

Downregulation of miR-486-5p in papillary thyroid carcinoma tissue: A study based on microarray and miRNA sequencing

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Abstract. Abnormal expression of microRNA (miR) is associated with the occurrence and progression of various types of cancers, including papillary thyroid carcinoma (PTC). In the present study, the aim was to explore miR-486-5p expression and its role in PTC, as well as to investigate the biological function of its potential target genes. The expression levels of miR-486-5p and its clinicopathological significance were examined in 507 PTC and 59 normal thyroid samples via The Cancer Genome Atlas (TCGA). Subsequently, the results were validated using data from Gene Expression Omnibus (GEO) and ArrayExpress. Receiver operating characteristic and summary receiver operating characteristic curves were used to assess the ability of miR-486-5p in distinguishing PTC from normal tissue. Furthermore, potential miR-486-5p mRNA targets were identified using 12 prediction tools and enrichment analysis was performed on the encoding genes using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes. The expression levels of miR-486-5p were consistently downregulated in PTC compared with in normal tissue across datasets from TCGA, GEO (GSE40807, GSE62054 and GSE73182) and ArrayExpress (E-MTAB-736). The results also demonstrated that miR-486-5p expression was associated with cancer stage (P=0.003), pathologic lymph node (P=0.047), metastasis (P=0.042), neoplasm (P=0.012) and recurrence (P=0.016) in patients with PTC. In addition, low expression of miR-486-5p in patients with

PTC was associated with a worse overall survival. A total of 80 miR-486-5p-related genes were observed from at least 9 of 12 prediction platforms, and these were involved in 'hsa05200: Pathways in cancer' and 'hsa05206: MicroRNAs in cancer'. Finally, three hub genes, CRK like proto-oncogene, phosphatase and tensin homolog and tropomyosin 3, were identified as important candidates in tumorigenesis and progression of PTC. In conclusion, it may be hypothesized that miR-486-5p contributes towards PTC onset and progression, and may act as a clinical target. However, *in vitro* and *in vivo* experiments are required to validate the findings of the present study.

Introduction

MicroRNAs (miRNAs/miRs) consist of a large family of non-coding, single-stranded RNA molecules that are 18-22 nucleotides long. These RNA molecules modulate gene expression post-transcriptionally by regulating the translation and stability of mRNAs. This regulation is achieved through complete or incomplete sequence matching between miRNAs and the 3'untranslated regions of the target mRNA (1). So far, thousands of mature miRNAs have been identified, of which ~2,600 human miRNAs have been annotated in the miRbase database (<http://www.mirbase.org/>).

The incidence of papillary thyroid carcinoma (PTC) continues to increase worldwide (2), and it has been demonstrated that miRNAs may contribute to occurrence and progression of the disease. Differential miRNA expression has been reported in PTC vs. normal tissue samples (3-5); certain miRNAs, including serum miR-34a, -155, -197, -221, -224 and -375, can be used as diagnostic and prognostic indicators of thyroid cancer (6,7). The mechanism of action for miR-150, -422a, -18a and -19a in human thyroid cancer has been studied to some extent (8-10); however, there are still a number of miRNAs that may be biologically relevant for the development of thyroid cancer.

Human miR-486 is situated on the short arm of chromosome 8p11 within the ankyrin 1 gene and is transcribed from an intron (11). Genomic deletion of miR-486-5p has been associated with various cancers, including colorectal, lung, pancreatic and gastric cancer (12-16). However, to the best of our knowledge, only one study to date has investigated the function of miR-486-5p in thyroid cancer. Ma *et al* (17) demonstrated that the fibrillin 1 (FBN1) gene is a distinct molecular target

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Abbreviations: PTC, papillary thyroid carcinoma; TCGA, the cancer genome atlas; GEO, gene expression omnibus; ROC, receiver operating characteristic; SROC, summary receiver operating characteristic; OS, overall survival; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes, PPI, protein-protein interaction

Key words: miR-486-5p, papillary thyroid carcinoma, bioinformatics analysis, hub genes

for miR-486-5p in PTC cells. In addition, reduced miR-486-5p expression levels were identified in PTC tissues and cell lines, and miR-486-5p was demonstrated to suppress cell growth and enhance apoptosis in PTC. A limitation of this study was the small sample size; therefore, it is necessary to further explore the expression of miR-486-5p in thyroid cancer in a larger cohort to better understand the underlying molecular mechanisms.

The Cancer Genome Atlas (TCGA; <https://cancergenome.nih.gov/>) is a comprehensive database of key genetic mutations responsible for different types of cancer, which is generated by the National Cancer Institute and the National Human Genome Research Institute (18). The Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) was released by the National Center for Biotechnology Information in 2000 and is a public functional genomics data repository, which contains gene chip expression data as well as data from non-chip technologies, including serial analysis of gene and mass spectrometry (19). ArrayExpress (<https://www.ebi.ac.uk/arrayexpress/>) is an archive of functional genomics data from microarray and sequencing platforms (20). These databases offer an excellent source of information for studying the importance of miRNAs in PTC.

TCGA database was used to evaluate the expression levels of miR-486-5p in 507 patients with PTC and to analyze its association with clinical parameters. In addition, miR-486-5p expression data were extracted, and verified by GEO and ArrayExpress. Subsequently, the miR-486-5p target mRNAs were identified through 12 miRNA-mRNA prediction platforms. Finally, the potential molecular mechanisms by which miR-486-5p may contribute to PTC were investigated using bioinformatics tools.

Materials and methods

TCGA miR-486-5p data analysis. TCGA contains miRNA sequencing data from 507 PTC and 59 normal thyroid samples (21). In the present study, miR-486-5p expression data, as well as clinicopathological features from PTC vs. normal thyroid samples, were extracted from TCGA by the end of October 1, 2017. Follow-up cases over a 5-year period from October 1, 2012 were also included. The expression data for miR-486-5p were log₂ transformed and values <1 were censored. Student's t-test was conducted to compare the expression levels of miR-486-5p in 507 PTC and 59 normal thyroid samples, as well as the association between miR-486-5p expression and the clinical parameters from TCGA. The cut-off value for high and low miR-486-5p mRNA expression was based on median expression in the PTC group. Receiver operating characteristic (ROC) and summary receiver operating characteristic (SROC) curves were generated to evaluate the accuracy of miR-486-5p in identifying cancer and normal tissue. Kaplan-Meier (K-M) analysis and log-rank test were used to assess the prognostic value of miR-486-5p. SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was utilized for statistical analyses and P<0.05 was considered to indicate a statistically significant difference.

