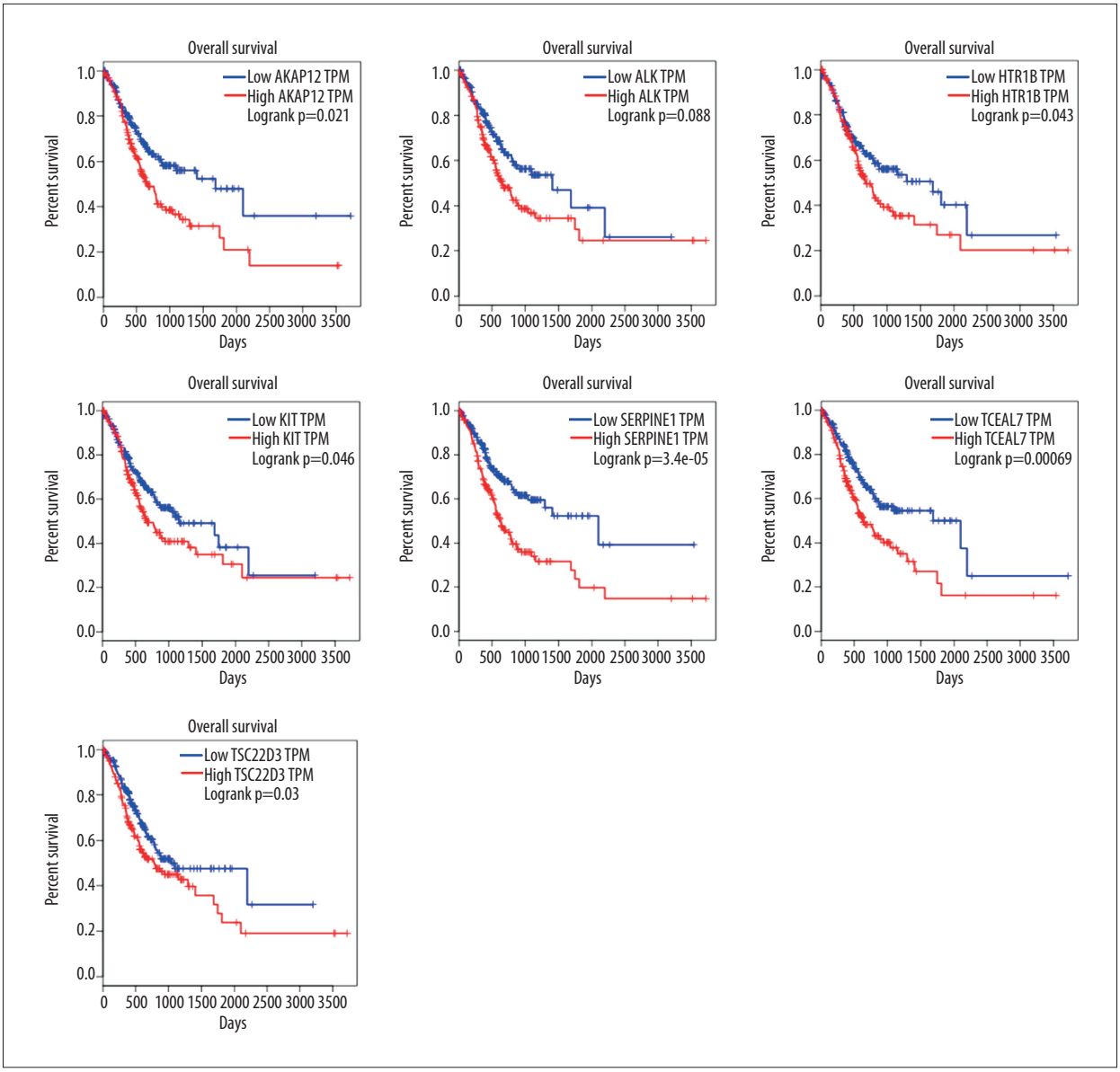

Figure 4. Kaplan-Meier survival curves for 7 protein-coding genes associated with overall survival in gastric cancer. (Seven DEmRNAs were presented (P<0.05), including AKAP12, ALK, HTR1B, KIT, SERPINE1, TCEAL7, and TSC22D3. Horizontal axis: overall survival time: days, Vertical axis: survival function).

et al. found that lncRNA MEG3 inhibits gastric cancer cell proliferation, invasion, and migration by competitively binding the miR-181 family, upregulating Bcl-2, and suppressing gastric carcinogenesis [34].

