ORIGINAL RESEARCH

Correlations of lncRNAs with cervical lymph node metastasis and prognosis of papillary thyroid carcinoma

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Background: Cervical lymph node metastasis is an important prognostic indicator for papillary thyroid carcinoma (PTC) and affects treatment strategies for PTC. lncRNAs essentially contribute to the biological functions of tumors. This study aimed to identify the lncRNAs associated with cervical lymph node metastasis and prognosis of PTC and their potential pathophysiological mechanisms.

Materials and methods: PTC-associated lncRNAs were selected from The Cancer Genome Atlas database, and correlations among lncRNAs, lymph node metastasis, tumor staging, and prognosis of PTC were analyzed in silico. These correlations were then validated through quantitative reverse transcription PCR (qRT-PCR) and immunohistochemistry (IHC).

Results: In silico analysis showed that *FAM95B1* and *UCA1* were significantly correlated with cervical lymph node metastasis, tumor staging, and PTC prognosis ($P<0.05$). qRT-PCR analysis revealed high UCA1 expression in PTC tissues and correlations between UCA1 expression levels and cervical lymph node metastasis and tumor staging in PTC, that is, higher *UCA1* expression resulted in poorer PTC prognosis. IHC analysis revealed that a high expression of *UCA1* was accompanied by a high expression of metastasis-related proteins (MMP-2 and MMP-9), thereby validating the correlation of *UCA1* expression with metastasis.

Conclusion: *FAM95B1* and *UCA1* expression was significantly correlated with the occurrence and progression of PTC. The expression levels of *UCA1* significantly affected the prognosis of PTC patients and were significantly correlated with tumor staging and cervical lymph node metastasis.

Keywords: papillary thyroid carcinoma, lncRNA, *FAM95B1*, *UCA1*

Introduction

The worldwide incidence of thyroid carcinomas has increased over the past decades. An estimated over 64,300 new cases of thyroid cancer occurred in men and women in 2016, leading to 1,980 deaths in USA.[1,2] Papillary thyroid carcinoma (PTC) is the most common pathologic type of thyroid carcinoma, accounting for ~60%–70% of all thyroid carcinomas, and patients are prone to cervical lymph node metastasis at an early stage of PTC.[3–6] With appropriate treatments such as operation and radioactive iodine ablation, the majority of PTCs have excellent prognoses with an overall survival rate of 97.7% at 5 years.[7,8] Nonetheless, up to 20% patients can suffer locoregional recurrence, and occasionally some progress to aggressive and lethal outcomes.[9] Molecular mechanisms contributing to its carcinogenesis and progression remain obscure. Thus, it is urgent to identify reliable biomarkers of PTC for its early diagnosis, effective therapy, and prognosis evaluation.

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With the rapid advancements in molecular biology techniques, greater emphasis has been gradually placed on the role of non-coding transcripts. lncRNAs, which are >200 nucleotides in length[10] and unable to be translated into proteins, may serve as master gene regulators in transcriptional or posttranscriptional level.[11] The involvement of lncRNAs in various biological processes such as cell proliferation, differentiation, apoptosis, metabolism, and signal transduction have been reported in numerous studies.[12–14] The association of lncRNAs with tumor diseases such as lung cancer, colon cancer, stomach cancer, and breast cancer has been reported previously.[15–19] Recently, a few lncRNAs were found to be important regulators of PTC. For instance, a study indicated that lncRNAs (*ENST00000426615* and *ENST00000537266*) might be important regulators of PTC cell proliferation and migration.[20] Another study identified lncRNAs that were associated with *BRAFV600E* oncogenic mutation in PTC.[21] However, the understanding of the expression and function of lncRNAs is still at a preliminary stage. The present study aimed to identify significant prognostic lncRNAs using data from public database, followed by experimental validation and investigation of association between lncRNAs expression level and clinicopathologic characteristics of PTC. The lncRNAs identified may assist us in evaluating disease status and prognosis for PTC patients.

Materials and methods
In silico analysis of gene expression data

Clinical data on PTC and gene expression data (RNASeqV2) of PTC were downloaded from The Cancer Genome Atlas (TCGA) database.[22] Known lncRNAs and their expression profiles were screened on the basis of records from the GENCODE database.[23] Then, the RNA-seq count data were normalized by the voom function[24] of the R package limma.[25] The voom method estimates the mean–variance relationship of the log-counts, generates a precision weight for each individual normalized observation, and enters these into the limma analysis pipeline.[24] lncRNAs with significant differential expression in paracancerous and cancerous tissue were subsequently screened using empirical Bayes method of limma.[25] Fold change >2 and corrected *P*-value <0.05 were set as the threshold for significant differential expression. A volcano plot for differential expression was drawn using R plot function.[26] The optimal cutoff points for each lncRNA with expression value were determined using receiver operating characteristic (ROC) curves. Patients were then divided into the high-expression group and low-expression

group based on the optimal cutoff points, and differences in prognosis between the two groups were compared. Kaplan–Meier (KM) survival curves were plotted on the basis of test results of each lncRNA, and the log-rank test was performed to calculate the *P*-values for KM curves. Differences in survival between the two groups were considered significant when *P*<0.05. In addition, lncRNAs were identified with the area under the ROC curve (AUC) value >0.6 and *P*-values for KM curves as the threshold; these lncRNAs could well distinguish adjacent noncancerous control tissues from tumor tissues and their expression levels had significant impact on patient prognosis.