Validation of miR-486-5p expression using GEO and ArrayExpress. The GEO and ArrayExpress databases were searched for PTC-relevant miRNA chip or sequencing data using the following phrases: [(thyroid OR papillary OR follicular OR medullary) AND (tumor OR tumour OR cancer

OR carcinoma OR neopla* OR malignan*) AND (miRNA OR miR OR microRNA) AND (profil* OR array OR chip OR microarray OR microchip)]. "*" refers to wildcard. All expression and clinical data related to miR-486-5p were extracted. Meta-analysis of GEO (GSE40807 (22), GSE57780 (unpublished data), GSE62054 (unpublished data), GSE73182 (23), ArrayExpress (E-MTAB-736) (24) and TCGA miRNA data was carried out in Stata version 12.0 (StataCorp LP, College Station, TX, USA). The pooled standard mean difference (SMD) with 95% confidence interval (CI) was utilized to assess miR-486-5p expression in PTC vs. normal thyroid tissue. χ^2 and I² statistics were calculated to measure the heterogeneity within the meta-analysis. The Mante-Haenszel fixed-effects model was applied if there was no obvious heterogeneity among the pooled studies (χ^2 test P>0.1 and I² <50%); conversely, a random-effects model was applied when obvious heterogeneity was identified (χ^2 test P<0.1 and I²>50%) (25).

Predicting miR-486-5p target genes. To identify potential miR-486-5p target genes, 12 miR-target prediction programs, including TDIANA-microT_{v4.0} (26), DIANA-microT-CDS (27), miRanda-rel2010 (28), miRBridge (29), miRDB4.0 (30), miRmap (31), miRNAMap (32), doRiNA (33), PITA (34), RNA22v2 (35), RNAhybrid2.1 (36) and Targetscan6.2 (37) were used. The 80 mRNAs predicted by at least nine programs were considered potential miR-486-5p target mRNAs in PTC and used for further mRNA functional analysis.

Predicting miR-486-5p biological function. To evaluate the potential molecular mechanisms that are regulated by miR-486-5p-associated genes, FunRich version 3.0 software (<http://www.funrich.org/>) was used for Gene Ontology (GO) functional enrichment analysis (38). The software supports enrichment analysis of GO terms, including those involved in biological process, cellular component and molecular function, which can also be visualized. The results were considered statistically significant when the false discovery rate (FDR) was <0.05. The Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (<https://david.ncifcrf.gov/>) and STRING version 10.5 (<https://string-db.org/>) were used for Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis (39,40). Genes in the top KEGG pathway were considered to be miR-486-5p hub genes.

Validation of the hub gene expression levels and their relationship with miR-486-5p. The cBioPortal for Cancer Genomics (<http://www.cbioportal.org/>), which is a public resource for large-scale cancer genomics data, was used to explore hub gene expression in PTC tissues (41). To ascertain the correlation between miR-486-5p and hub genes, a Pearson's correlation analysis was conducted; P<0.05 was considered to indicate a statistically significant difference. The expression of the hub genes in PTC and normal thyroid tissue was examined by immunohistochemistry via The Human Protein Atlas (<http://www.proteinatlas.org/>) (42).

Results

Downregulated miR-486-5p expression in PTC and its clinical significance. Data on miR-486-5p gene expression

Table I. Association between miR-486-5p expression and clinicopathological factors in PTC.

Variable	No.	Relative miR-486-5p expression		P-value
		Mean \pm standard deviation	t-value	
Tissue				
Normal	59	11.08 \pm 1.18	13.81	<0.001
PTC	507	8.47 \pm 1.30		
Age (years)				
\leq 60	393	8.61 \pm 1.27	0.33	0.740
>60	113	8.56 \pm 1.36		
Sex				
Female	370	8.64 \pm 1.31	1.28	0.203
Male	136	8.48 \pm 1.24		
Stage				
I-II	336	8.72 \pm 1.30	2.94	0.003
III-IV	168	8.36 \pm 1.26		
Grade				
I-II	311	8.69 \pm 1.29	1.92	0.056
III-V	195	8.46 \pm 1.29		
Pathological lymph node				
No	231	8.72 \pm 1.28	1.99	0.047
Yes	225	8.49 \pm 1.20		
Metastasis				
No	496	8.61 \pm 1.29	2.04	0.042
Yes	9	7.73 \pm 0.99		
Tumor				
No	450	8.63 \pm 1.29	2.51	0.012
Yes	44	8.12 \pm 1.17		
Recurrence				
No	462	8.64 \pm 1.32	2.41	0.016
Yes	44	8.15 \pm 0.90		

miR, microRNA; PTC, papillary thyroid carcinoma; SD, standard deviation.

from 507 PTC and 59 normal thyroid samples were extracted from TCGA. The expression levels of miR-486-5p were significantly decreased in PTC compared with in normal tissue [8.47 \pm 1.30 vs. 11.08 \pm 1.18; $P < 0.001$; Fold change (FC)=0.76; Table I; Fig. 1A]. ROC curve analysis of miR-486-5p in discriminating PTC from normal tissue was calculated with an area under curve (AUC) of 0.918 ($P < 0.001$; 95% CI: 0.892-0.939; Fig. 1B). The miR-486-5p expression levels and clinicopathological factors are summarized in Table I. miR-486-5p expression levels were decreased in stage III-IV cancers (8.36 \pm 1.26) compared with in stage I-II cancers (8.72 \pm 1.30; $P = 0.003$). In addition, PTC tissues with pathological lymph nodes had decreased expression levels of miR-486-5p (8.49 \pm 1.20) compared with PTC tissues without pathological lymph nodes (8.72 \pm 1.28; $P = 0.047$). Furthermore, miR-486-5p was downregulated in tissues with metastasis (7.73 \pm 0.99) compared with in tissues without metastasis (8.61 \pm 1.29; $P = 0.042$). Decreased expression levels of miR-486-5p were observed in PTC group (8.12 \pm 1.17)

compared with the normal thyroid group (8.63 \pm 1.29; $P = 0.012$). miR-486-5p expression was also reduced in recurrent cancer (8.15 \pm 0.90) compared with non-recurrent cancer (8.64 \pm 1.32; $P = 0.016$). Differential miR-486-5p expression was not observed for the remaining clinicopathological factors analyzed (Table I). Subsequently, the prognostic value of miR-486-5p expression was investigated. The K-M survival curves indicated that the median overall survival (OS) for the high expression group was 1,443 days, whereas the median OS for the low expression group was 1,015 days. The curves suggested that PTC cases with higher miR-486-5p expression levels were likely to have an improved clinical outcome. However, there was no statistically significant difference in the OS [log-rank $P = 0.355$; hazard ratio (HR)=0.617; 95% CI: 0.221-1.718; Fig. 2A] and the disease-free survival (DFS) (log-rank $P = 0.132$; HR=0.454; 95% CI: 0.162-1.269; Fig. 2B) of samples with high vs. low expression levels of miR-486-5p (cut-off =8.469). Therefore, the ability for miR-486-5p expression to predict OS and DFS is limited.

