Progression Risk Assessment of Post-Surgical Papillary Thyroid Carcinoma Based on Circular RNA-Associated Competing Endogenous RNA Mechanisms

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Abstract

Background

Accurate risk assessment of post-surgical progression in papillary thyroid carcinoma (PTC) patients is critical. Exploring key differentially expressed mRNAs (DE-mRNAs) regulated by differentially expressed circRNAs (DE-circRNAs) via the ceRNA mechanism could help establish a novel assessment tool.

Methods

tRNA network was established based on differentially expressed RNAs and correlation analysis. DE-mRNAs within the tRNA network associated with progression-free interval (PFI) of PTC were identified to construct a prognostic tRNA regulatory subnetwork. LASSO-Cox regression was applied to identify hub DE-mRNAs and establish a novel DE-mRNA signature in predicting PFI of PTC.

Results

Six hub DE-mRNAs, namely CLCNKB, FXBO27, FXYD6, RIMS2, SPC24, and CDKN2A, were identified to be most significantly related to the PFI of PTC and a prognostic DE-mRNA signature was proposed. A nomogram incorporating the DE-mRNA signature and clinical parameters was established to improve the progression risk assessment in post-surgical PTC, which was superior to the ATA risk stratification system and MACIS score AJCC staging system.

Conclusions

Based on the circRNA-associated ceRNA RNA mechanism, a DE-mRNA signature and prognostic nomogram was established, which may improve the progression risk assessment in post-surgical PTC.

Background

Thyroid cancer, originating from thyroid follicular epithelial cells, is the most common malignancy of the endocrine system [1]. Thyroid cancer is currently the eighth most common malignancy around the world [2] and its incidence is rapidly increasing worldwide. There are four main types of thyroid cancer, including papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, anaplastic thyroid carcinoma, and medullary thyroid carcinoma. Of these, PTC accounts for more than 80% of all thyroid cancer cases. Among all types of thyroid cancer, PTC has the highest incidence with a higher proportion of young patients. Genetic mutations and environmental exposures are the major risk factors for PTC in which BRAF V600E is the most common mutation. The primary treatment for PTC is thyroidectomy. Most PTCs grow slowly and 90% of patients have a relatively good prognosis after treatment, with a low postoperative recurrence rate less than 10% [3]. However, a small percentage of patients experience high risk of recurrence and metastasis after surgery, resulting in a poor prognosis.
The molecular mechanisms leading to PTC recurrence remain unclear. Moreover, there is a lack of satisfactory assessment tools to precisely evaluate the post-surgical risk of recurrence in PTC. The American Thyroid Association (ATA) currently recommends the use of American Joint Committee on Cancer (AJCC) staging system and MACIS score to predict mortality after PTC, and proposed the risk stratification system for risk assessment of post-surgical progression in PTC [3]. However, the accuracy of current available assessment tools is inadequate to meet the requirements of clinical work. The challenge of treating PTC lies in balancing side effects and benefits of treatment [4]. Patients with low-risk and high-risk PTC should be differentiated to adopt different treatment strategies. Patients with high risk of recurrence require more extensive surgical resection and adjuvant radioactive iodine therapy to improve post-surgical prognosis and prevent recurrence. Secondary surgery for recurrence can result in additional surgical trauma with a higher risk of recurrent laryngeal nerve injury. Alternatively, PTC patients with low risk of recurrence should receive a more conservative treatment to reduce surgical trauma and improve quality of life. Unnecessary post-surgical radioactive iodine therapy and thyroid stimulating hormone (TSH) suppression therapy should also be avoided. Long-term subclinical hyperthyroidism caused by a high dose of TSH suppression can lead to multiple potential side effects, including osteoporosis, atrial fibrillation, heart discomfort, and an increased risk of fractures and heart disease in elderly patients [5]. Unnecessary radioactive iodine therapy also increases the risk of developing other cancers. Therefore, more accurate assessment tools to assess the risk of progression in post-surgical PTC patients are urgently required.