Moreover, using the lncRNA threshold obtained from the ROC curve, patients were divided into high-expression and low-expression groups. Patients were divided into lymph node metastasis group and non-metastasis group according to clinical information, and then chi-squared test of independence was used to determine if there was a significant relationship between lncRNA expression and lymph node metastasis. On the other hand, patients were grouped into stage I, II, III, and IV according to the clinical information. Chi-squared test was performed to assess the association between gene expression and tumor stage. The *P*-value by chi-squared test was obtained.

The target genes for the prognosis-related lncRNAs were predicted based on co-expression relationship. Genes with absolute value of the correlation coefficient greater than 0.5 and false discovery rate <0.01 were predicted as potential targets for lncRNA. Subsequently, functional enrichment analysis was performed on the target genes of prognosis-related lncRNAs using the database for annotation, visualization, and integrated discovery (DAVID) tool with the cutoff of *P*-value being <0.05.

Quantitative reverse transcription PCR (qRT-PCR) validation

A total 40 pairs of cancerous tissue and matched paracancerous tissue specimens and 15 metastatic cervical lymph node tissue specimens were collected from patients with PTC, who underwent surgical treatment at the Affiliated Cancer Hospital of Harbin Medical University between January 2016 and May 2016. All specimens were collected after obtaining written informed consent from the patients in accordance with the tenets of the Declaration of Helsinki and approval from the ethics committee of the hospital. After resection, specimens were immediately placed in RNA protection buffer and stored at −80°C freezer until use. The PTC patients

included 30 female and 10 male patients aged 27–60 years, and complete clinical data (age, sex, family history, past medical history, clinical stage, pathologic diagnosis, tumor size, number, morphology, pathologic grade, metastatic cervical lymph nodes, surgical procedures) were collected. Clinical staging of thyroid cancer was performed in accordance with the American Joint Committee on Cancer Staging System for Thyroid Cancer published in 2010.

Total RNA was extracted from fresh frozen tissue specimens using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with manufacturer's instructions. Based on the instructions provided with the Transcriptor First Strand cDNA Synthesis Kit (Roche, Brussels, Belgium), the reverse transcription reagent was added, and total RNA was reverse transcribed into cDNA using a conventional PCR machine (Thermo Fisher Scientific). Using the synthesized cDNA as a template, qPCR was performed with the addition of an RNA fluorescence reagent in accordance with the manufacturer's instructions. The following primers were used: UCA1 forward primer: 5′-ACGCTAACTGGCACCTTGTT-3′, reverse primer: 5′-TGGGGATTACTGGGGTAGGG-3′; GAPDH forward primer: 5′-GAAGGTGAAGGTCGGAGTC-3′, reverse primer: 5′-GAAGATGGTGGGGATTTC-3′. The cycling conditions were as follows: 95°C for 5 minutes, followed by 45 cycles of 95°C for 5 seconds, 60°C for 15 seconds, and 70°C for 15 seconds. The expression level of *UCA1* was normalized to the internal control GAPDH and the relative expression level of *UCA1* in cancerous tissue, paracancerous tissue, and metastatic lymph nodes was calculated using the $2^{-\Delta\Delta CT}$ method.

Immunohistochemistry (IHC) analysis

Tissue samples were fixed, dehydrated, cleared, and processed into conventional paraffin blocks, which were then cut into 5 µm sections and adhered to polylysine-coated glass slides. The sections were then deparaffinized. Benzene was removed, and the antigens were retrieved using citric acid. Then 3% of H_2O_2 was used for blocking the sections and MMP-2 or MMP-9 antibodies, both diluted to 1:200, were added (anti-MMP-2 antibody [ab37150], anti-MMP-9 antibody [ab73734]; Abcam, Cambridge, MA, USA). The sections were incubated at 4°C overnight, washed with PBS for three times, and incubated with the working solution of the secondary antibody (Beijing Zhongshan gold bridge Biotechnology Co., Ltd) at room temperature (25°C) for 30 minutes. Diaminobenzidine was used as a chromogen

(Beijing Zhongshan gold bridge Biotechnology Co., Ltd), hematoxylin was used for counterstaining, and neutral resin was used to seal the film.