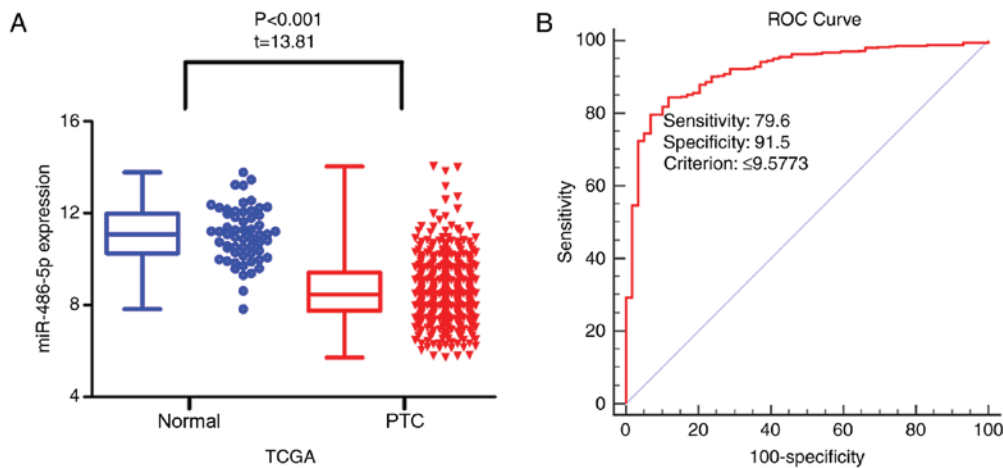


Figure 1. (A) Relative expression levels of miR-486-5p in PTC vs. normal thyroid tissues using the dataset from TCGA. (B) ROC curve analysis of miR-486-5p in discriminating PTC from normal controls. miR, microRNA; PTC, papillary thyroid carcinoma; ROC, receiver-operating characteristic; TCGA, The Cancer Genome Atlas.

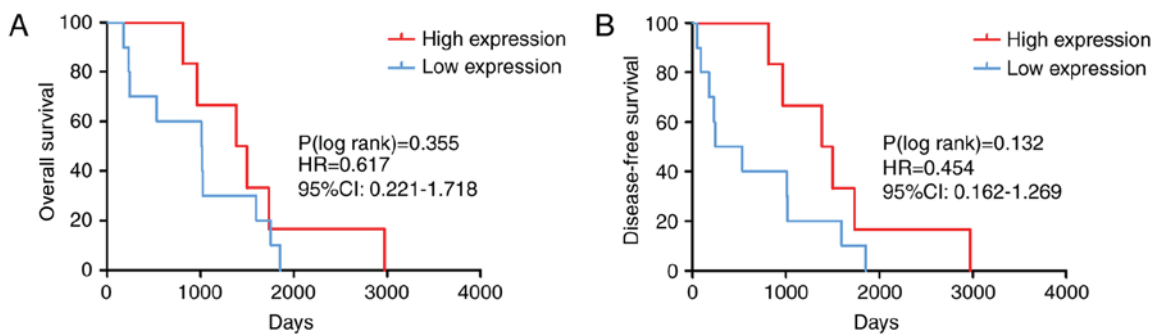


Figure 2. Survival curves in patients with PTC according to miR-486-5p expression levels (cut-off=8.469). Kaplan-Meier survival analysis of (A) overall survival and (B) disease-free survival in 507 PTC samples. CI, confidence interval; HR, hazard ratio; miR, microRNA; PTC, papillary thyroid carcinoma.

Verification of miR-486-5p downregulation in PTC using GEO and ArrayExpress data. GSE40807, GSE57780, GSE62054 and GSE73182 were obtained from the GEO database, which provided miR-486-5p expression levels in 77 PTC and 56 normal thyroid tissues (Fig. 3). The expression levels of miR-486-5p were lower in PTC compared with in normal samples in the following datasets: GSE40807 (6.426 ± 0.992 vs. 7.450 ± 1.819 ; $P=0.003$; $FC=1.159$; Fig. 3A), GSE62054 (5.000 ± 1.003 vs. 6.176 ± 1.349 ; $P=0.022$; $FC=0.810$; Fig. 3E) and GSE73182 (9.716 ± 0.171 vs. 9.952 ± 0.173 ; $P=0.012$; $FC=0.976$; Fig. 3G). The expression levels of miR-486-5p did not differ significantly between PTC and normal tissue in the GSE57780 dataset (10.648 ± 0.133 vs. 12.195 ± 0.994 ; $P=0.056$; $FC=0.873$; Fig. 3C). Chip array data from a single study (E-MTAB-736) was obtained from ArrayExpress and the data also indicated that miR-486-5p expression levels were downregulated in the 12 PTC samples compared with in the 10 normal tissue samples (8.048 ± 0.593 vs. 8.914 ± 0.579 ; $P=0.003$; $FC=0.903$; Fig. 4A). To evaluate the ability of miR-486-5p to distinguish cancer from normal thyroid samples, ROC curve analyses were performed. The AUC values for miR-486-5p calculated using the GSE40807, GSE57780, GSE62054, GSE73182 and E-MTAB-736 datasets were 0.684 ($P<0.001$; cut-off ≤ 7.217 ; Fig. 3B), 1.000 ($P<0.001$; cut-off ≤ 10.747 ; Fig. 3D), 0.746 ($P=0.036$; cut-off ≤ 5.644 ; Fig. 3F), 0.884 ($P<0.001$; cut-off

≤ 9.779 ; Fig. 3H) and 0.867 ($P<0.001$; cut-off ≤ 8.203 ; Fig. 4B), respectively.

Further verification of miR-486-5p downregulation in PTC using SMD and SROC. miR-486-5p expression data from TCGA, GEO (GSE40807, GSE57780, GSE62054 and GSE73182) and ArrayExpress (E-MTAB-736) were combined for meta-analysis, which included a total of 603 PTC and 125 normal tissue samples (Table II). The pooled SMD of miR-486-5p was -1.358 [95% CI, -1.950 – (-0.766) ; $P<0.001$; Fig. 5A] by the random effects model, and the P-value of the heterogeneity test was 0.001 ($I^2=75.6\%$). From meta-analysis, the AUC of the SROC was 0.84 (95% CI, 0.81–0.87; $P<0.001$; Fig. 5B).

Prediction of miR-486-5p target mRNAs and GO analysis. From at least nine of the 12 miRNA target prediction databases, 80 mRNA targets of miR-486-5p were identified. To understand the biological function of miR-486-5p in PTC, GO enrichment analysis of genes encoding for miR-486-5p target mRNAs was performed in FunRich. 'Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism' ($P=0.008$; Fig. 6A), 'Cytoplasm' ($P=6.158 \times 10^{-8}$; Fig. 6B) and 'RNA binding' ($P=0.004$; Fig. 6C) were the top functions for biological process, cellular component and molecular function, respectively.