In our study, 11 DElncRNAs were identified in gastric cancer samples compared with the normal samples. We found that 7 of them were significantly associated with overall survival, could be considered as a prognostic marker for gastric cancer. Moreover, we noted that the lncRNA ADAMTS9-AS2 and DLX6-AS1 were included in the ceRNA network. Therefore, we think these lncRNAs may play an important role in the pathogenesis and prognosis of gastric cancer. ADAMTS9-AS2 is an antisense overlapping lncRNA to ADAMTS9 which is mostly located upstream from ADAMTS9, and is considered as a new tumor suppressor. Expression of ADAMTS9-AS2 was down-regulated, which has been experimentally confirmed to be associated with glioma and non-small cell lung cancer cells (NSCLC), and ADAMTS9-AS2 expression may be correlated with poor prognosis of NSCLC and glioma through interaction with DNMT1(DNA methyltransferase 1) [35,36].

In our research, expression of ADAMTS9-AS2 was down-regulated in 375 patients with gastric cancer compared to 32

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Table 5. Top 10 KEEG pathways enriched by the coding genes involved in ceRNA network.

Pathway ID	Description	P-value	Number of DERNAs
hsa05206	MicroRNAs in cancer	1.38E-14	11
hsa05202	Transcriptional misregulation in cancer	8.87E-10	7
hsa05200	Pathways in cancer	7.64E-09	8
hsa05161	Hepatitis B	4.94E-07	5
hsa05214	Glioma	7.98E-07	4
hsa05212	Pancreatic cancer	8.46E-07	4
hsa05218	Melanoma	1.12E-06	4
hsa04933	AGE-RAGE signaling pathway in diabetic complications	4.30E-06	4
hsa04066	HIF-1 signaling pathway	4.63E-06	4
hsa04630	Jak-STAT signaling pathway	2.38E-05	4

non-tumor tissues. Interesting, our analysis confirmed that decreased expression of ADAMTS9-AS2 was associated with good prognosis in patients with gastric cancer. Based on the above results, we think that lncRNA may compete with 3 key DEmiRNAs (miR-96 miR-145, and miR-182) to mediate the expression of TCEAL7, ZEB1, and ILK miRNAs target gene. We noted that these target genes were also significantly associated with overall survival. Then, we performed a regression analysis between the expression levels of ADAMTS9-AS2 and miRNAs-targeted genes TCEAL7, ZEB1, and ILK that were involved in the newly identified ceRNA network. The results revealed a very strong positive correlation between ADAMTS9-AS2 and TCEAL7, ZEB1, and ILK expression levels.

Furthermore, we found that DLX6-AS1 with high expression demonstrated a poor prognosis in patients with gastric cancer. Research has confirmed that the expression level of DLX6-AS1 was also up-regulated in lung adenocarcinoma tissues, and high expression levels of DLX6-AS1 were significantly associated with both histological differentiation and TNM stage [37].

Although lncRNA has received wide attention in recent years, miRNAs also warrant increased attention. There is no doubt that tumorigenic-related pathways of research based on regulation of miRNAs are indispensable. Disrupting those miRNAs may result in a permissive tumorigenic state. Dysregulated expression miRNAs in tumors is reported to play various roles in carcinogenesis. Studies found that miR-23b was overexpressed in gastric cancer patients compared with healthy controls and was associated with multiple clinical parameters, including T stage, distant metastasis, and differentiation [38].

In our study, we identified tumor initiation-related miRNAs in gastric cancer. In addition, we found that 4 of the miRNAs involved in ceRNA network are closely associated with survival in gastric cancer. Several studies have shown that an evolutionarily conserved miRNA cluster (miR-96, miR-182, and miR-183) was closely related to the occurrence and progress of gastric cancer, which are also considered as potential therapeutic targets [39,40]. Our survival analysis results indicated that the prognosis of gastric cancer patients with low expression of miR-96 and miR-183 is poor. Previous reports have revealed that miR-145 can inhibit invasion of gastric cancer cells by down-regulating cytoplasmic catenin delta-1 (CTNND1) expression and inducing the translocation of CTNND1 and E-cadherin [41]. Additionally, survival analysis demonstrated that low expression of mir-145 can prolong patient survival time. However, those findings need more research to identify whether those miRNAs have a specific role in tumorigenesis and prognosis of gastric cancer.