Expression of MMP-2 and MMP-9 was considered positive when yellowish-brown and/or brown particles were observed in the cytoplasm. Samples without color were considered negative. Five high magnification fields or 500 cells were counted. A score was given according to the staining intensity: 0 for absence of color (no staining), 1 for light yellow color (weak staining), 2 for yellowish-brown color (medium staining), and 3 for brown color (strong staining). A score was calculated based on the percentage of positively stained tumor cells in a sample: 0 represented absence of positive tumor cells, 1 represented <10% of positive tumor cells, 2 represented 10%–50% of positive tumor cells, and 3 represented >50% of positive tumor cells. The scores for staining intensity and those for percentages of positive tumor cells were multiplied, and the product was used as the staining index. Using this method, the staining indices for MMP-2 and MMP-9 expression in PTC were 0, 1, 2, 3, 4, 6, or 9. High expression was defined as an index ≥4 and low expression as an index <4.

Statistical analysis

SPSS 20.0 software (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. The paired *t*-test was performed to compare differences in *UCA1* expression levels between matched cancer tissues and adjacent normal tissues, while unpaired *t*-test was performed to compare *UCA1* expression levels in cancerous tissues with those in metastatic lymph nodes. Chi-squared test was performed to analyze the association between *UCA1* expression levels in patients and their clinical characteristics in a 2×2 contingency table. Chi-squared test was also performed to analyze the association between *UCA1* expression levels in patients and the expression of metastasis-related proteins (MMP-2 and MMP-9). Differences were considered statistically significant when $P<0.05$.

Results
In silico analysis of lncRNAs associated with PTC
Characteristics of gene expression data and differential expression analysis

A total of 557 samples comprising 500 cancerous tissue samples and 57 paracancerous tissue samples were collected from the TCGA database. Clinical data were available for

Table I Identification of differentially expressed lncRNAs

lncRNA	logFC	P-value	Adj. P-value
UCAI	1.368752077	3.68E-07	2.15E-06
DLEU2	1.05591211	1.18E-18	3.66E-17
EGOT	1.032212489	4.03E-05	0.000139274
FAM157B	−1.057921492	7.89E-06	3.28E-05
EMX2OS	−1.241155959	1.30E-06	6.29E-06
CASC2	−1.242623565	2.66E-26	2.71E-24
SCARNA2	−1.255535464	5.48E-12	5.88E-11
DKFZP434L187	−1.259477809	1.49E-06	6.91E-06
DIO3OS	−1.262327003	6.64E-07	3.57E-06
LOH12CR2	−1.301957431	5.59E-26	3.80E-24
KC6	−1.328305834	0.00018469	0.0005887
RFPL1S	−1.365642278	5.13E-12	5.81E-11
PART1	−1.441819524	4.38E-05	0.000148885
HYMAI	−1.450653297	2.89E-14	4.91E-13
CECR7	−1.45495488	4.19E-09	3.05E-08
KIAA0125	−1.467912556	0.000718533	0.00212436
TCL6	−1.5356365	8.90E-06	3.63E-05
SMCR5	−1.65200412	1.72E-17	4.40E-16
FAM95B1	−1.820552657	2.26E-32	4.60E-30
FAM182A	−2.05223951	3.85E-16	7.85E-15
TERC	−2.439049743	7.79E-23	3.97E-21

506 samples (survival data and tumor expression samples were available for 499 patients). Based on the records from the GENCODE database, 110 lncRNAs with expression values were identified (Table S1).

Then, 21 differentially expressed lncRNAs were identified using limma software (Table 1). The volcano plot of lncRNAs with differential expression is shown in Figure S1.

lncRNAs significantly affecting PTC prognosis

Using AUC ≥0.6 and $P<0.05$ for KM survival curves as the screening criteria, three lncRNAs (*FAM95B1*, *HCG22*, and *UCA1*) were identified which could well distinguish paracancerous tissue from cancerous tissue, and their expression level significantly affected patient prognosis (Table 2). In particular, *FAM95B1* was downregulated in tumors, and KM plot showed that lower expression values of *FAM95B1* were associated with lower survival probabilities. However, *UCA1* was upregulated in tumors, and KM plot showed that

Table 2 Three lncRNAs identified to affect prognosis of patients with papillary thyroid carcinoma

lncRNA	KM P-value	DEG.log2FC	DEG.FDR
FAM95B1	0.038211079	−1.820552657	4.60E-30
SNHG6	0.01220598	0.315120308	0.004787686
UCA1	0.027307018	1.368752077	2.15E-06

Abbreviations: FDR, false discovery rate; KM, Kaplan–Meier.

higher expression values of *UCA1* were associated with lower survival probabilities (Figure 1).