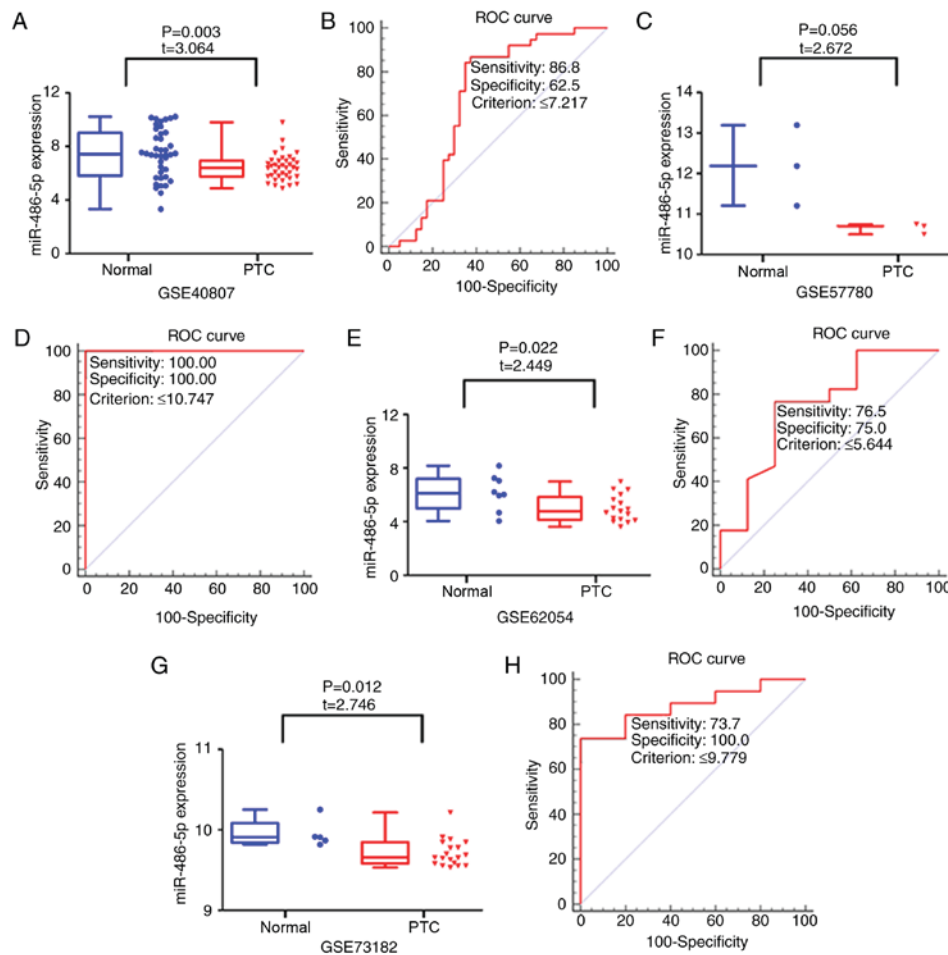


Figure 3. Relative miR-486-5p expression levels in PTC and normal tissue using the datasets (A) GSE40807, (C) GSE57780, (E) GSE62054 and (G) GSE73182. ROC curve analysis of miR-486-5p in discriminating PTC from normal controls using the datasets (B) GSE40807, (D) GSE57780, (F) GSE62054 and (H) GSE73182. miR, microRNA; PTC, papillary thyroid carcinoma; ROC, receiver-operating characteristic.

Identification of miR-486-5p-regulated pathways. The KEGG pathway enrichment analysis was performed on miR-486-5p-relevant genes using DAVID. The results revealed the most significantly enriched pathway was ‘hsa05200: Pathways in cancer’ with six genes including CRK like proto-oncogene (CRKL), C-terminal binding protein 2, forkhead box O1, phosphatase and tensin homolog (PTEN), phosphoinositide-3-kinase regulatory subunit 1 and tropomyosin 3 (TPM3) (P=0.010; Fig. 7). In addition, protein-protein interaction network analysis was performed on the 80 relevant genes using STRING: ‘hsa05206: MicroRNAs in cancer’ was identified, with six genes including CRKL, pim-1 proto-oncogene, sirtuin 1, PTEN, SRY-box 4 and TPM3 (Fig. 8). The three common genes (CRKL, PTEN and TPM3) of the ‘hsa05200: Pathways in cancer’ and ‘hsa05206: MicroRNAs in cancer’ pathways from the analyses were considered hub genes (Fig. 9A).

Validation of hub genes. To validate the relationship between miR-486-5p and the hub genes CRKL, PTEN and TPM3, their gene expression levels were examined using the cBioPortal for Cancer Genomics. The expression levels of the three hub genes (CRKL, PTEN and TPM3) in PTC were markedly higher than in non-cancerous thyroid samples (Fig. 9B). Pearson's correlation analysis was performed to assess the relationship between miR-486-5p and the hub genes. A statistically significant

negative correlation between miR-486-5p and CRKL ($r=-0.111$; $P=0.013$; Fig. 9C) and TPM3 ($r=-0.089$; $P=0.044$; Fig. 9D) was observed. Finally, the protein expression levels of CRKL, PTEN and TPM3 in PTC tissues were visualized using immunohistochemical staining images from The Human Protein Atlas database. In line with the gene expression data, protein expression of CRKL, PTEN and TPM3 was increased in PTC compared with in normal thyroid tissue (Fig. 10).

Discussion

In the present study, significantly decreased expression levels of miR-486-5p in PTC were demonstrated by analysis of TCGA, GEO (GSE40807, GSE57780, GSE62054 and GSE73182) and ArrayExpress (E-MTAB-736) data, which included 603 PTC and 125 normal thyroid tissue samples. Downregulation of miR-486-5p was associated with cancer stage, pathological lymph node, metastasis, neoplasm and disease recurrence, all of which are clinical indicators of poor disease outcome. SROC curve analysis of miR-486-5p data from TCGA, GEO (GSE40807, GSE57780, GSE62054 and GSE73182) and ArrayExpress (E-MTAB-736) demonstrated that expression levels could provide high diagnostic value for PTC with an AUC of 0.84 (95% CI: 0.81-0.87). In addition, downregulation of miR-486-5p in patients with PTC may lead to worse

OS. The biological functions of miR-486-5p in facilitating the onset and progression of PTC were also explored. The potential miR-486-5p target mRNAs were found to be enriched in diverse signaling pathways, including 'hsa05200: Pathways in cancer' and 'hsa05206: MicroRNAs in cancer'. Furthermore, three hub genes, CRKL, PTEN and TPM3, were identified, all of which were notably upregulated in PTC samples based on analysis using the cBioPortal for Cancer Genomics. Therefore, it may be hypothesized that downregulated miR-486-5p serves as a clinical biomarker for PTC and exerts a potential biological role in progression of the disease.