The KEGG pathway involved in ceRNA network analysis results showed that miRNAs-targeted genes were mainly enriched "microRNAs in cancer, transcriptional misregulation in cancer, and pathways in cancer" pathways. Seventeen of 40 miRNA-targeted genes were included in these 3 pathways and 3 of them (RECK, ZEB1, and KIT) were associated with overall survival. Several studies have shown that RECK and ZEB1 play an important role in the pathogenesis of gastric cancer [42,43].Our study revealed how specific lncRNAs interact with miRNAs and coding genes through the successful construction of lncRNA – miRNAs – mRNA ceRNA network in gastric cancer.

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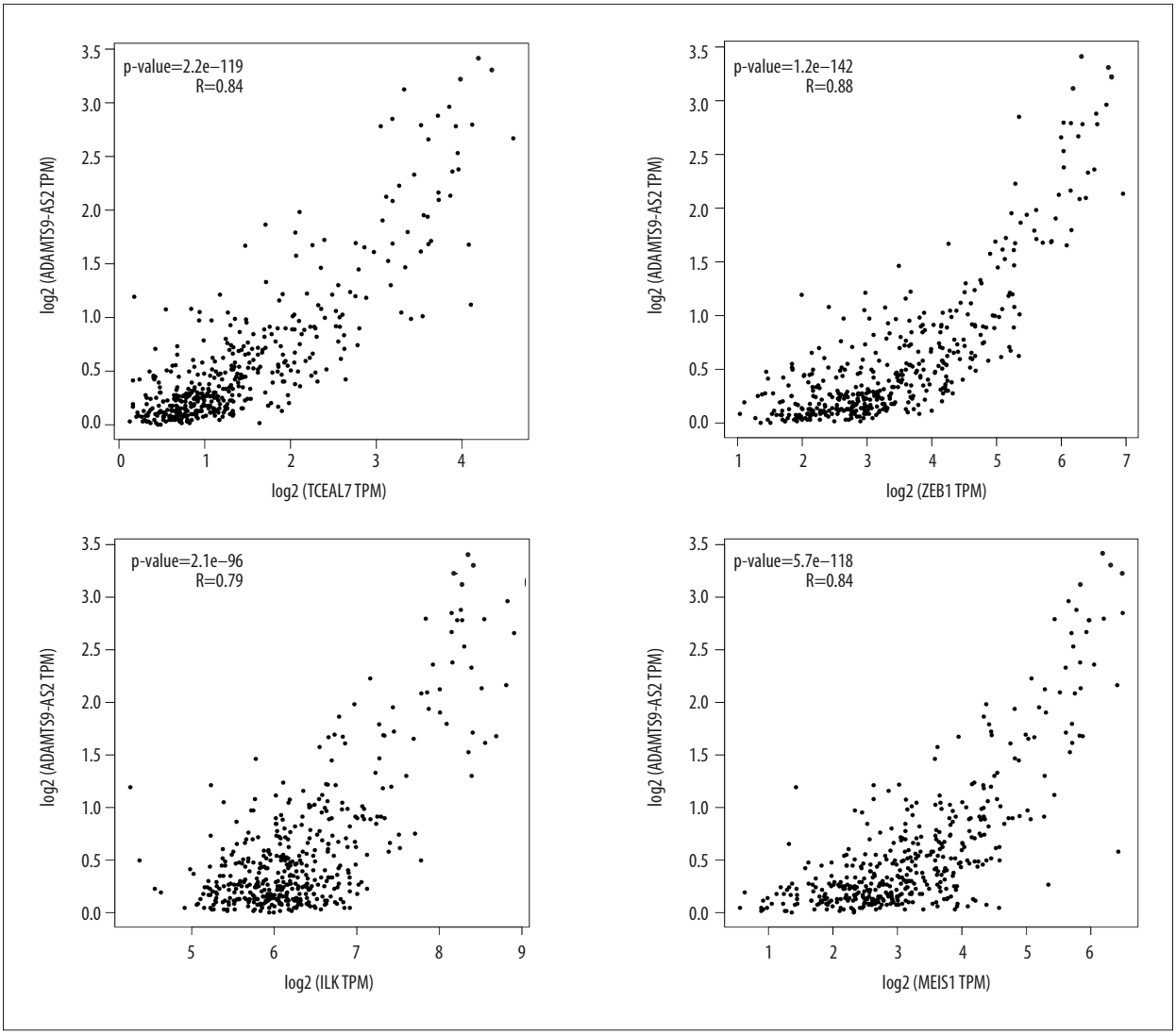

Figure 5. Regression analysis between the expression levels of DElncRNA and DERNAs in ceRNA network. (R: correlation coefficient.)