Association between lncRNA expression levels and lymph node metastasis

A significant correlation ($P<0.05$) was obtained between expression levels and lymph node metastasis in 66 lncRNAs (Table S2), including 2 of the 3 lncRNAs (*FAM95B1* and *UCA1*) with a significant effect on prognosis (Table 3).

Lymph node metastasis was unknown in 49 of the 499 patients, with an effective data rate of 90.2%. Among the remaining 450 patients, 223 patients had lymph node metastasis, including 40 cases (17.9%) in the *FAM95B1* high-expression group and 183 (82.1%) cases in the *FAM95B1* low-expression group. Also, 227 cases were included in the lymph node non-metastasis group, including 67 cases (29.5%) in the *FAM95B1* high-expression group and 160 cases (70.5%) in the *FAM95B1* low-expression group. Chi-squared test showed that the expression level of *FAM95B1* was associated with lymph node metastasis ($P<0.05$). Similarly, for *UCA1*, in the lymph node metastasis group, there were 135 (60.5%) cases with high expression of *UCA1* and 88 (39.5%) cases with low expression of *UCA1*; in the lymph node non-metastasis group, there were 103 (45.4%) cases with high expression of *UCA1* and 124 cases (54.6%) with low expression of *UCA1*. Chi-squared test showed that the expression of *UCA1* was associated with lymph node metastasis ($P<0.05$).

Association between lncRNA expression level and tumor staging

Expression levels of 34 lncRNAs were associated with tumor staging ($P<0.05$; Table S3), which included *FAM95B1* and *UCA1* (Table 4). Among the 499 patients, 2 patients had unknown tumor stage, and the data efficiency was 99.6%. Among the remaining 497 patients, there were 282 patients in stage I, including 75 (26.6%) in the *FAM95B1* high-expression group and 207 (73.4%) in the low-expression group; there were 52 patients in stage II, including 14 (26.9%) in the *FAM95B1* high-expression group and 38 cases (73.1%) in the low-expression group; there were 110 patients in stage III, including 18 cases (16.4%) in the *FAM95B1* high-expression group and 92 cases (83.6%) in the low-expression group. Of the 53 patients in stage IV, 14 (26.4%) had high expression of *FAM95B1* and 39 (73.6%) had low expression of *FAM95B1*. Chi-squared test showed that the expression level of *FAM95B1* was related to tumor
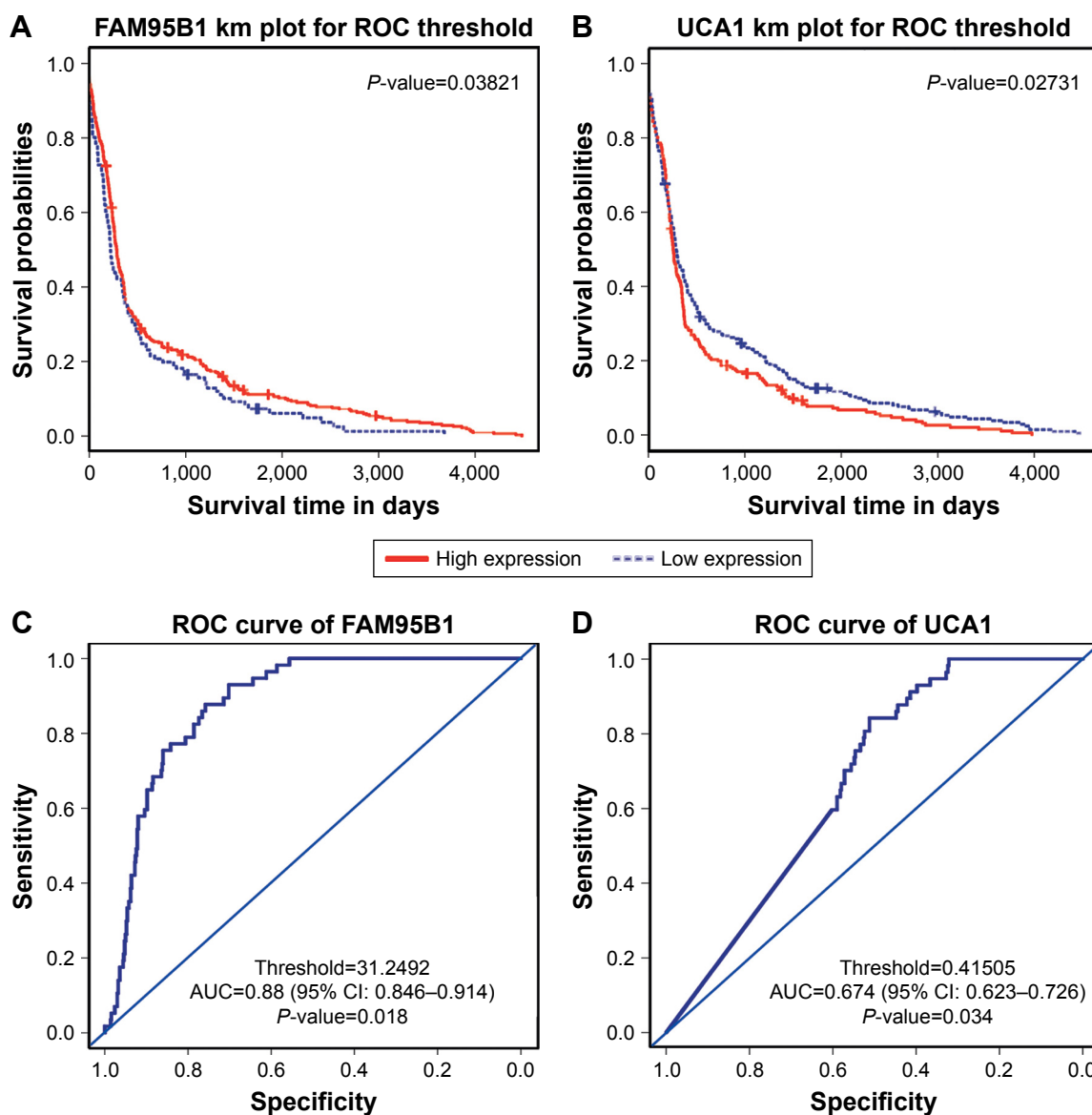
Figure 1 The relationship between lncRNA expression and prognosis in PTC.
Notes: (**A**) Low expression of *FAM95B1* correlates with worse overall survival. (**B**) High expression of *UCA1* correlates with worse overall survival. (**C** and **D**): ROC curves for *FAM95B1* and *UCA1*.
Abbreviations: AUC, area under the ROC curve; PTC, papillary thyroid carcinoma; ROC, receiver operating characteristic.

