Construction and investigation of lncRNA-associated ceRNA regulatory network in papillary thyroid cancer

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Abstract. Increasing evidence has experimentally proved the competitive endogenous RNA (ceRNA) hypothesis that long non-coding RNA (lncRNA) can affect the expression of RNA targets by competitively combining microRNA (miRNA) via miRNA response elements. However, an extensive ceRNA network of thyroid carcinoma in a large cohort has not been evaluated. We analyzed the RNAseq and miRNAseq data of 348 cases of primary papillary thyroid cancer (PTC) patients with clinical information downloaded from The Cancer Genome Atlas (TCGA) project to search for potential biomarkers or therapeutic targets. A computational approach was applied to build an lncRNA-miRNA-mRNA regulatory network of PTC. In total, 780 lncRNAs were detected as collectively dysregulated lncRNAs in all 3 PTC variants compared with normal tissues (fold change >2 and false discovery rate <0.05). The interactions among 45 lncRNAs, 13 miRNAs and 86 mRNAs constituted a ceRNA network of PTC. Nine out of the 45 aberrantly expressed lncRNAs were related to the clinical features of PTC patients. However, the expression levels of 3 lncRNAs (LINC00284, RBMS3-AS1 and ZFX-AS1) were identified to be tightly correlated with the patients overall survival (log-rank, P<0.05). The present study identified a list of specific lncRNAs associated with PTC progression and prognosis. This complex ceRNA interaction network in PTC may provide guidance for better understanding the molecular mechanisms underlying PTC.

Introduction

Thyroid cancer (TC) is the major endocrine malignancy with estimated 298,000 new cases and 40,000 deaths worldwide in 2012 (1). Thyroid carcinoma is the fastest-growing cancer with the increasing morbidity rate >5% due to the advanced diagnostic techniques as well as multiple risk factors. Although the 5-year survival rate for patients with thyroid carcinoma is relatively higher than other malignant neoplasms, the mortality rate of TC has remained ever-increasing over the past decade (2,3). Thyroid cancer is a heterogeneous disease of 3 histological subtypes: papillary TC (PTC), follicular TC (FTC) and anaplastic TC (ATC) (4). As the most primary malignant thyroid tumor, PTC accounts for up to 80% of total cases. Although PTC is generally effectively treated with thyroidectomy and hormone therapy, large numbers of unresponsive patients suffer from progressive disease and metastases (5). Thus, it is urgent to explore the molecular mechanisms underlying PTC for developing effective diagnostic and therapeutic targets.

Non-coding RNAs (ncRNAs) are generally defined as a wide class of RNAs without protein-coding function, but with universal expression in organisms. ncRNAs are loosely split into small ncRNAs and long non-coding RNAs (lncRNAs), both of which have regulatory functions in the various biological processes (6). The well-documented microRNAs (miRNAs; ~22 nucleotides long) belong to small ncRNAs and are regarded as vital regulators of cellular gene expression network (7). lncRNAs are transcripts >200 nucleotides with tissue-specific expression, but neither has significant open reading frames nor translate into proteins (8). Accumulating studies over the past decades have altered the understanding of lncRNA from 'dark matter' to important transcriptional and post-transcriptional regulators. Abnormally expressed lncRNAs participate in tumorigenesis by interrupting biological processes of both oncogenic and tumor suppressive pathways (9). In 2011, Pandolfi *et al* put forward the competitive

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endogenous RNA (ceRNA) hypothesis that specific RNAs (including lncRNA, mRNA, pseudogene and circular RNA) can impair miRNA activity by sponging miRNA via common miRNA response elements (MREs) and upregulate target RNA expression subsequently (10). This hypothesis introduced a new RNA-RNA crosstalk theory that can generally regulate gene expression. In addition, the promising area of disclosing the landscape of the ceRNA mechanism has evoked much interest. Therefore, the lncRNA-miRNA-mRNA networks that may lead to tumor development and progression have already been constructed in many malignancies, including gastric cancer (11), hepatocellular carcinoma (12), glioblastoma (13) and lung adenocarcinoma (14).

Recent studies have preliminarily revealed several ceRNA regulatory interactions and corresponding mechanisms in thyroid carcinoma. lncRNA H19 was certified to act as ceRNA by sponging miR-17-5p to upregulate the target gene YES1, demonstrating a potential ceRNA crosstalk including H19, miR-17-5p, and YES1 in thyroid cancer pathogenesis (15). A bioinformatic ceRNA analysis revealed that stem cell factor SOX2 could be functionally co-regulated with other genes by crosstalk mediated by several miRNAs in anaplastic thyroid carcinoma (16). Furthermore, both a ceRNA network built by microarray analysis of 5-paired clinical samples and a protein-coding modulatory network that regulated immune responses have been reported in PTC (17,18). However, a whole genome-wide analysis of the lncRNA-miRNA-mRNA regulatory network of papillary thyroid cancer with large-scale sample size is still lacking.

In the present study, we comprehensively investigated both RNA and miRNA sequencing data of 348 PTC primary tumor tissues and 58 non-cancerous thyroid samples from The Cancer Genome Atlas (TCGA) data matrix to obtain the aberrantly expressed lncRNAs, miRNAs, and mRNAs. Next, an lncRNA-associated ceRNA network of papillary thyroid cancer was built based on ceRNA theory. The expression levels of the important lncRNAs involved in ceRNA crosstalk were also assessed for their prognostic values in patients with papillary thyroid cancer.