Non-coding RNAs such as microRNAs (miRNAs), long non-coding RNAs, and circular RNAs (circRNAs) are major components of the human transcriptome. circRNAs were first discovered in human cells in 1986 and are critical in the pathophysiology of several human diseases including cancer [6]. circRNAs are products of a reverse splicing mechanism and are resistant to RNase R [7]. circRNAs contain a single-stranded covalent closed-loop structure that have neither a 5'-3' polarity nor a polyadenylated tail. circRNAs are primarily located in the cytoplasm and regulate gene expression by acting as a miRNA sponge at the transcriptional or post-transcriptional stage, affecting protein translation and thus the regulation of cellular processes. According to the competitive endogenous RNA (ceRNA) hypothesis, noncoding RNAs like circRNAs can positively regulate mRNA expression by competitive binding of its shared miRNAs [8]. Recent studies further reveal that several circRNAs differentially expressed in cancers can exert a tumor promoting or suppressing role via ceRNA mechanism, thus regulating apoptosis, proliferation, invasion and metastasis, etc. mRNAs regulated by differentially expressed circRNAs (DE-circRNAs) may be more likely to play a functional role in cancer. Particularly in PTC, the upregulated SOX2 by circ_0005273/hsa-miR-1183 axis promotes the proliferation, invasion, and metastasis of PTC [9]. Transforming growth factor alpha, upregulated by hsacirc0060060/hsa-miR1443p axis in PTC, was identified to promote the proliferation and autophagy of PTC and was associated with poor prognosis [10]. The establishment of circRNA-miRNA-mRNA regulatory networks in PTC may provide key targets and novel regulatory pathways in understanding the progression of PTC.

The primary objective of this study is to explore key differentially expressed mRNAs (DE-mRNAs) regulated by DE-circRNAs associated ceRNA regulatory mechanism in PTC to elucidate the post-surgical
progression of PTC and establish a novel assessment tool.

**Methods**

**Identification of DE-circRNAs, DE-miRNAs, and DE-mRNAs in PTC**

Expression data of circRNAs and related clinical data for PTC were downloaded from the GSE93522 dataset based on GPL19978 platform [11]. An annotation file provided by the manufacturers was used to match the probes with corresponding circRNA IDs. The median ranking value was used to determine the expression value if multiple probes matched a single circRNA ID. The ‘LIMMA’ R package was used to identify DE-circRNAs between cancer and normal tissues [12]. The cutoff value was set at $|\log_2\text{FC}| > 1$ and $p < 0.05$. The basic characteristics of DE-circRNAs were obtained from circBase (https://www.circbase.org/) and the corresponding structures were analyzed using the cancer-specific circRNA database (CSCD) (https://gb.whu.edu.cn/CSCD/#/).

Normalized RNA sequencing data in the form of millions of transcripts (TPM) for miRNA and mRNA and related clinical data of post-surgical PTC were downloaded from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/), up to July 1, 2020). TCGA-THCA dataset originally included 507 cases, 510 tumor samples, and 58 normal tissue samples. A total of 495 cases of PTC with matching tumor tissue and clinical information, and 58 normal samples of thyroid tissue were eventually included in the current analysis following the removal of five cases without matching tumor samples, two cases with poorly differentiated oncolytic carcinoma or follicular carcinoma, five cases with history of neoadjuvant therapy and eight samples of metastases. The ‘LIMMA’ R package was used to identify DE-miRNAs and DE-mRNAs between cancer and normal tissues with a cutoff value set at $|\log_2\text{FC}| > 1$ and a false discovery rate (FDR) < 0.05. GEPIA (http://gepia.cancer-pku.cn) incorporating RNA sequencing expression data of tumors and normal samples from TCGA and the Genotype-Tissue Expression (GTEx) projects was used to validate the differential expression level of a specific DE-mRNA [13]. Data of copy number alterations and mutation were downloaded from Cbioportal (http://www.cbioportal.org/).