stage (*P*<0.05). For *UCA1*, in the stage I group, there were 140 cases (49.7%) with high expression of *UCA1* and 142 cases (50.3%) with low expression of *UCA1*. In the stage II group, there were 18 cases (34.6%) with high expression of *UCA1* and 34 cases (65.4%) with low expression of *UCA1*. In the stage III group, there were 64 cases (58.2%) with high expression of *UCA1* and 46 cases (41.8%) with low expression of *UCA1*. In the stage IV group, there were 34 cases (64.2%) with high expression of *UCA1* and 19 patients (35.8%) with low expression of *UCA1*. Chi-squared test showed that the expression of *UCA1* was related to tumor stage (*P*<0.05).

Table 3 Two lncRNAs with significant effect on prognosis and lymph node metastasis

lncRNA	Chi-square *P*-value	KM *P*-value	DEG.log2FC	DEG.FDR
FAM95B1	0.016263465	0.0382110787064344	−1.820552657	4.60E-30
UCA1	0.019784618	0.0273070182501289	1.368752077	2.15E-06

Abbreviations: FDR, false discovery rate; KM, Kaplan–Meier.

Table 4 Two lncRNAs with significant effect on prognosis and tumor staging

lncRNA	Chi-square *P*-value	KM *P*-value	DEG.log2FC	DEG.FDR
FAM95B1	0.012929509	0.0382110787064344	−1.820552657	4.60E-30
UCA1	0.021512139	0.0273070182501289	1.368752077	2.15E-06

Abbreviations: FDR, false discovery rate; KM, Kaplan–Meier.

Target gene prediction and lncRNA functional analysis

A total of 316 target genes and 6 target genes were predicted for *FAM95B1* and *UCA1*, respectively. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were carried out based on the target genes of *FAM95B1* and *UCA1*. No significant GO terms and pathways were identified for *UCA1*. On the other hand, the functional enrichment analysis revealed that *FAM95B1* was associated with various pathways, such as oncogenic pathways, lipid metabolic pathways, glycerophospholipid metabolic pathways, and lysine degradation pathways (Figure 2).

qRT-PCR validation

UCA1 expression levels were significantly higher in PTC tissues than in the matched paracancerous tissues ($P<0.05$).

In addition, *UCA1* expression levels were significantly higher in metastatic cervical lymph node tissues than in the primary tumor tissues ($P<0.01$; Figure 3). Therefore, *UCA1* was highly expressed in PTC tissues, and its expression levels correlated with cervical lymph node metastasis.