There is accumulating evidence suggesting that aberrant miRNA expression levels contribute towards PTC development and progression. Several studies have identified a crucial role for miR-486-5p during onset and progression of various types of cancer; for example, miR-486-5p is markedly downregulated in non-small cell lung cancer, osteosarcoma, breast cancer, myxoid liposarcoma and pancreatic ductal adenocarcinomas (43-47). Conversely, miR-486-5p is upregulated in chronic myelocytic leukemia and myeloid leukemia of Down syndrome (48,49). Therefore, miR-486-5p may be differentially expressed depending on cancer type. To the best of our knowledge, only one study has explored miR-486-5p expression levels in PTC. Ma *et al* (50) demonstrated that miR-486-5p was predominantly downregulated in PTC tissues and cell lines, whereas miR-486-5p upregulation resulted in suppression of proliferation and growth by targeting FBN1. However, a limitation to this study is that it only analyzed 20 paired PTC and normal thyroid samples by reverse transcription-quantitative polymerase chain reaction (50). In the present study, integration of results from multiple studies via bioinformatics analysis revealed that miR-486-5p expression levels were decreased in PTC, and may have diagnostic and prognostic value for patients with PTC. Further investigations *in vivo* and *in vitro* are required to identify the underlying molecular mechanism of miR-486-5p in PTC. Exploring miR-486-5p expression levels in neoplastic tissues from patients with PTC will aid in understanding its clinical value.

miR-486-5p is upregulated by miR-660-5p via the miR-660-5p-mouse double minute 2 homolog-*proto-oncogene-p53* axis in lung cancer (51). In gastric cancer, miR-486-5p acts as a tumor suppressor and its downregulation facilitates tumor growth by increasing transcription of the anti-apoptotic factor, olfactomedin 4 (52). Similarly, miR-486-5p enhances hepatocellular carcinoma chemosensitivity to sorafenib and regulates metastasis by targeting citron rho-interacting serine/threonine kinase and claudin 10 (53). *In vitro*, Borjigin *et al* (46) revealed that the FUS RNA binding protein-DNA damage inducible transcript 3 fusion protein suppressed miR-486-5p expression leading to induction of plasminogen activator inhibitor-1 expression in human myxoid liposarcoma. Overall, these results suggest that miR-486-5p may act as a tumor suppressor in various types of cancer. However, most of the aforementioned studies only investigated individual mRNA targets of miR-486-5p, whereas the present study utilized pathway enrichment and network analysis to identify possible targets of miR-486-5p. Certain target genes were associated with 'Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism', 'Cytoplasm' and 'RNA binding' in the GO functional enrichment analysis. Notably, the three genes CRKL,

Table II. Microarray and RNA sequencing datasets.

Contributor (s)	Country	Year	Dataset	PTC			Normal			TN	FN	TP	FP	TN	(Refs.)
				No.	Mean	SD	No.	Mean	SD						
Lassalle <i>et al</i>	France	2014	GSE40807	38	6.426	0.992	40	7.450	1.819	33	15	5	25	(22)	
Saiselet <i>et al</i>	Belgium	2015	GSE57780	3	10.648	0.133	3	12.195	0.994	3	0	0	3	Unpublished data	
Stokowy <i>et al</i>	Norway	2014	GSE62054	17	5.000	1.003	8	6.176	1.349	13	2	4	6	Unpublished data	
Grazia Borrello <i>et al</i>	Italy	2016	GSE73182	19	9.716	0.171	5	9.952	0.173	14	0	5	5	(23)	
Rossing <i>et al</i>	Denmark	2014	E-MTAB-736	12	8.048	0.593	10	8.914	0.579	8	0	4	10	(24)	
TCGA	USA	2017	TCGA	514	8.606	1.304	59	11.058	1.179	409	4	105	55	-	

Mean indicates the average expression of miR-486-5p. FN, false negative; FP, false positive; PTC, papillary thyroid carcinoma; SD, standard deviation; TCGA, The Cancer Genome Atlas; TN, true negative; TP, true positive.

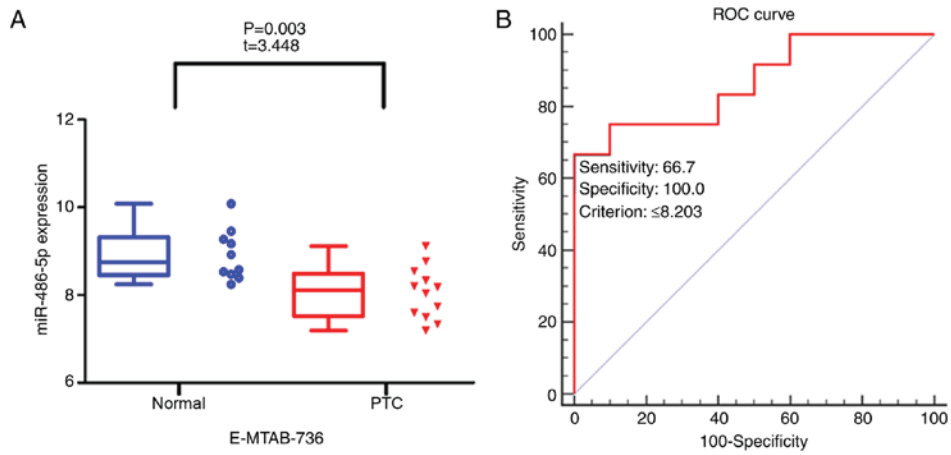


Figure 4. (A) Relative expression levels of miR-486-5p in PTC vs. normal thyroid tissue using the E-MTAB-736 dataset from ArrayExpress. (B) ROC curve analysis of miR-486-5p in discriminating PTC from normal controls. CI, confidence interval; miR, microRNA; PTC, papillary thyroid carcinoma; ROC, receiver-operating characteristic; SENS, sensitivity; SMD, standard mean difference; SPEC, specificity; TCGA, The Cancer Genome Atlas.