Materials and methods

Date collection. PTC patients (502) were obtained from the TCGA consortium. Inclusion criteria included: i) patients diagnosed with PTC but with no other malignancies; ii) patients with complete clinical data, including age, sex, race, the American Joint Committee on Cancer (AJCC) TNM and pathologic stage; iii) patients with overall survival time <2,000 days; and iv) patients with complete lncRNA, mRNA and miRNA expression profiles. In total, 348 patients (cohort T) who were pathologically diagnosed as PTC and 58 normal samples (cohort N) were enrolled in downstream analysis. The RNAseq and miRNAseq data (level 3) generated from IlluminaHiseq_RNASeq and IlluminaHiseq_miRNASeq sequencing platform was downloaded from TCGA data portal. The present study followed the publication guidelines of TCGA Research Network (http://cancergenome.nih.gov/publications/publicationguidelines). Thus, no further ethical approvals were required.

Analysis of differentially expressed genes. Differential expression analysis was carried out to identify differentially expressed lncRNAs, mRNAs and miRNAs (DElncRNAs, DEmRNAs, DEmiRNAs) between cohort T and N, by the R/Bioconductor package of edgeR (19) with the cut-off value of |log$_2$FC|>1 and FDR <0.05 (FC, fold change; FDR, false discovery rate).

CeRNA network construction. lncRNA-miRNA interactions were predicted by miRcode (http://www.mircode.org/) (20). TargetScan (http://www.TargetScan.org/mamm_31/), miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/) and miRanda (http://www.microrna.org/microrna/home.do) were cooperatively utilized to predict the mRNAs targeted by miRNAs (21,22). According to the above lncRNA/miRNA and miRNA/mRNA interactions, the Cytoscape (version 3.5.1) was utilized to build and visualize the lncRNA-miRNA-mRNA network (23).

Functional annotation. The Database for Annotation Visualization and Integrated Discovery (DAVID) online tool (https://david.ncifcrf.gov/) was used to conduct the functional and pathway enrichment analyses. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to detect the potential biological functions and pathways of the DEmRNAs involved in the network (P-value <0.05).

Statistical analysis. Unpaired t-test was applied to identify differentially expressed genes between cohort T and N, and to find out DElncRNAs between different pathological subgroups of cohort T. The univariate Cox proportional hazards regression was used to estimate the expression of DElncRNAs in ceRNA network with overall survival. Kaplan-Meier survival analysis was used to analyze the correlation between lncRNA expression and PTC patient prognosis.

Results

Patient characteristics. The clinical and pathological information of patients in cohort T was summarized in Table I. The median age of all patients in cohort T was ~48 years. The distributions of sex and race showed that white female patients accounted for the majority in the male/female ratio of 2.8/1 and white race ratio of 80.2%. The 3 histopathological variants, classical PTC (CPTC), follicular PTC (FPTC) and tall-cell PTC (TCPTC) represented 75.9, 14.3 and 9.8%, respectively. The proportions and distributions of these patients clinical characteristics were consistent with previous studies (24,25).

Differentially expressed lncRNA, mRNA and miRNA. Considering the dissimilarity among the 3 PTC variants, RNA expression levels in the 3 PTC variants (cohort T) vs. normal tissues (cohort N) were investigated, respectively. Genes with an absolute fold change >2 and FDR value <0.05 were considered as discriminatively expressed. As a result, a total of 780 lncRNAs and 1,700 mRNAs were detected to be collectively dysregulated in carcinoma tissues of 3 PTC variants compared with normal tissues. Among them, 440 and 340 lncRNAs were commonly elevated and

Table I. Demographic and clinical characteristics of 348 patients with papillary thyroid cancer in cohort T.

Parameter/feature	Cohort T (n=348) (%)
Age, years (mean ± SD)	48.1±15.9
Sex	
Male	92 (26.4)
Female	256 (73.6)
Race	
Asian	46 (13.2)
Black or African American	22 (6.3)
American Indian or	1 (0.3)
Alaska native	
White	279 (80.2)
Subtype	
CPTC	264 (75.9)
FPTC	50 (14.3)
TCPTC	34 (9.8)
Pathologic stage	
I	192 (55.2)
II	28 (8.0)
III	88 (25.3)
IV	40 (11.5)
Tumor size	
T1	105 (30.2)
T2	108 (31.0)
T3	116 (33.3)
T4	17 (4.9)
TX	2 (0.6)
Lymph node	
N0	164 (47.2)
N1	157 (45.2)
NX	27 (7.6)
Metastasis status	
M0	231 (66.4)
M1	6 (1.7)
MX	111 (31.9)

SD, standard deviation; PTC, papillary thyroid cancer; CPTC, classical PTC; FPTC, follicular PTC; TCPTC, tall-cell PTC.