Correlation between *UCA1* expression levels and clinicopathologic characteristics of PTC

The association between *UCA1* expression levels in tumor tissues and clinicopathologic characteristics of PTC patients is shown in Table 5. There were no significant differences in *UCA1* expression levels between sexes and different age groups ($P>0.05$), and *UCA1* expression was not significantly correlated with tumor size ($P>0.05$). However, *UCA1*
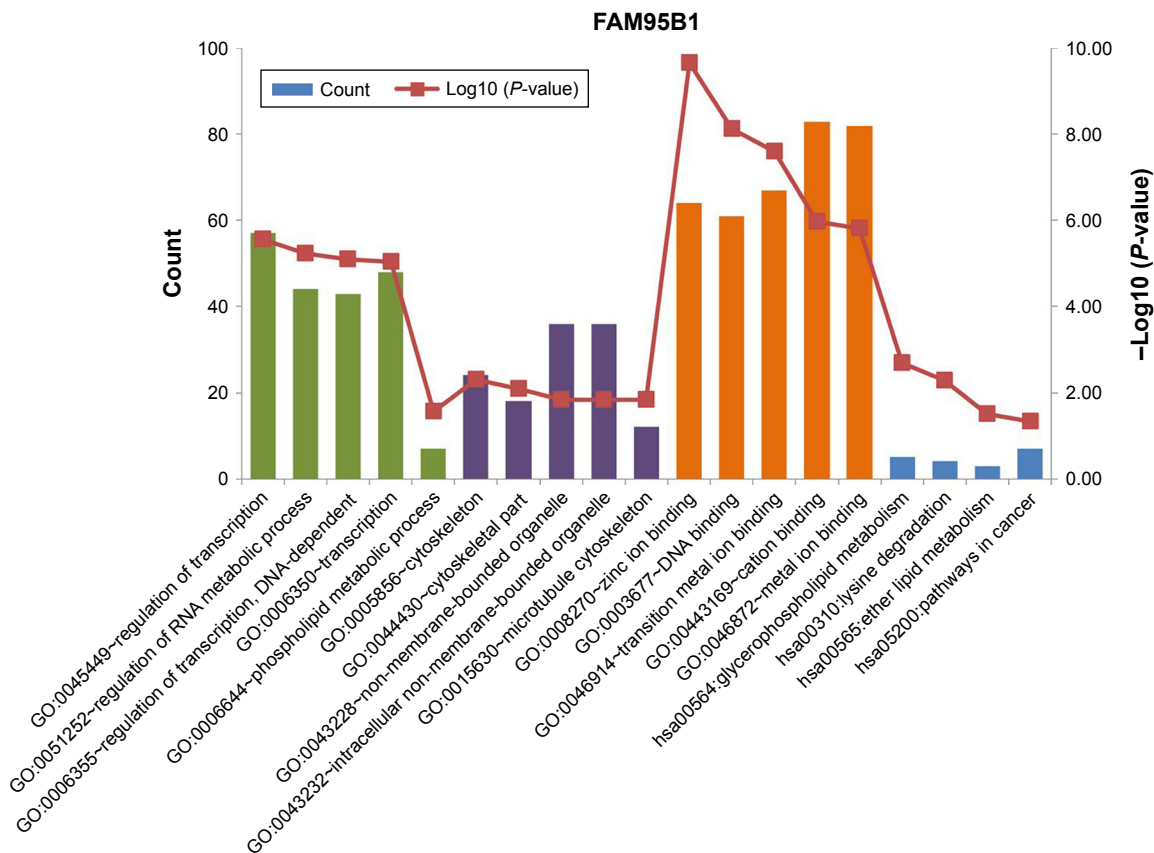

Figure 2 Functional enrichment analysis of FAM95B1.
Note: Green bars represent biological processes (GO BP), purple bars represent cellular components (GO CC), orange bars represent molecular functions (GO MF), and blue bars represent pathways (KEGG pathways).
Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological processes; CC, cellular components; MF, molecular functions.
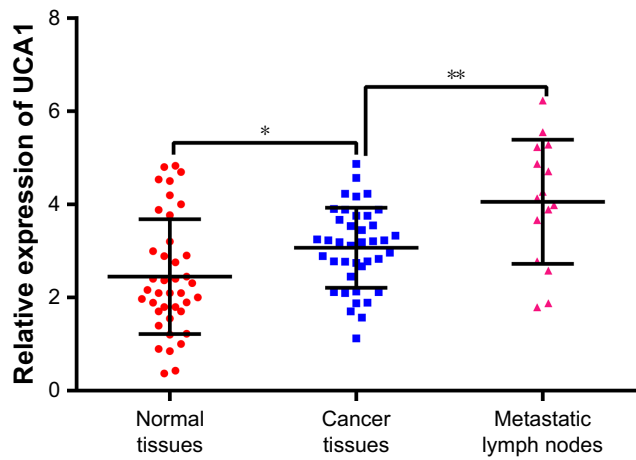
Figure 3 The UCA1 level in PTC tissues, normal tissues, and metastatic lymph node tissue identified by qRT-PCR.
Notes: The expression of UCA1 in normal tissues was significantly lowest among the three groups. The level of UCA1 in metastasis tissues was highest among the three groups. *$P<0.05$, **$P<0.01$.
Abbreviations: PTC, papillary thyroid carcinoma; qRT-PCR, quantitative reverse transcription PCR.

expression levels were significantly higher in the cervical lymph node metastasis group than in the non-metastasis group, and in clinical stage III/IV patients than in stage I and stage II patients ($P<0.05$).