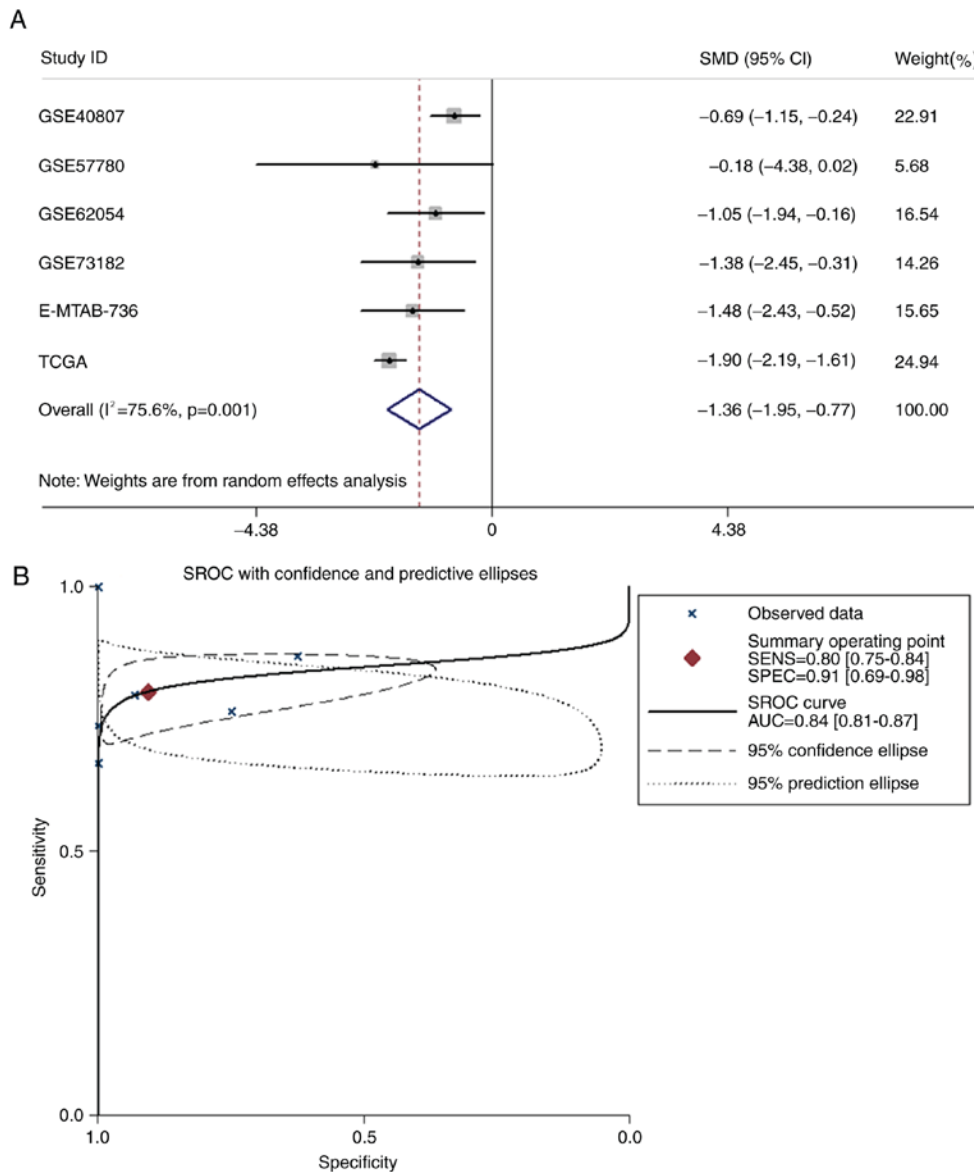


Figure 5. Meta-analysis of miR-486-5p expression levels in PTC. (A) Forest plot of all datasets evaluating miR-486-5p expression between PTC and normal thyroid tissue. (B) SROC curve for miR-486-5p in the diagnosis of PTC for all studies. Datasets from TCGA, GSE40807, GSE57780, GSE62054, GSE73182 and E-MTAB-736 were included in the meta-analysis. AUC, area under curve; CI, confidence interval; miR, microRNA; PTC, papillary thyroid carcinoma; SROC, summary receiver operating characteristic; TCGA, The Cancer Genome Atlas.

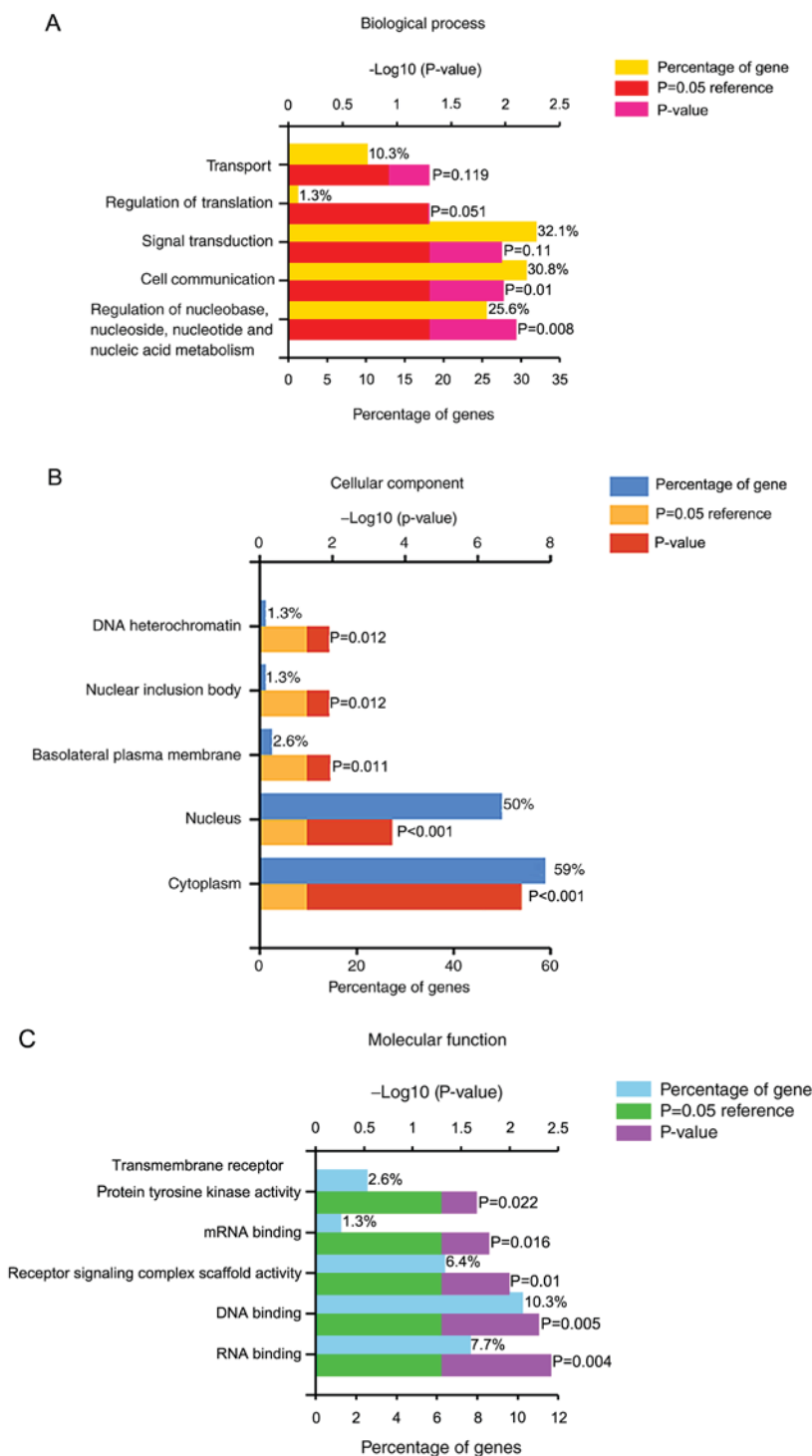


Figure 6. Functional enrichment analysis of potential microRNA-486-5p target genes in the Gene Ontology categories (A) biological process, (B) cellular component and (C) molecular function.

PTEN and TPM3 were identified in both 'hsa05200: Pathways in cancer' and 'hsa05206: MicroRNAs in cancer' through KEGG pathway enrichment analysis; therefore, these genes may be essential to the pathogenesis and progression of PTC.