downregulated, meanwhile, 973 and 727 mRNAs were collectively upregulated and downregulated, respectively. Filtering analysis with the same criteria ($|log_2FC|>1$ and FDR <0.05) also identified 43 miRNAs differentially expressed between 3 PTC variants and normal thyroid tissues, among them 27 upregulated and 16 downregulated (Fig. 1) (data not shown). To visualize the most significant lncRNAs, we selected the top 50 upregulated and top 50 downregulated lncRNAs to construct expression heatmaps (Fig. 2) (data not shown). The data suggested that the expression profiles of DElncRNAs can distinguish PTC tissues from normal tissues.
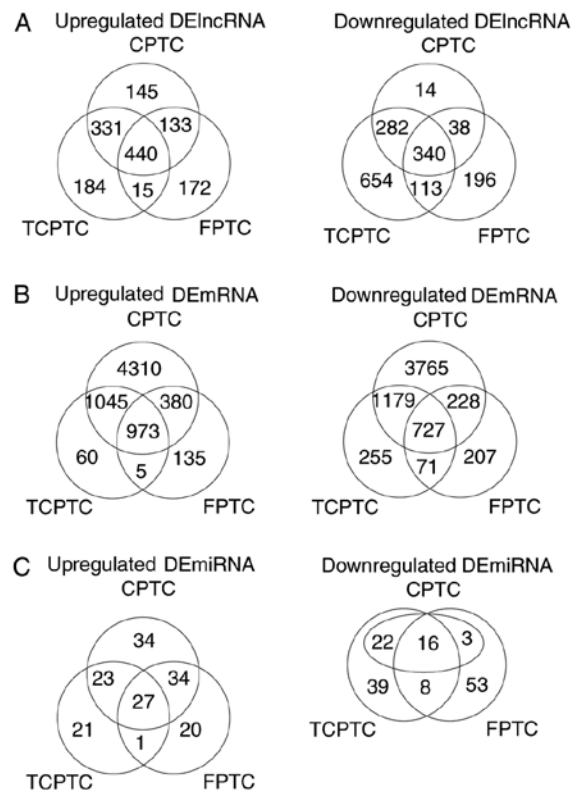

Figure 1. Venn analysis of differentially expressed (DE) lncRNA (A), mRNA (B), and miRNA (C) in 3 PTC variants (CPTC, FPTC and TCPTC), compared with normal tissues.

ceRNA network in PTC. In the next step, we predicted the potential interactions among the above collectively dysregulated genes based on ceRNA hypothesis by computational analysis to further understand the function of DElncRNAs. Thirteen specific DEmiRNAs were identified to interact with 45 DElncRNAs through miRNA response elements by miRcode online tools (Table II). To improve the predictive accuracy, TargetScan, miRTarBase and miRanda databases were combined to predict the candidate mRNA targets of the 11 DEmiRNAs. After excluding the mRNAs that were not involved in the 1,700 DEmRNAs, 86 mRNAs were enrolled to create the ceRNA network (Table III). Finally, the ceRNA network that involved 45 lncRNAs, 13 miRNAs and 86 mRNAs was visualized using Cytoscape software based on the interactions among lncRNAs, miRNAs and mRNAs in Tables II and III (Fig. 3).