Patients with high expression of *UCA1* had high expression levels of MMP-2 and MMP-9

MMP-2 and MMP-9 were mainly expressed in the cytoplasm, and their expression levels were positively correlated with lymph node metastases in PTC ($P=0.0022$ and $P=0.0072$, respectively; Table 6). Results from IHC showed that

Table 5 Correlation between *UCA1* expression and clinico-pathologic characteristics of PTC patients

Patient characteristics	Low UCA1 expression group	High UCA1 expression group	P-value
Age (years)			1.0
≤45	11	13	
>45	8	8	
Sex			0.2812
Male	3	7	
Female	16	14	
Tumor diameter (cm)			0.5254
≤2	10	8	
>2	9	13	
Lymph node metastasis			0.0098
Negative	16	9	
Positive	3	12	
Stage			0.0270
I/II	15	9	
III/IV	4	12	

Abbreviation: PTC, papillary thyroid carcinoma.

Table 6 Correlation between MMP-2 and MMP-9 expression and cervical lymph node metastasis

Protein expression	Lymph node metastasis		P-value
	Positive	Negative	
MMP-2			0.0022
High expression	14	11	
Low expression	1	14	
MMP-9			0.0072
High expression	13	10	
Low expression	2	15	

MMP-2 and MMP-9 were highly expressed in samples with high expression of *UCA1*. Contrarily, MMP-2 and MMP-9 expression were low in patients with low expression of *UCA1* ($P=0.0457$ and $P=0.0238$, respectively; Table 7). These results indicated that the high expression of *UCA1* was accompanied by the high expression of metastasis-related proteins (MMP-2 and MMP-9), suggesting that *UCA1* expression was correlated with metastasis (Figure 4).

Discussion

The role of lncRNA in tumorigenesis and tumor development has received increasing interest in oncology research. Numerous lncRNAs display altered expression levels in human cancers, including lung cancer, colon cancer, gastric cancer, and breast cancer.[15–19] In this study, in silico analyses were performed to identify PTC-related lncRNA. Based on the analyses, lncRNAs that significantly affected prognosis, lymph node metastases, and tumor stage were respectively selected. This study focused on lncRNAs (*FAM95B1* and *UCA1*) that significantly affected prognosis while being concurrently associated with lymph node metastases and tumor stage. Of these two lncRNAs, *FAM95B1* was downregulated in PTC and its expression level was positively correlated with the survival rates of patients. However, *UCA1* was upregulated in cancer and its expression level was negatively correlated with the survival rates of PTC patients.

Table 7 Correlation between MMP-2 and MMP-9 expression and *UCA1* expression

	Low UCA1 expression group	High UCA1 expression group	P-value
MMP-2 expression			0.0457
Low expression	10	4	
High expression	9	17	
MMP-9 expression			0.0238
Low expression	12	5	
High expression	7	16	

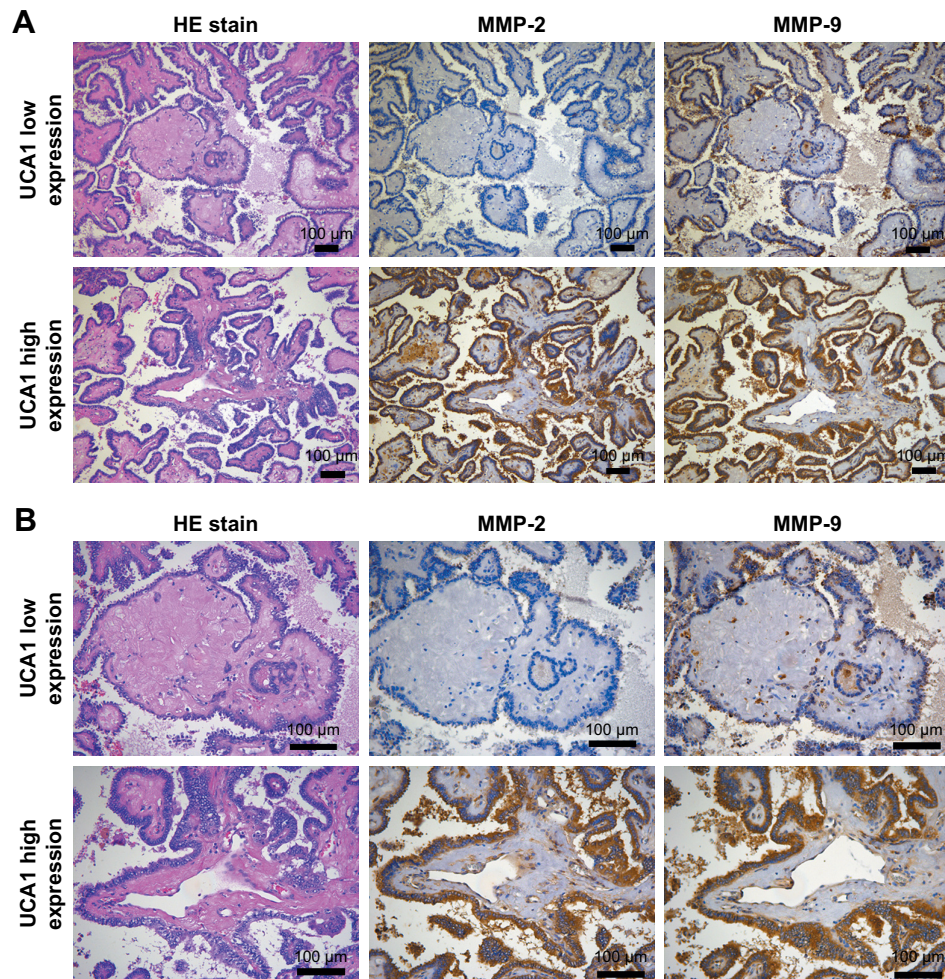**Figure 4** The relationship of MMP-2 and MMP-9 expression with *UCA1* expression (**A** ×100 magnification; **B** ×200 magnification).