**Construction of the circRNA-miRNA-mRNA network**

CIRCinteractome is a computational tool that enables the prediction and mapping of binding sites for RNA-binding proteins and miRNAs on reported circRNAs [14]. circRNA-miRNA relationships were predicted using CIRCinteractome. miRWalk is an open-source platform that provides predicted and validated miRNA-binding sites with a machine learning algorithm, incorporating data from TargetScan (conserved site context scores, version 7.1), miRDB (release 5.0), and validated information from miRTarBase (version 7.0) [15]. miRNA-mRNA relationships were predicted using miRWalk 3.0 with score $\geq 0.95$. Based on differential expression data, only relationship pairs with negative correlation were retained. The correlation between DE-miRNA and DE-mRNA expression with potential ceRNA regulatory relationships was further analyzed using Pearson's correlation coefficient in TCGA-THCA dataset. Only miRNA-mRNA relationships with $r < -0.2$ and $p < 0.05$ were retained in the final circRNA-miRNA-mRNA network. To construct a ceRNA regulatory subnetwork associated with PFI of PTC, univariate Cox regression analysis
was performed based on the DE-mRNAs involved. Only DE-mRNAs associated with PFI of PTC and the corresponding circRNA-miRNA and miRNA-mRNA pairs were retained in the PFI related subnetwork. The ceRNA network was visualized using Cytoscape v.3.8.0 (https://www.cytoscape.org/). The Sankey diagram that represented the ceRNA regulatory relationship was drafted using Origin 2020 (https://www.originlab.com/).

Bioinformatic analysis of the circRNA-miRNA-mRNA network

To investigate the underlying biological function of ceRNA regulatory relationships in PTC, DAVID (https://david.ncifcrf.gov/) was employed to explore enriched biological processes, cellular components, molecular functions, and significantly relevant signal pathways of DE-mRNAs involved, using default parameters [16]. P < 0.05 was regarded statistically significant.

Identification of PFI related hub DE-mRNAs and establishment of the prognostic DE-mRNA signature

In this study, PFI was selected as the main endpoint. DE-mRNAs associated with PFI were identified based on TCGA-THCA dataset using a univariate Cox proportional hazards regression model. Normalized gene expression data were transformed on the base-2 logarithm for further survival analysis. DE-mRNAs with p < 0.05 were considered statistically significant for further analysis. Only cases with follow-up > 30 days were included for survival analysis. The 492 eligible TCGA cases were subsequently randomly divided into a training dataset and a validation dataset in a 7:3 ratio. LASSO penalized Cox regression analysis was performed to select prognostic hub DE-mRNAs related to PFI, and constructed a prognostic DE-mRNA signature in patients with PTC based on a linear combination of the regression coefficient derived from LASSO-Cox regression model coefficients (β) multiplied with its normalized mRNA expression level in the training dataset. Patients were divided into the high-risk and the low-risk group based on the optimal cut off value determined by X-Tile [17]. Performance of the prognostic DE-mRNA signature was assessed using Harrell’s concordance index (C-index), area under the curve (AUC) of the receiver operating characteristic (ROC) curve, and Kaplan–Meier analysis. The validation dataset and TCGA-THCA dataset were further utilized for validation.

Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) was performed to compare the molecular alteration in the high-risk group and the low-risk group against previously annotated gene sets [18]. Samples from TCGA-THCA dataset were divided into the high-risk group and the low-risk group according to the optimal cut off value determined by X-Tile. Thereafter, GSEA analysis was run on javaGSEA v4.0.3 based on the Molecular Signatures Database v7.1. H: Hallmark gene sets, C2: curated gene sets and C5: gene ontology gene sets were searched to identify enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, biological processes, cellular components, molecular functions, and specific well-defined biological states or processes associated with poorer survival of the high-risk group. Gene sets with |NES| > 1 and FDR < 0.25 were considered statistically significant.
Validation of the independent prognostic role of the DE-mRNA signature

To validate the independent prognostic role of the DE-mRNA signature, univariate and multivariate Cox regression analyses were performed on the prognostic DE-mRNA signature and clinical parameters, including age, PFI status, mutational status of \textit{BRAF} V600E, \textit{RAS}, \textit{RET}, \textit{NTRK1}, and \textit{TERT}, sex, histological subtype, T stage, N stage, M stage, AJCC stage, residual tumor status, extrathyroidal extension, multifocality, and anatomic site in TCGA dataset. Parameters with p < 0.25 in univariate analysis were further included in the multivariate Cox regression analysis. P < 0.05 was considered statistically significant.