Functional analysis of DEmRNAs in the ceRNA network. The function analysis revealed that the 86 DEmRNAs in the above ceRNA network were enriched in 60 GO biological process categories and 7 KEGG categories (P<0.05). The significant GO biological processes of dysregulated genes were 3 cell adhesion terms (GO:0007155, GO:0007160 and GO:007156) (Fig. 4). Table IV contains the significantly enriched pathways of these DEmRNAs by KEGG analysis. There were two cancer-related pathways, including pathways in cancer and miRNAs in cancer. Notably, transcriptional factor E2F1 had an elevated expression in PTC tissues and was involved as the key component in both cancer-related pathways.
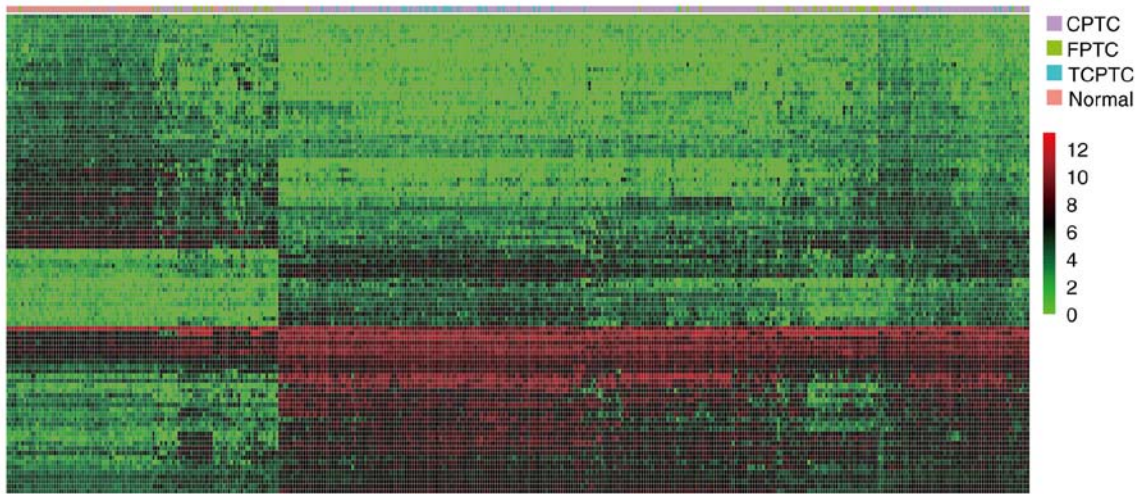
Figure 2. Heatmap of the top 50 collectively upregulated and top 50 collectively downregulated DElncRNAs in 3 PTC variants, compared with normal tissues. Each column represents one sample, and each row indicates one lncRNA. Purple, green, blue and red stripes represent CPTC, FPTC, TCPTC and non-tumor samples, respectively. Ascending normalized expression level is colored from green to red.
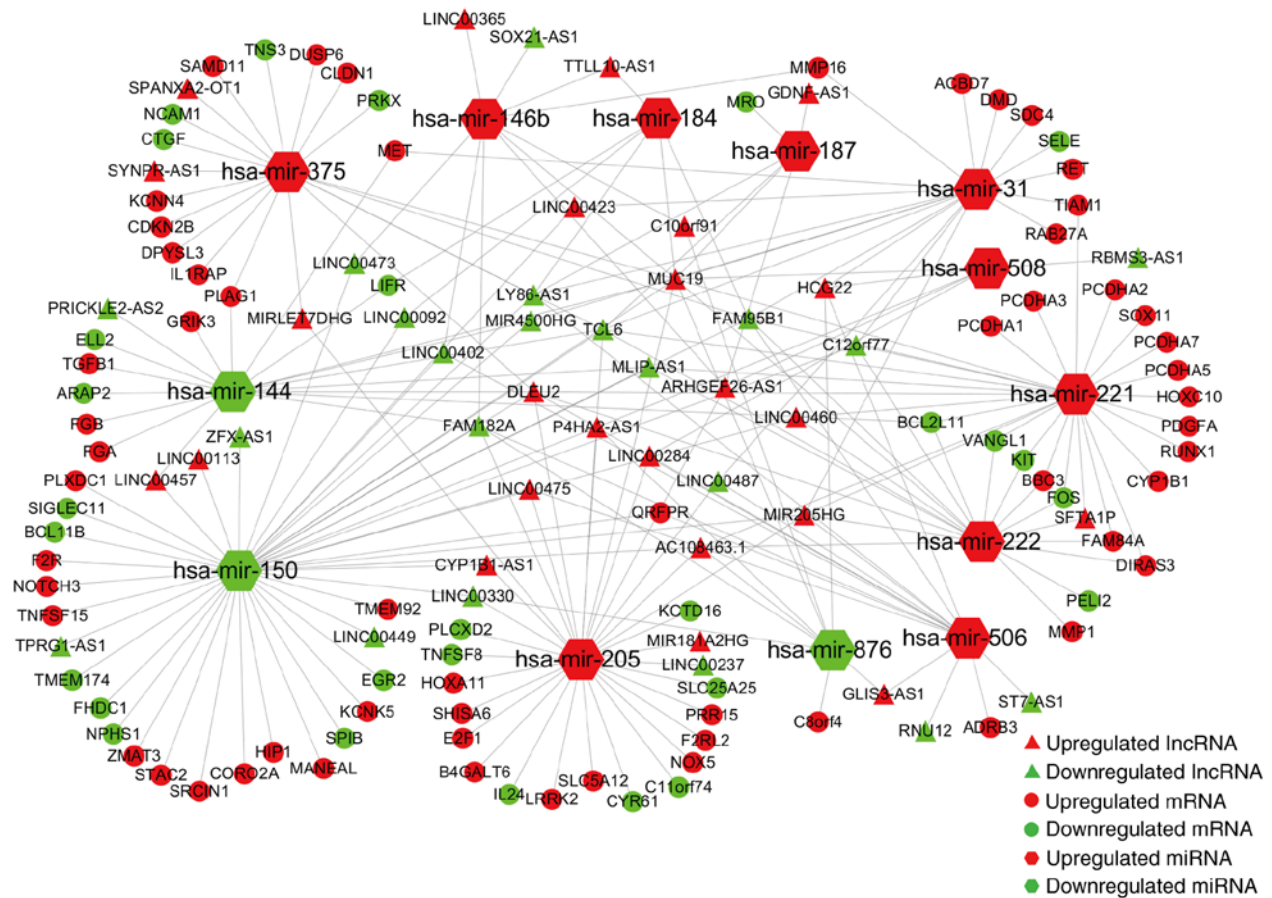

Figure 3. The lncRNA-miRNA-mRNA ceRNA network of PTC. Different colors represent expression levels and different shapes represent different RNA types.

lncRNAs in relation to clinical outcome. To further understand whether dysregulated lncRNAs were correlated with patient outcome, the 45 DElncRNAs involved in the network were analyzed according to clinical characteristics including sex, race, TNM staging system and pathologic stage. The expression levels of 9 lncRNAs were aberrantly expressed in clinical feature comparisons of cohort T ($|log_2FC|>1$ and FDR<0.05) (Table V). Among them, we identified 3 lncRNAs (FAM182A, LINC00402 and LINC00473) differentially expressed in race subgroup. Four upregulated lncRNAs (MIR205HG, C12orf7, TCL6 and LINC00460) and 4 downregulated lncRNAs (GDNF-AS1, LINC00402,