CRKL encodes for a substrate of the BCR-ABL proto-oncogene fusion gene, and has a pivotal role in fibroblast transformation and may be oncogenic (54). Yang *et al* (55) reported that CRKL is associated with cervical lymph node metastasis and invasion of capsules in PTC, which has clinical implications on treatment. PTEN acts as an oncogene and is

highly prevalent in various cancers (56-58). Fang *et al* (59) demonstrated that ribonucleotide reductase catalytic subunit M1 induces PTEN expression and decreases Akt serine/threonine kinase phosphorylation in a ribonucleotide reductase activity-independent manner in PTC. In addition, Zhao *et al* (60) demonstrated that PTEN mRNA levels may serve as a biomarker for PTC onset and act as a molecular diagnostic index. TPM3 encodes a member of the tropomyosin family of actin-binding proteins and is frequently involved in genetic rearrangements resulting in fusion with the neurotrophic

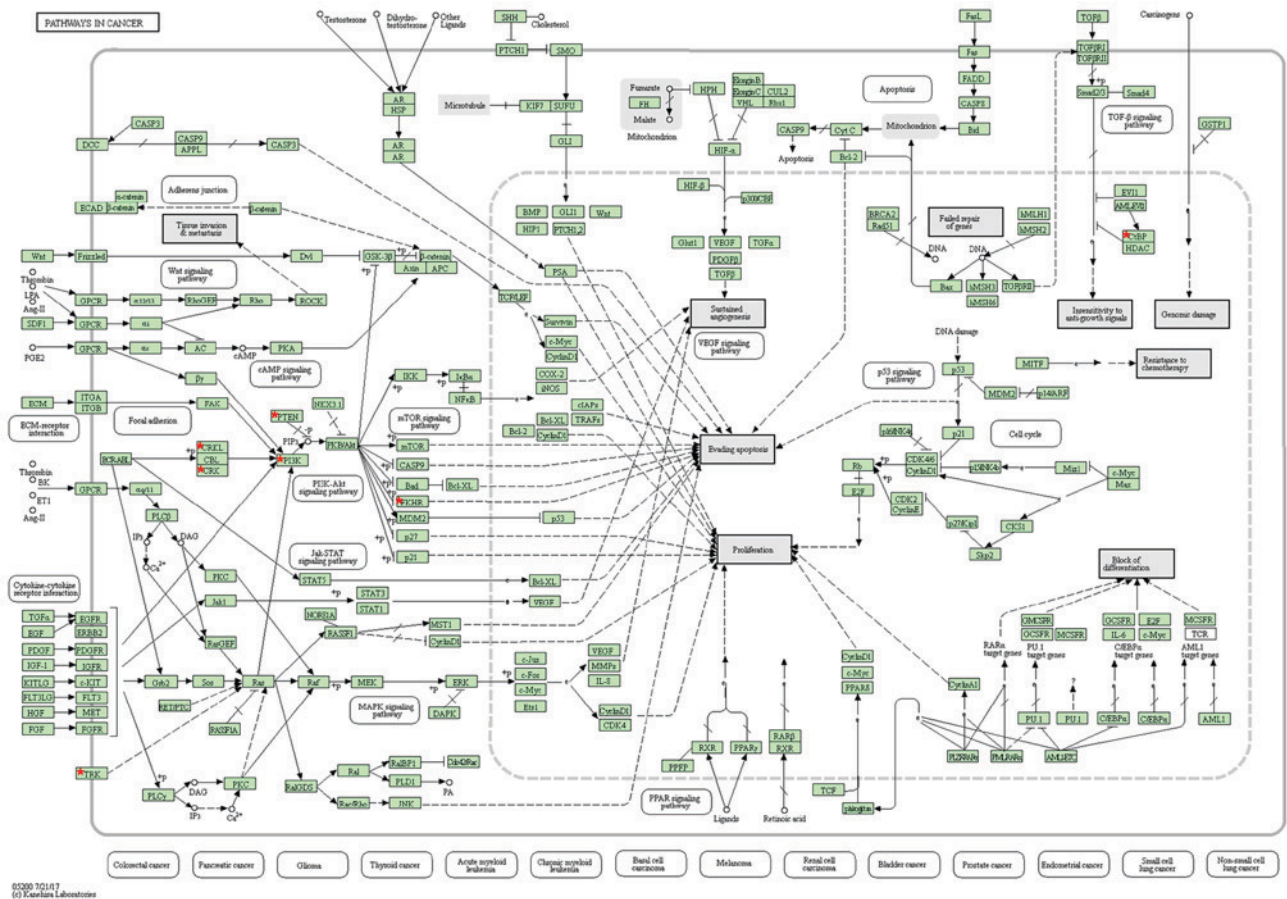


Figure 7. Kyoto Encyclopedia of Genes and Genomes pathway map illustrating 'hsa05200: Pathways in cancer', as identified by pathway enrichment analysis using the Database for Annotation, Visualization and Integrated Discovery. Enriched target genes of miR-486-5p are shown in red. miR, microRNA.

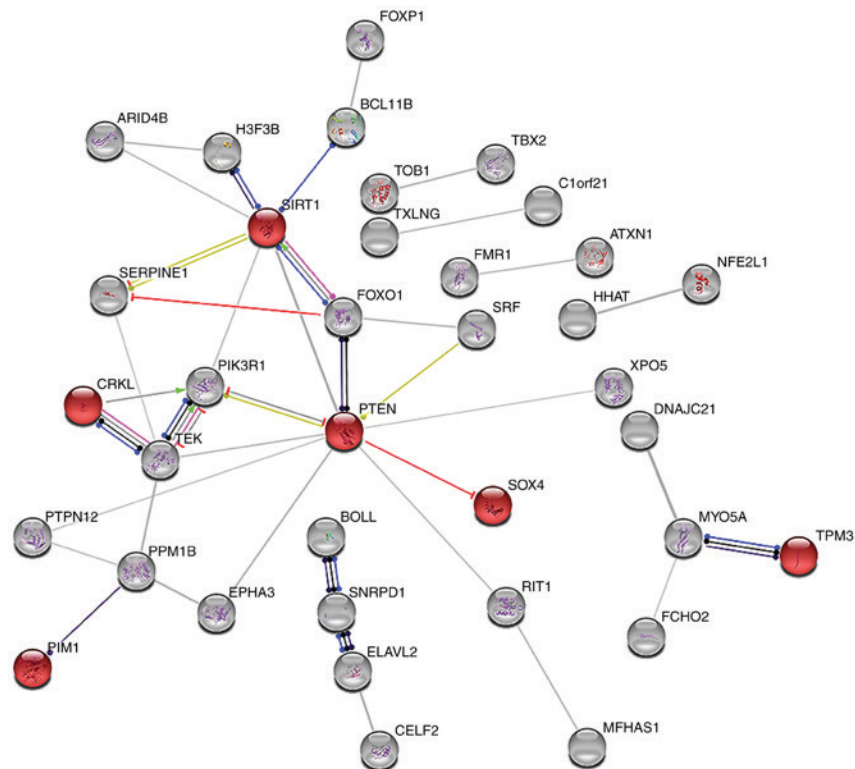


Figure 8. Kyoto Encyclopedia of Genes and Genomes pathway map illustrating 'hsa05206: MicroRNAs in cancer', as identified by protein-protein interaction network analysis using STRING. The network map was generated in the Database for Annotation, Visualization and Integrated Discovery 6.8. Enriched target genes of miR-486-5p are shown in red. miR, microRNA.