Conclusions

In conclusion, 11 cancer-specific lncRNAs were identified from hundreds of candidate lncRNAs in large-scale gastric cancer samples. The research indicates that dysregulation of the ceRNA network can lead to tumorigenesis [44]. We found that some lncRNA were remarkably associated with overall survival in patient with gastric cancer. Importantly, we have successfully constructed a lncRNA-associated ceRNA network, which brings to light an unknown ceRNA regulatory network in gastric cancer. Our study will contribute to increased understanding of the pathogenesis of gastric cancer and provide novel lncRNAs as candidate prognosis biomarkers or potential therapeutic targets.

Conflicts of interest

None.

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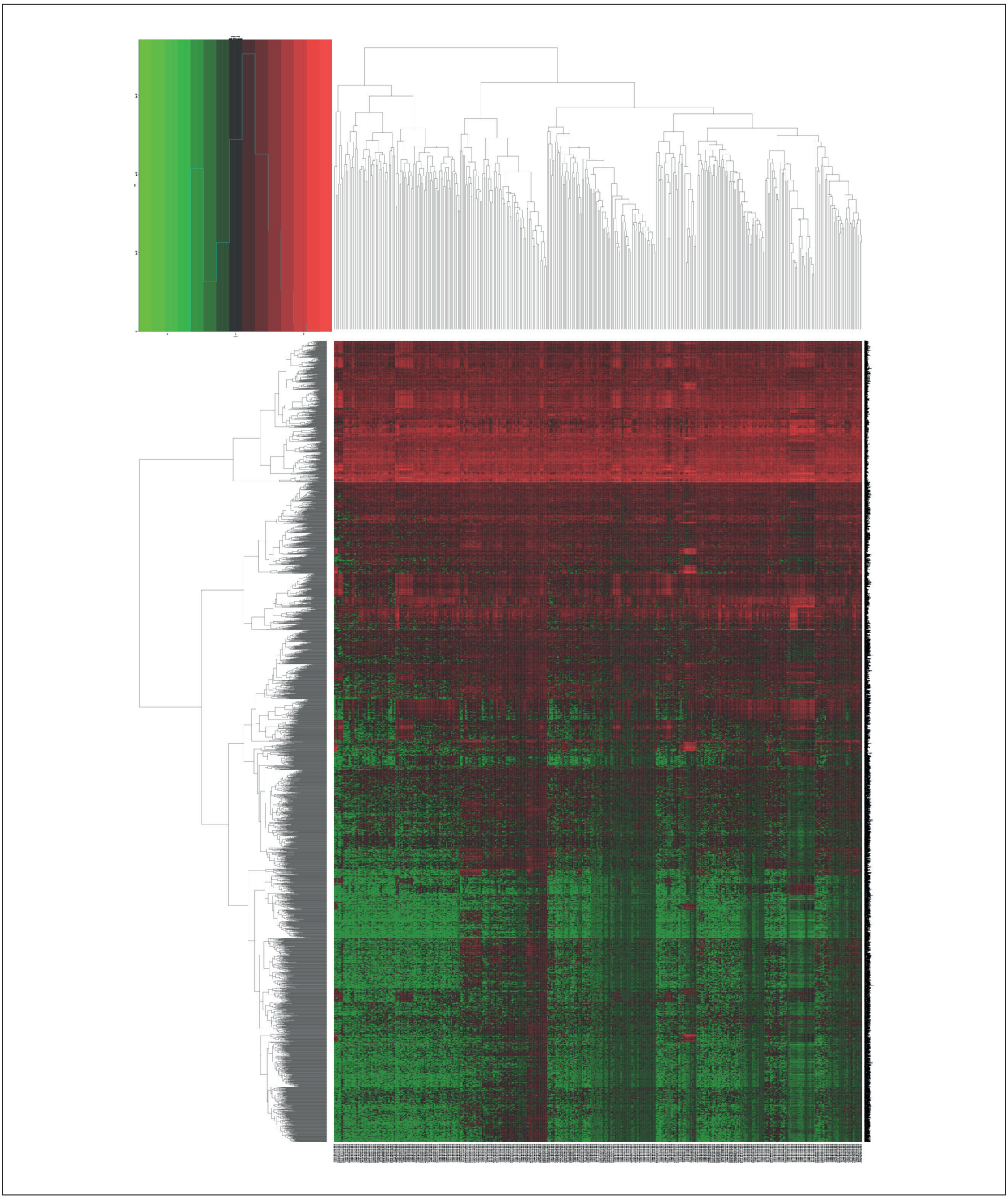
Supplementary Figures



Supplementary Figure 1. Hierarchical cluster dendrogram of DEmRNAs. The horizontal axis represents sample names and the above horizontal axis shows clusters of samples. The left vertical axis shows clusters of DEmRNAs and right vertical axis represents mRNA names. Red represents up-regulated genes and green represents down-regulated genes.