Table II. Putative miRNAs that may target lncRNAs by miRNA response elements.

lncRNA	miRNAs	lncRNA	miRNAs
AC108463.1	miR-221, miR-222, miR-876, miR-150, miR-205	LINC00475	miR-506, miR-150, miR-205
ARHGEF26-AS1	miR-146b, miR-187, miR-508, miR-205	LINC00487	miR-31, miR-506, miR-205
C10orf91	miR-146b, miR-876	LY86-AS1	miR-375, miR-31, miR-187, miR-506, miR-150, miR-184
C12orf77	miR-221, miR-222, miR-31, miR-150	MIR181A2HG	miR-205
CYP1B1-AS1	miR-150, miR-205	MIR205HG	miR-221, miR-222, miR-31, miR-506, miR-150, miR-205
DLEU2	miR-221, miR-222, miR-144, miR-375, miR-506, miR-150, miR-205	MIR4500HG	miR-144, miR-31, miR-150
FAM182A	miR-146b, miR-144, miR-876, miR-506, miR-150, miR-205	MIRLET7DHG	miR-375, miR-205
FAM95B1	miR-221, miR-222, miR-375, miR-31, miR-506, miR-150, miR-184	MLIP-AS1	miR-221, miR-222, miR-144, miR-150
GDNF-AS1	miR-187	MUC19	miR-146b, miR-221, miR-222, miR-144, miR-375, miR-31, miR-187, miR-508, miR-876, miR-150, miR-205, miR-184
GLIS3-AS1	miR-876, miR-506	P4HA2-AS1	miR-508, miR-876, miR-150, miR-205
HCG22	miR-31, miR-508, miR-876, miR-506	PRICKLE2-AS2	miR-144
LINC00092	miR-150, miR-184	RBMS3-AS1	miR-508
LINC00113	miR-150	RNU12	miR-506
LINC00237	miR-205	SFTA1P	miR-221, miR-222
LINC00284	miR-508, miR-506, miR-205	SOX21-AS1	miR-146b
LINC00330	miR-876, miR-150, miR-205	SPANXA2-OT1	miR-375
LINC00365	miR-146b	ST7-AS1	miR-506
LINC00402	miR-146b, miR-506, miR-150	SYNPR-AS1	miR-375
LINC00423	miR-31, miR-150	TCL6	miR-221, miR-222, miR-144, miR-375, miR-31, miR-187, miR-150, miR-205
LINC00449	miR-150	TPRG1-AS1	miR-150
LINC00457	miR-144, miR-150	TTLL10-AS1	miR-146b, miR-184
LINC00460	miR-221, miR-222, miR-150	ZFX-AS1	miR-150
LINC00473	miR-146b, miR-150		

Table III. miRNAs targeting mRNAs in papillary thyroid carcinoma.

miRNA	mRNAs targeted by miRNA
miR-144	FGA, ARAP2, PLAG1, FGB, MET, TGFB1, LIFR, BCL2L11, ELL2, GRIK3
miR-146b	MMP16
miR-150	BCL11B, STAC2, CORO2A, TMEM174, SPIB, F2R, PLXDC1, KCNK5, HIP1, NOTCH3, FHDC1, SRCIN1, TNFSF15, TMEM92, EGR2, NPHS1, MANEAL, QRFPR, ZMAT3, SIGLEC11
miR-184	LIFR
miR-187	MRO
miR-205	NOX5, TNFSF8, KCTD16, CYR61, SLC25A25, PLCXD2, SLC5A12, C11orf74, F2RL2, LRRK2, E2F1, HOXA11, IL24, B4GALT6, PRR15, SHISA6
miR-221	PCDHA2, SOX11, PCDHA1, FOS, PCDHA3, PCDHA7, FAM84A, RUNX1, PDGFA, KIT, PCDHA5, TIAM1, DIRAS3, BBC3, BCL2L11, VANGL1, HOXC10, CYP1B1
miR-222	BBC3, DIRAS3, FOS, VANGL1, KIT, PELI2, FAM84A, MMP1, BCL2L11
miR-31	DMD, RET, ACBD7, SELE, MMP16, RAB27A, TIAM1, MET, SDC4
miR-375	KCNN4, CTGF, DUSP6, TNS3, IL1RAP, PLAG1, DPYSL3, NCAM1, PRKX, SAMD11, CLDN1, CDKN2B
miR-506	ADRB3, QRFPR
miR-876	C8orf4

Table IV. KEGG pathways enriched by DEmRNA involved in the ceRNA network.

Pathway ID	Description	P-value	Numbers of DEmRNAs
hsa05200	Pathways in cancer	8.99E-05	E2F1, FOS, RET, CDKN2B, PDGFA, MET, KIT, RUNX1, TGFB1, MMP1, F2R
hsa04060	Cytokine-cytokine receptor interaction	4.12E-04	PDGFA, IL1RAP, MET, TNFSF15, LIFR, KIT, TGFB1, TNFSF8
hsa04144	Endocytosis	0.0043302	ADRB3, RET, MET, KIT, ARAP2, TGFB1, F2R
hsa05206	MicroRNAs in cancer	0.0070069	NOTCH3, E2F1, CYP1B1, PDGFA, MET, MMP16, BCL2L11
hsa05166	HTLV-I infection	0.018641	E2F1, FOS, EGR2, CDKN2B, PDGFA, TGFB1
hsa05144	Malaria	0.0354242	MET, SELE, TGFB1
hsa04015	Rap1 signaling pathway	0.0377107	TIAM1, PDGFA, MET, KIT, F2R

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table V. The correlation between lncRNA in the ceRNA network and patient characteristics.