The upregulation of *UCA1* promotes invasion and migration of numerous types of cancer, including gastric cancer, lung cancer, hepatocellular carcinoma, and pancreatic cancer.[27–31] Recently, the investigation of Liu et al showed that *UCA1* was upregulated in PTC tissues and cell lines and knockdown of *UCA1* significantly inhibited proliferation and migration of PTC cells through regulating miR-204/IGFBP5 axis.[32] Two other studies also indicated the role of *UCA1* in stimulating proliferation of PTC cells.[33,34] In line with these previous studies, we found that *UCA1* was upregulated in PTC tissues and associated with prognosis by public data investigation. To further explore the clinical significance of *UCA1* in PTC, qRT-PCR analysis was performed to determine *UCA1* expression levels in PTC tissue, adjacent noncancerous control tissues, and metastatic cervical lymph nodes. *UCA1* expression levels were significantly higher in PTC tissue than in adjacent noncancerous control tissues. In addition, *UCA1* expression levels were the highest in the metastatic cervical

lymph nodes and were higher than those in the primary tumor. This finding suggested that *UCA1* expression was significantly correlated with cervical lymph node metastases. Statistical analyses of the relationships between *UCA1* expression levels and the clinicopathologic features of PTC also revealed that *UCA1* expression levels correlated with lymph node metastases and tumor stage. Thus, these results suggest that *UCA1* may be an effective target for PTC treatment.

Degradation of the extracellular matrix and basement membrane is necessary for local invasion or metastases of malignant tumors.[35] MMP-2 and MMP-9, members of MMP family, are mainly involved in the decomposition of type IV collagen, thereby degrading the basement membrane and promoting tumor invasion and metastases.[36] Studies have shown that the expression of MMP-2 and MMP-9 is positively correlated with lymph node metastases and degree of tumor invasion in PTC.[37,38] In order to further verify the correlation of *UCA1* expression with cervical lymph node metastasis, IHC

experiments were carried out to verify the correlation between *UCA1* and lymph node metastases. We detected high levels of MMP-2 and MMP-9 in samples with high *UCA1* expression. Our results indicate that upregulation of *UCA1* is associated with an increased expression of metastasis-related proteins, suggesting its relation with lymph node metastases in PTC.

FAM95B1 is a new type of lncRNA and its relationship with cancer has not been previously reported. In silico analysis revealed that *FAM95B1* was downregulated in PTC, which was also correlated with cervical lymph node metastases and tumor stage. Furthermore, patients with lower expression of *FAM95B1* had lower survival rates. GO and KEGG analyses of *FAM95B1* revealed that *FAM95B1* was related to cancer signaling pathways and metabolic pathways. The biological functions and detailed mechanisms of *FAM95B1* were not elucidated, and more investigation is underway.

Overall, the comprehensive study of *FAM95B1* and *UCA1* in this work broadens the oncogenic lncRNA landscape of PTC. This study revealed *FAM95B1* and *UCA1* as potential biological markers, and may aid in the prognosis and the comprehensive management of PTC.

Ethical approval

The project was approved by the Ethics Committees of Harbin Medical University Cancer Hospital, Harbin Medical University

Informed consent

Informed consent was obtained from all individual participants included in the study. This article does not contain any study with animals performed by any of the authors.

Disclosure

The authors report no conflicts of interest in this work.

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