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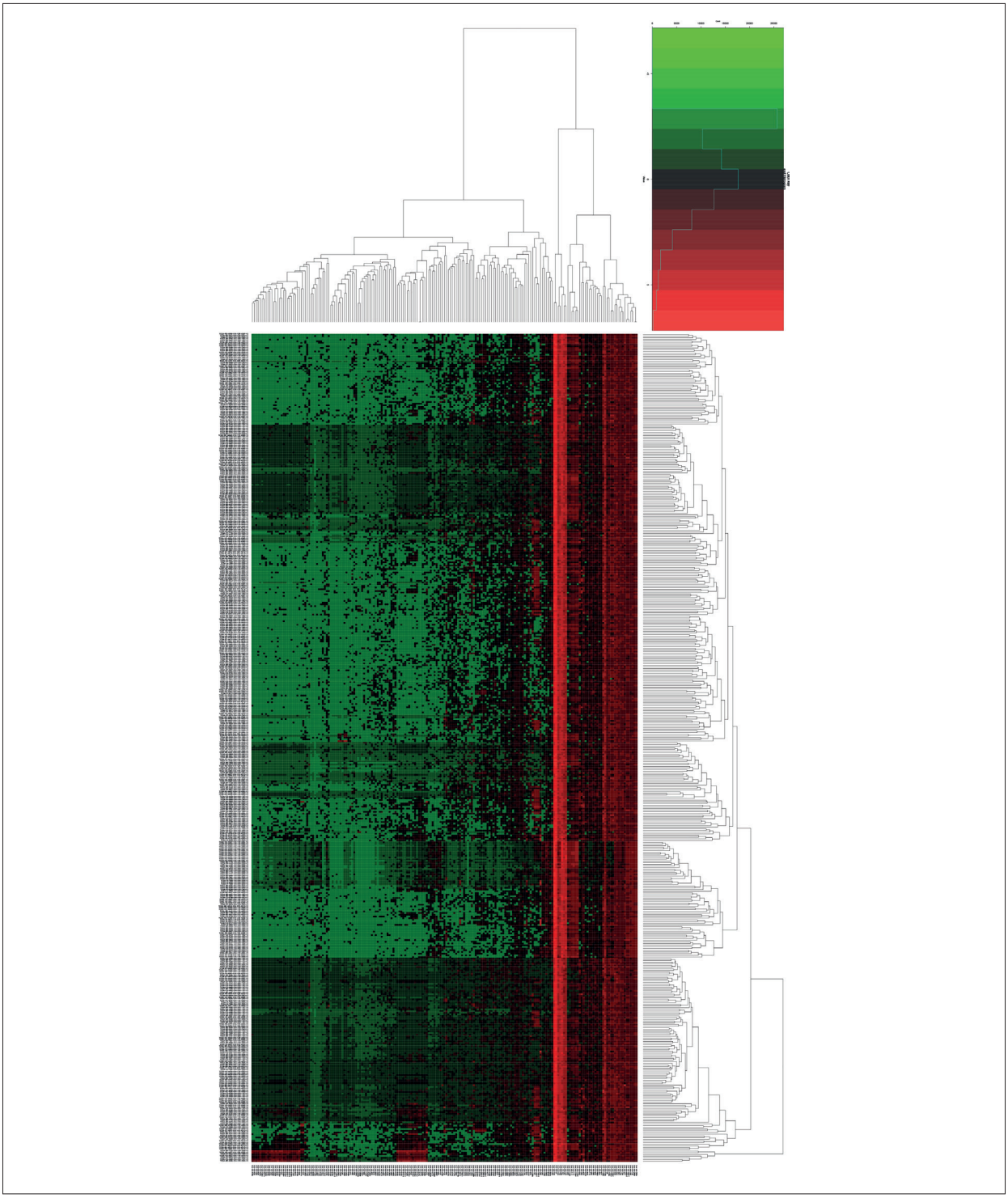

Supplementary Figure 2. Hierarchical cluster dendrogram of DEmiRNAs. The horizontal axis represents sample names and the above horizontal axis shows clusters of samples. The left vertical axis shows clusters of DEmiRNAs and right vertical axis represents miRNA names. Red represents up-regulated miRNAs and green represents down-regulated miRNAs.

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