Comparisons	Downregulated	Upregulated
Race (Asian vs. White)		FAM182A, LINC00402 LINC00473
Tumor size (T3+T4 vs. T1+T2)	GDNF-AS1, LINC00402, LINC00473	
Lymph node (N1 vs. N0)	LINC00237	MIR205HG, C12orf77, TCL6
Pathologic stage (Stage III+IV vs. stage I+II)	LINC00473	LINC00460

LINC00473 and LINC00237) were markedly related to tumor progression. LINC00473 may not only inhibit tumor growth (T3+T4 vs. T1+T2), but also decrease in individuals with poor pathologic stage, suggesting its potential negative role in tumor
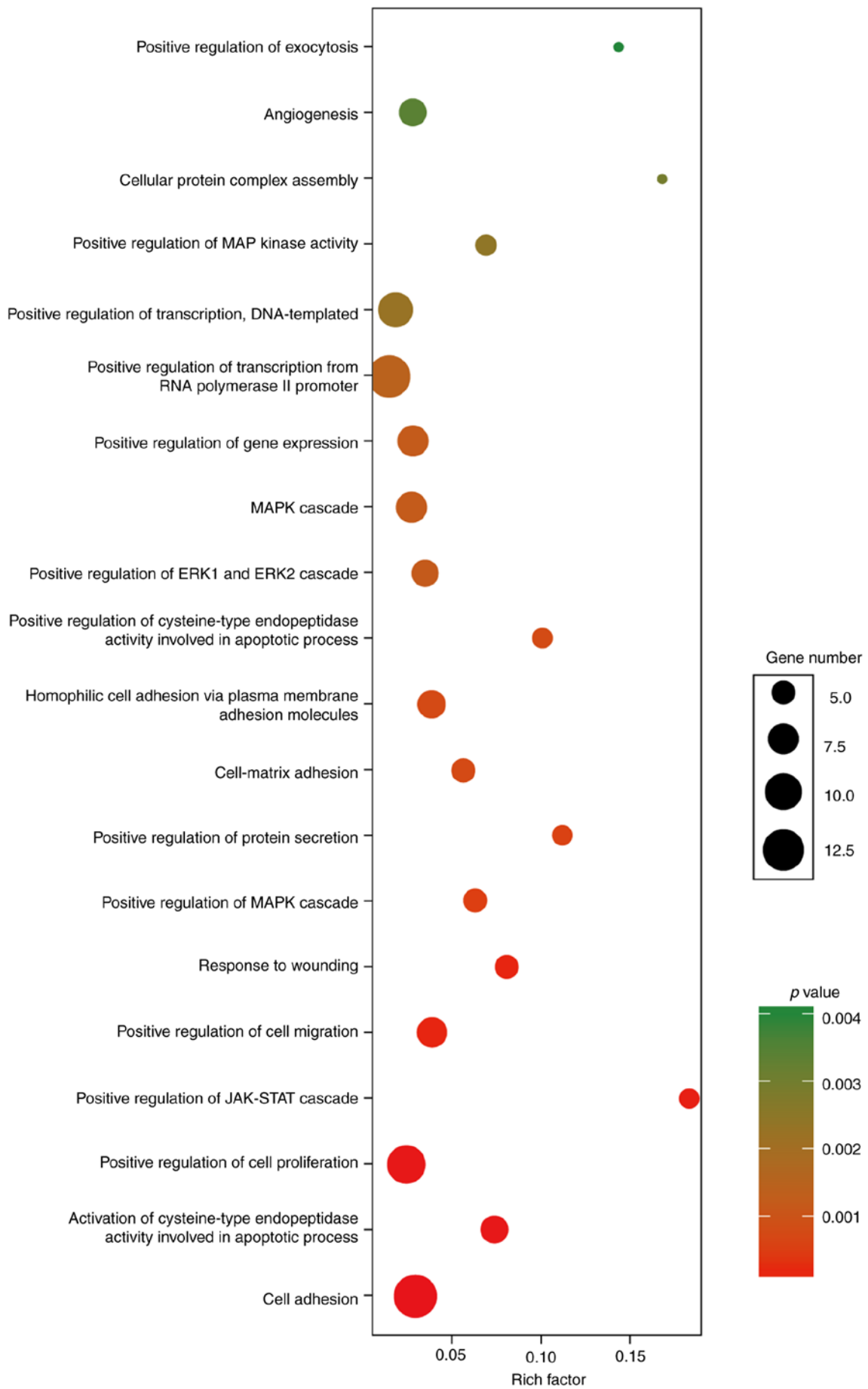