Building and validation of a prognostic nomogram

Following a test for collinearity, independent prognostic parameters and relevant clinical parameters were included to construct a prognostic nomogram to predict 1-year, 2-year, 3-year, 4-year, and 5-year PFI of PTC patients in the entire TCGA dataset with the stepwise Cox regression model. Discrimination of the nomogram was assessed with the C-index calculated by a bootstrap method with 1000 resamples. The predicted progression-free survival of nomogram against observed survival rates was plotted using the calibration curve. The performance of the prognostic nomogram was further assessed with ROC curves and Kaplan–Meier analysis. The ATA risk stratification system, MACIS score and AJCC staging system were used as references for comparison of AUCs. Information of ATA risk stratification and MACIS Score for each case was obtained from the official TCGA publication [19].

Statistical analysis

Statistical analysis was conducted using R software v3.4.3 (https://www.r-project.org/) and GraphPad Prism v8.01 (https://www.graphpad.com/). Categorical variables were analyzed with $\chi^2$ test or Fisher's exact test. Continuous variables of two groups were analyzed with Student's t test. Multiple groups of continuous variables were analyzed with a one-way ANOVA test. DE-mRNAs associated with progression-free survival were identified based on univariate and multivariate Cox regression analysis. Hazard ratio (HR) and 95% confidence interval (CI) were calculated. Correlation between two variables was analyzed with Pearson's correlation coefficient. ROC curves were analyzed using the ‘timeROC’ R package and the AUCs were compared with the method described by Paul Blanche et al [20]. A two-tailed p value <0.05 was considered statistically significant if there was no special statement.

Results

Identification of DE-circRNAs, DE-miRNAs, and DE-mRNAs in PTC

Workflow of the current study is shown in Fig. 1A. Differentially expressed circRNAs, miRNAs, and mRNAs between PTC and normal thyroid tissues were identified after normalization of expression data. A total of 138 DE-circRNAs were obtained from GSE93522, of which 117 DE-circRNAs were upregulated and 21 DE-
circRNAs were downregulated (Table S1). DE-miRNAs and DE-mRNAs were obtained from TCGA-THCA dataset. Altogether, 113 DE-miRNAs and 2252 DE-mRNAs were identified (Table S2 and Table S3). Among them, 32 DE-miRNAs and 905 DE-mRNAs were upregulated, and 81 DE-miRNAs and 1347 DE-mRNAs were downregulated.

**Prediction of potential circular RNA-associated ceRNA regulatory relationships and functional enrichment analysis**

circRNA-miRNA relationships were predicted using Circular RNA Interactome. miRNA-mRNA relationships were predicted using mirWalk 3.0. Based on the targeted relationship combined with differential expression data, 93 potential circRNA-miRNA pairs and 1105 miRNA-mRNA pairs were identified, which involved 54 DE-circRNAs, 14 DE-miRNAs, and 698 DE-mRNAs (Fig. 1B–1D, Table S4 and Table S5).

To investigate the underlying biological function of ceRNA regulatory relationships in PTC, functional enrichment analysis was performed based on the 698 DE-mRNAs involved (Fig. 2A–2D and Table S6). Biological processes associated with malignant phenotype were significantly enriched, which included regulation of cell adhesion, extracellular matrix disassembly and organization, positive regulation of cell proliferation, and angiogenesis. Processes associated with intracellular signal transduction were also significantly enriched, particularly the positive regulation of the mitogen-activated protein kinase cascade. In terms of KEGG pathways, DE-mRNAs were significantly associated with transcriptional mis-regulation and miRNAs in cancer. Multiple tumor-associated signaling pathways were also enriched, including p53 and PI3K-Akt signaling pathways.