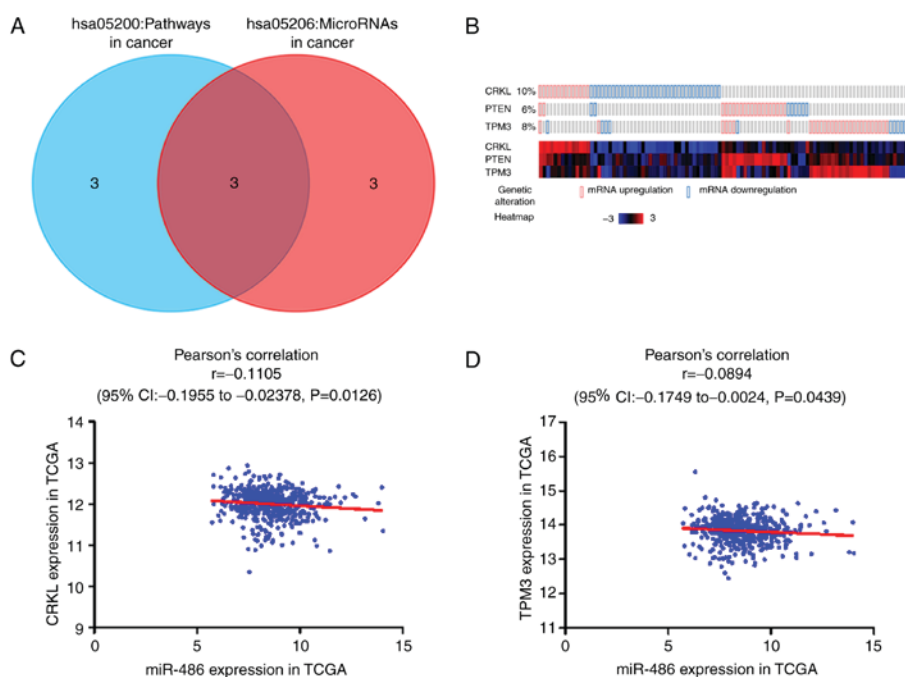


Figure 9. Expression of the hub genes CRKL, PTEN and TPM3 in PTC. (A) Venn diagram showing overlap between predicted miR-486-5p target genes in the 'hsa05200: Pathways in cancer' and 'hsa05206: MicroRNAs in cancer'. (B) Expression levels of CRKL, PTEN and TPM3 using the cBioPortal for cancer genomics using data from TCGA. The percentages indicate the proportion of HCC patients ($n=371$) with genetic alterations detected. Pearson's correlation between (C) CRKL or (D) TPM3 and miR-486-5p expression using data from TCGA. CI, confidence interval; CRKL, CRK like proto-oncogene; miR, microRNA; PTC, papillary thyroid carcinoma; PTEN, phosphatase and tensin homolog; TCGA, The Cancer Genome Atlas; TPM3, tropomyosin 3.

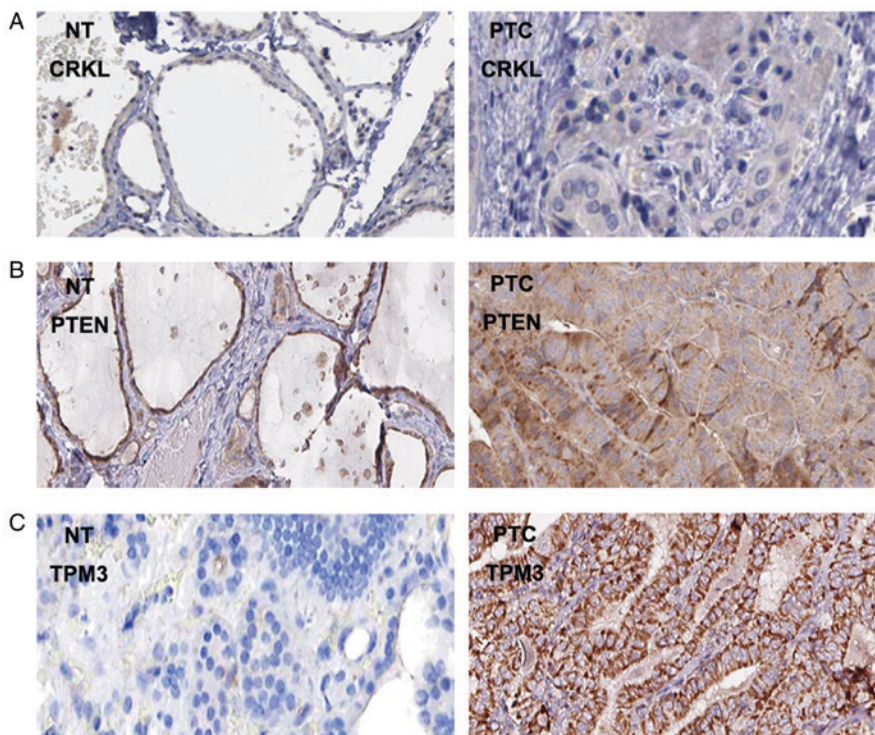


Figure 10. Immunohistochemical staining images from The Human Protein Atlas of (A) CRKL (<https://www.proteinatlas.org/search/CRKL>), (B) PTEN (<https://www.proteinatlas.org/search/PTEN>) and (C) TPM3 (<https://www.proteinatlas.org/search/TPM3>) in PTC vs. NT tissue. CRKL, CRK like proto-oncogene; NT, normal thyroid; PTC, papillary thyroid carcinoma; PTEN, phosphatase and tensin homolog; TPM3, tropomyosin 3.

tyrosine kinase receptor type 1 (NTRK1) gene, which then acts as an oncogene (61). Numerous studies that have demonstrated the association of NTRK1 with genetic rearrangements in PTC (62,63).

Notably, CRKL, PTEN and TPM3 were more highly expressed in PTC, and the results suggested that these genes may be regulated by miR-486-5p, whose expression is lower in PTC. It may be hypothesized that CRKL, PTEN and TPM3 have an

important role in PTC and are potential molecular targets in the clinic. Nevertheless, *in silico* target gene prediction algorithms have limited specificity, and further *in vitro* and *in vivo* investigations are required to confirm the function of miR-486-5p in PTC.

In conclusion, the present study revealed that the expression of miR-486-5p was significantly downregulated in PTC. The clinical implications of miR-486-5p were also evaluated using results from multiple datasets. Bioinformatics analysis was performed to elucidate the molecular mechanisms of miR-486-5p in facilitating the onset and progression of PTC. Three hub genes CRKL, PTEN and TPM3, were identified, which may be potential targets of miR-486-5p in PTC. The clinical importance of miR-486-5p in PTC and its underlying molecular mechanisms require further investigation; however, the results indicated that miR-486-5p may be involved in the pathogenesis of PTC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

D-YW, HY, GC and YH designed the study and performed the experiments. D-HP, PL, Q-YM, Y-HL, J-QC, GC and Y-PW participated in data processing and statistical analysis. D-YW and Y-HL wrote the manuscript. D-YW, Y-HL, YH and HY critically revised the manuscript. All authors 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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