Figure 4. Top 20 enrichment Gene Ontology (GO) biological process terms of DEmRNAs involved in the ceRNA network. The size of balls represents gene number and color represents P-value.
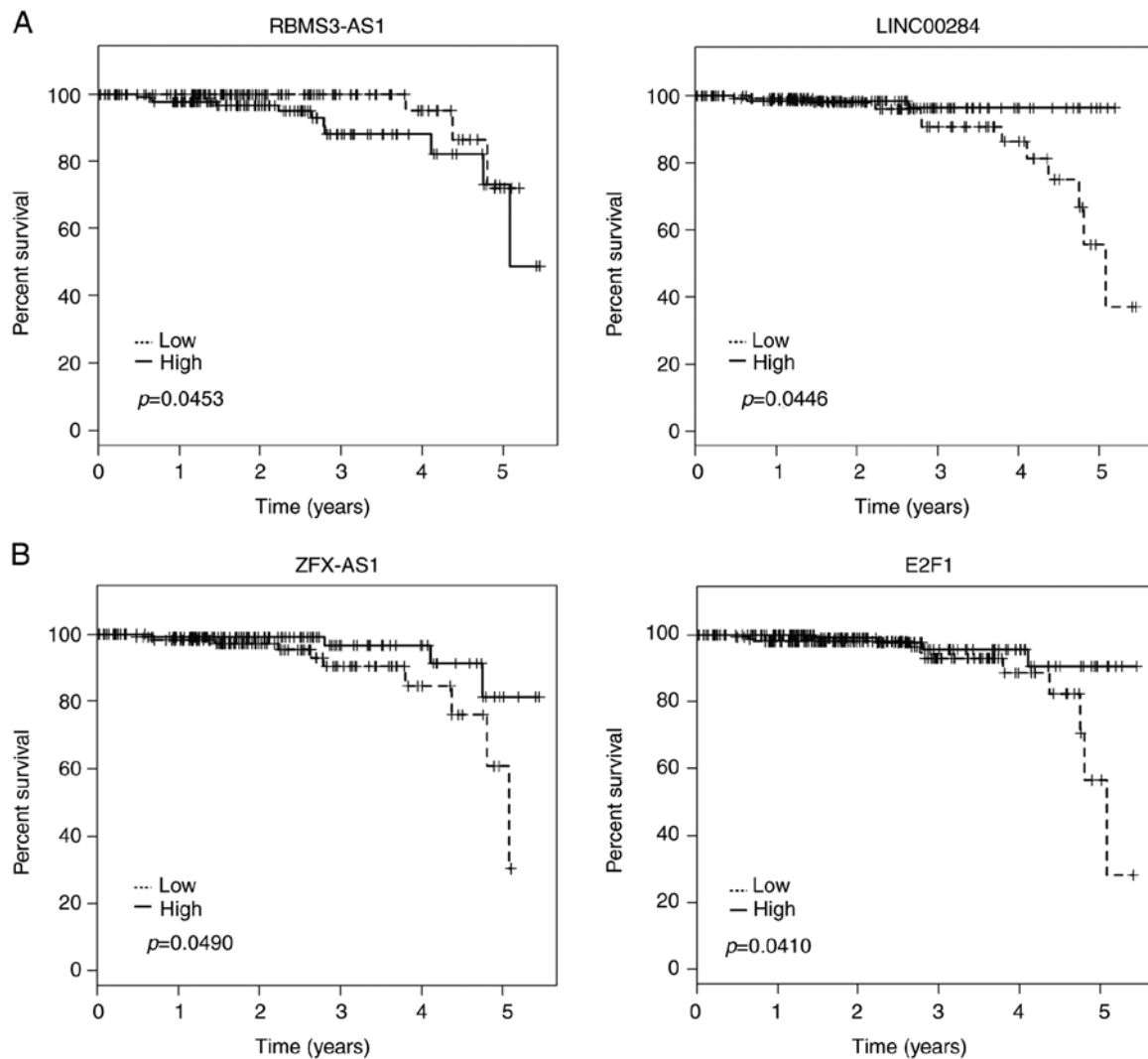
Figure 5. Kaplan-Meier curves for (A) 3 DElncRNAs and (B) one mRNA associated with overall survival. Horizontal axis, overall survival time (years); Vertical axis, survival function. Tumor samples were divided into 'high' group (solid lines, ≥median) and 'low' group (dotted lines, <median) according to expression levels.