**Discussion**

Accumulating evidence has shown that circRNAs and their mediated ceRNA regulatory networks play a vital role in pathogenesis and progression of various human cancers, including PTC. hsacirc0058124 was previously identified to be upregulated in PTC and associated with poor prognosis. By sponging hsa-miR2185p, hsacirc0058124 promoted proliferation, invasion, and metastasis of PTC via the **NOTCH3**/**GATAD2A** signaling axis [21]. Through regulation of **ABCA9** and **MTA1** via sponge hsa-miR-1179 and hsa-miR-1205, upregulated hsa_circ_0039411 promoted proliferation, migration, and invasion of PTC cells and inhibited cell apoptosis [22]. CircRNAs are resistant to exonase and RNase R, enabling them to be more stable than other types of ncRNAs as well as play an important regulatory role in cancer cell. The current understanding of the circRNA-related ceRNA network in PTC is limited, requiring further study.

In this study, DE-circRNAs, DE-miRNAs, and DE-mRNAs with potential ceRNA regulatory relationships in PTC were identified through comprehensive analysis of targeting relationship prediction and differential expression data. Based on the further correlation analysis of expression between DE-miRNAs and DE-mRNAs with potential ceRNA regulatory relationships, a circRNA-miRNA-mRNA regulatory network was constructed in PTC. DE-mRNAs within the ceRNA network associated with PFI of PTC were further identified to construct a ceRNA regulatory subnetwork associated with PFI. Six hub DE-mRNAs, namely
CLCNKB, FXBO27, FXYD6, RIMS2, SPC24, and CDKN2A, were identified to be most significantly related to the PFI of PTC, which were regulated by three DE-miRNAs, including hsa-miR-146b-3p, hsa-miR-139-5p, and hsa-miR-139-3p. They were subsequently regulated by eleven DE-circRNAs via the ceRNA mechanism, including hsa_circ_0003645, hsa_circ_0089153, hsa_circ_0005699, hsa_circ_0007146, hsa_circ_0038718, hsa_circ_0001658, hsa_circ_0008784, hsa_circ_0000965, hsa_circ_0001917, hsa_circ_0008354, and hsa_circ_0049271. These newly identified key circRNA-miRNA-mRNA regulatory relationships may help elucidate the molecular mechanism of progression in post-surgical PTC. Moreover, they may provide novel therapeutic targets for treatment of recurrence in PTC. Hub DE-mRNAs identified in the ceRNA network were potential predictors of PFI in PTC.

Although most patients with PTC have a relatively good prognosis, a portion of patients will eventually develop post-surgical recurrence. These high-risk PTC patients should be distinguished early enough to adopt more aggressive treatment options, additional adjuvant radioactive iodine therapy, thyroid hormone suppression therapy with a higher dosage, and timely intervention to prevent post-surgical progression if necessary. In contrast, for the remaining low-risk PTC patients, aggressive treatment options should be avoided to improve quality of life. Therefore, it is critical to accurately predict the post-surgical risk of progression in patients with PTC. Currently, the ATA guidelines recommend the use of AJCC staging system and MACIS score to predict the risk of postoperative mortality. ATA risk stratification system was recommended to assess post-surgical risk of recurrence. The existing risk assessment tools are not sufficiently accurate and a novel prediction system should be established to more accurately predict the prognosis of PTC patients. In the current study, we established a circRNA associated ceRNA network in the PTC. Based on this, six hub PFI related DE-mRNAs of the PTC were identified. A six-DE-mRNA signature was subsequently established, which was able to distinguish high-risk PTC patients from the low-risk ones and could accurately predict the PFI. Nomogram has been widely applied in oncology to assess the prognosis of cancer patients [23]. Nomogram can integrate various prognostic factors, including molecular and clinical parameters, and provide a visual graphical interface for personalized prediction of clinical events. In this study, to establish a more accurate assessment tool for prognosis evaluation in post-surgical PTC, a prognostic nomogram was established incorporating the DE-mRNA signature and clinicopathological parameters. This nomogram was able to accurately predict PFI of PTC and was better than the ATA risk stratification system, MACIS score and AJCC staging system recommended by ATA guidelines.

Currently, 11 DE-circRNAs were identified to regulate the six hub DE-mRNAs via the ceRNA mechanism in the study. The role of hsa_circ_0089153 in PTC has previously been reported. hsa_circ_0089153 was upregulated in clinical specimens of PTC [24]. In vitro experiments indicated that downregulation of hsa_circ_0089153 expression could inhibit proliferation, migration, and invasion of PTC cells. The luciferase reporter assay confirmed that hsa_circ_0089153 sponged hsa-miR-145 to mediate upregulation of ZEB2 expression, thereby playing a carcinogenic role in PTC. hsa_circ_0089153 was also reported to be upregulated in gastric cancer and bladder cancer [25, 26]. Our study indicated that upregulated hsa_circ_0089153 may sponge hsa-miR-139-3p, and upregulated SPC24 and CDKN2A expression to promote PTC progression. hsa_circ_0003645 was previously identified to be upregulated in non-small cell
lung cancer tissue [27]. hsa_circ_0038718 was reported to be upregulated in hepatocellular carcinoma [28]. Our result suggested that hsa_circ_0003645 and hsa_circ_0038718 may also play an oncogenic role as a sponge of hsa-miR-139-3p. The function of hsa_circ_0001917 in PTC has not been reported. However, hsa_circ_0001917 was also identified to be upregulated in hepatocellular carcinoma [28]. Our result suggested that the upregulated hsa_circ_0001917 may sponge hsa-miR-139-5p, promoting PTC via upregulation of RIMS2. The function of hsa_circ_0005699 in cancer was controversial. hsa_circ_0001917 was previously reported to downregulate MCM8 and NCPAPD2 expression by acting as a sponge of hsa-miR-504, functioning as tumor suppressor in gastric cancer [29]. Our study suggested that hsa_circ_0005699 may promote PTC as a sponge of hsa-miR-139-3p and upregulate SPC24 and CDKN2A expression. The function of the remaining six circRNAs in cancer is still known. Our study suggests that the upregulation of hsa_circ_0007146 may promote PTC through the sponge of hsa-miR-139-3p. hsa_circ_0001658, hsa_circ_0008784, and hsa_circ_0000965 may upregulate RIMS2 expression as a sponge of hsa-miR-139-5p, playing an oncogenic role in PTC. Moreover, downregulated hsa_circ_0008354 and hsa_circ_0049271 may sponge hsa-miR-146b-3p and suppress the expression of CLCNKB, FBXO27, and FXYD6. The function of these circRNAs in PTC require further experimental validation.