development of PTC. Overexpression lncRNA LINC00460 was identified to be correlated with the poor pathologic stage.

Kaplan-Meier analysis was applied to investigate overall survival for DElncRNAs in cohort T. Among the 45 DElncRNAs in the ceRNA network, 3 lncRNAs (LINC00284, RBMS3-AS1 and ZFX-AS1) were identified to be associated with overall survival by univariate Cox regression analysis (log-rank, P<0.05). Kaplan-Meier plots indicated that individuals with relatively higher RBMS3-AS1 expression tended to have shorter overall survival time. On the contrary, LINC00284 and ZFX-AS1 could prolong patient survival time (Fig. 5A). Kaplan-Meier survival analysis was also assessed to estimate overall survival for the potentially associated DEmRNAs of these lncRNAs. Notably, high expression of E2F1 was also predicted to be positively associated with patient survival time (Fig. 5B).

Discussion

PTC is the most frequent endocrine neoplasia characterized by a variety of aberrant molecular events driving tumori-

genesis (26). Currently, much effort has been made to elucidate the roles of ncRNAs involved in tumorigenesis, disease progression and metastasis of PTC (17,27,28). According to the ceRNA hypothesis, increasing evidence has demonstrated that lncRNAs regulated target genes through microRNAs (miRNAs) competition (29). To comprehensively identify the landscape regarding how lncRNA-associated ceRNA network affects PTC, the present study analyzed the large-scale sequencing data of PTC patient cohort in TCGA database. We identified the aberrantly expressed lncRNAs, mRNAs and miRNAs, and further successfully constructed the dysregulated lncRNA-associated ceRNA network in PTC by biological prediction. We also identified several lncRNAs and their related mRNAs to be potential prognostic indicators for PTC patients.

In previous studies, several groups have identified numerous abnormally expressed lncRNAs in PTC compared with non-tumor tissues. Two small cohort studies based on microarray sequencing of 3 and 5 pairs of PTC samples, respectively, have identified thousands of significantly dysregulated lncRNAs and validated several DElncRNAs by

real-time PCR (18,30). Xie *et al* reported a ceRNA network in PTC containing 29 lncRNAs, 9 miRNAs and 67 mRNAs by microarray analysis of 5-paired clinical samples (31). However, lncRNA-associated ceRNA network based on whole-genome gene expression profiling with large study population in PTC has not been described.

In the present study, 780 lncRNAs were detected as commonly dysregulated lncRNAs in 3 PTC variants (CPTC, FPTC and TCPTC) compared with normal tissues, including 440 upregulated and 340 downregulated. Among them, 45 aberrantly expressed lncRNAs were identified in the ceRNA network, and 8 out of them were related to tumor progression. The low expression of an intergenic lncRNA LINC00473 in PTC tumor tissues (~10-folds) may have potential capacity to promote tumor growth and pathologic stage. It has been identified to have prognostic value for lung cancer in a cyclic AMP-dependent manner (32). However, whether LINC00473 can utilize similar mechanism to lung cancer in PTC needs further research. LINC00460, a human gene transcribed from chromosome 13, is upregulated in various cancers, including thyroid cancer (33). The upregulation of LINC00460 was also observed in the present study and was positively correlated with the pathologic stage of PTC. It was reported that LINC00460 participated in a series of biological processes (e.g., metabolic process, chromatin modification, cell cycle and cell death) and showed prognostic prediction power for head and neck squamous cell carcinoma (33,34). These findings implied that LINC00460 may functionally be involved in the tumorigenesis of PTC. Moreover, RBMS3-AS1 and ZFX-AS1 also had potential values for PTC patients prognosis. Since these lncRNAs are reported for the first time as prognostic predictors, much effort deserves to be made to elucidate their biological function in neoplasms. Although the lncRNAs listed in Table V had no statistical significance in Kaplan Meier analysis (data not shown), both the lnRNAs that were associated with the progression of PTC and with patients' overall survival may have potential roles in PTC and are worth studying in the future.

However, having identified several specific lncRNAs for PTC, the present study drew a comprehensive ceRNA network, which revealed the complexity of the regulatory relationships among lncRNAs, miRNAs and mRNAs. E2F1, the best-studied E2F member, was significantly upregulated in PTC tumor tissues, which was consistent with a previous study (35). E2F1 was involved in both cancer-related pathways: pathways in cancer and miRNAs in cancer. Although previously E2F1 was found to promote thyroid carcinogenesis (35), our results demonstrated that high expression of E2F1 could prolong survival time of PTC patients, which strongly suggested its tumor-suppressive activity in thyroid cancer. Thus, we supposed that E2F1 may exhibit both oncogenic and tumor suppressive properties in thyroid cancer resulting from its capacity to induce tumor cell proliferation and apoptosis (36-38). The ceRNA network predicted that E2F1 was a target gene of miR-205. It was consistent with the previous study that miR-205 induced cancer senescence by regulating E2F1 and ultimately suppressed melanoma development (39). Our bioinformatic analysis predicted that miR-205 may interact with LINC00284, the increased expression of which was also positively correlated with the prognosis of PTC patients. Although the function of LINC00284 is poorly annotated, it is conceivable that LINC00284 may act as a ceRNA to interact with miR-205 and E2F1 in PTC.

There are several limitations in the present study. First, ceRNA hypothesis indicates that lncRNAs can serve as ceRNAs to regulate expression of the target genes by merging miRNAs. Therefore, the complex ceRNA network of PTC was built based on this hypothesis using biological prediction. Second, the ceRNA network with hundreds of connections suggested that one lncRNA connected with a number of miRNAs and relevant mRNAs. In this regard, our further study may focus on conducting experiments to validate several crucial interactions, particularly to verify the key interactions we discussed above and determine the roles of these lncRNAs as ceRNA in PTC. Third, the 3 lncRNAs that were aberrantly expressed in race need extensive validation studies, which must be based on a large-scale multiracial cohort.

In conclusion, we identified various dysregulated lncRNAs, miRNAs and mRNAs between tumor and non-tumor samples by genome-wide analysis of a large PTC patient cohort from TCGA. The lncRNA-related ceRNA network will be useful to guide further investigation concerning the involved lncRNAs of their definite mechanisms and functions in the tumorigenesis of PTC.

Acknowledgements

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