Six hub DE-mRNAs markedly associated with the PFI of PTC were identified in the current study. Upregulated SPC24 and CDKN2A were identified as targets of hsa-miR-139-3p. The tumor suppressing role of hsa-miR-139-3p has been identified in multiple tumors [30]. SPC24 is an important component of kinetochore-associated NDC80 complex. This complex mediates chromosome segregation and spindle checkpoint activity. SPC24 plays an important role in maintaining the integrity of kinetochore [31]. SPC24 was previously identified to be highly expressed in anaplastic thyroid cancer. Knockdown of SPC24 expression inhibited cell growth and invasion and promoted tumor cell apoptosis [32]. SPC24 was also reported to be highly expressed in hepatocellular carcinoma and was an independent predictor of survival [33]. Downregulation of SPC24 inhibited growth, invasion of tumor cells, and promoted apoptosis. The oncogenic role of SPC24 was also identified in breast cancer and lung cancer [34, 35]. Here, SPC24 was identified to be negatively regulated by hsa-miR-139-3p. This regulatory role between SPC24 and hsa-miR-139-3p was previously observed in bladder cancer [36]. CDKN2A is traditionally known as a tumor suppressor gene coding for two proteins, including the p16INK4a and p14arf [37]. CDKN2A is involved in cell cycle regulation. However, mounting data suggest that CDKN2A may play a dual role in multiple tumors. The p14arf that it codes plays an important role in invasion and metastasis and is associated with a poor prognosis [38]. Upregulation of p14arf has been identified in multiple hematological malignancies, aggressive types of B-cell lymphomas and bladder cancers. [39–41]. The oncogenic function of p14arf is associated with the autophagy regulation [38]. Downregulation of p14arf was shown to inhibit progression of lymphomas with the MYC mutation [42]. Particularly in PTC, p16INK4a and p14arf coded by CDKN2A were both identified to be upregulated in thyroid tumorigenesis [43]. Wild type p14arf has been observed to delocalize into the cytoplasm in aggressive PTC. Here, we further identified that CDKN2A was upregulated in PTC and was associated with shorter PFI, and hsa-miR-139-3p was the potential negative regulator of CDKN2A. The function of CDKN2A in PTC progression requires further study. RIMS2 was also identified to be upregulated in PTC and was potentially regulated by hsa-
miR-139-5p. hsa-miR-139-5p was identified to be downregulated in the primary tumor and further in PTC metastasis [44]. hsa-miR-139-5p was also able to be sponged by circBACH2 and relieved the suppression of the target gene LMO4 in PTC [45]. RIMS2 was identified as a novel target of hsa-miR-139-5p in this study. RIMS2 is a Rab effector and scaffold protein associated with exocytosis [46]. It was recently reported to have a high mutation rate in melanoma and mantle cell lymphoma [47, 48]. In this study, RIMS2 was identified as a hub DE-mRNA in the ceRNA network and was associated with PFI of PTC. The function of RIMS2 in PTC requires further experimental validation.

In this study, downregulated CLCNKB, FBXO27, and FXYD6 were identified to be novel targets of hsa-miR-146b-3p in PTC. hsa-miR-146b-3p was previously reported to be upregulated in PTC and positively associated with central lymph node metastases [49]. hsa-miR-146b-3p may promote invasion and metastasis of PTC by targeting NF2 [50]. hsa-miR-146b-3p also targeted PAX8 in modulating the differentiated phenotype of PTC [51]. FXYD6 is known as a specific modulator of Na, K-ATPase, and is expressed in multiple epithelial cells of the inner ear [52]. In accordance with the current study, FXYD6 was previously identified to be downregulated in PTC and was associated with poor prognosis [53]. CLCNKB is a voltage-gated chloride channel participating in the regulation of cell volume, membrane potential stabilization, signal transduction, and transepithelial transport [54]. CLCNKB was previously reported to be downregulated in renal carcinoma [55]. Hypermethylation in deletions of CLCNKB in renal carcinoma further indicated its tumor suppressing role in cancer [56]. FBXO27 is a component for substrate-recognition in the SCF-type E3 ubiquitin ligase complex [57]. Its role in cancer is currently unknown. In this study, CLCNKB, FBXO27, and FXYD6 were identified to be negatively regulated by hsa-miR-146b-3p. Together with hsa_circ_0008354 and hsa_circ_0049271 formed an important part of the PFI related ceRNA subnetwork we established. The potential tumor suppressing role of CLCNKB, FBXO27, and FXYD6 in PTC requires further validation.

To the best of our knowledge, the ceRNA network we established and the prognostic DE-mRNA signature we proposed has not been reported previously. The nomogram incorporating the DE-mRNA signature and clinical parameters was robust in predicting PFI of PTC. DE-circRNAs, DE-miRNAs, and DE-mRNAs were potential therapeutic targets for prevention and treatment of recurrent PTC. We acknowledge that our study inevitably has some limitations. Firstly, sequencing data and follow-up data of PTCs in our study were based on TCGA-THCA dataset. Most patients were from North America. Therefore, caution should be exercised in extrapolating this conclusion to other populations. Secondly, the targeting relationship of ceRNA is based on bioinformatic speculation and requires further experimental validation. Finally, the biological function of certain DE-circRNAs, DE-miRNAs, and DE-mRNAs require investigation with experiments in PTC.

**Conclusions**

In summary, this study revealed a circRNA associated ceRNA network in PTC. Based on survival analysis, a ceRNA subnetwork associated with PFI in PTC was identified. The molecules within the PFI related subnetwork may represent a promising target of treatment for patients of recurrent PTC. With the hub DE-
mRNAs identified within the subnetwork, a prognostic six DE-mRNA signature was established. The nomogram incorporating the DE-mRNA signature and clinical parameters is robust in predicting the PFI of post-surgical PTC.

**Abbreviations**

ATA: American Thyroid Association; AJCC: American Joint Committee on Cancer; AUC: area under the curve; CSCD: cancer-specific circRNA database; circRNAs: circular RNAs; ceRNA: competitive endogenous RNA; CI: confidence interval; DE-circRNAs: differentially expressed circRNAs; DE-miRNAs: differentially expressed miRNAs; DE-mRNAs: differentially expressed mRNAs; FDR: false discovery rate; GTEx: Genotype-Tissue Expression; GSEA: Gene Set Enrichment Analysis; C-index: Harrell's concordance index; HR: Hazard ratio; KEGG: Kyoto Encyclopedia of Genes and Genomes; miRNAs: microRNAs; TPM: millions of transcripts; PTC: papillary thyroid carcinoma; PFI: progression-free interval; TCGA: The Cancer Genome Atlas; TSH: thyroid stimulating hormone.

**Declarations**

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and materials:** The datasets analyzed during the current study are available in the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) and The Cancer Genome Atlas (https://portal.gdc.cancer.gov/).

**Competing interests:** The authors declare that they have no competing interests.

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**Authors' contributions:** ZL, XL and XX: conception and design. MW and RL: development of methodology. MW and HY: analysis and interpretation of data. MW: writing of the manuscript. XX and XL: review of the manuscript. ZL: study supervision.

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**Tables**
### Table 1. Basic characteristics of the 11 differently expressed circRNAs

| CircRNA ID       | logFC | Regulation | Position                  | Strand | Spliced length | Parental gene symbol |
|------------------|-------|------------|---------------------------|--------|----------------|----------------------|
| hsa_circ_0003645 | 2.46  | Up         | chr16:19656207-19663412   | +      | 356            | C16orf62             |
| hsa_circ_0089153 | 2.37  | Up         | chr9:134011326-134022971  | +      | 1102           | NUP214               |
| hsa_circ_0005699 | 2.32  | Up         | chr16:19627435-19663412   | +      | 1198           | C16orf62             |
| hsa_circ_0001658 | 1.57  | Up         | chr6:157357968-157406039  | +      | 48071          | ARID1B               |
| hsa_circ_0007146 | 1.48  | Up         | chr16:5077135-5078186     | -      | 420            | NAGPA                |
| hsa_circ_0038718 | 1.22  | Up         | chr16:27351506-27353580   | +      | 227            | IL4R                 |
| hsa_circ_0008784 | 1.22  | Up         | chr13:24164288-24200931   | +      | 479            | TNFRSF19             |
| hsa_circ_0000965 | 1.17  | Up         | chr19:58472758-58476205   | -      | 3447           | C19orf18             |
| hsa_circ_0001917 | 1.02  | Up         | chrX:41519691-41530783    | -      | 402            | CASK                 |
| hsa_circ_0008354 | -1.03 | Down       | chr19:34981280-34986676   | +      | 485            | WTIP                 |
| hsa_circ_0049271 | -1.34 | Down       | chr19:10610070-10610756   | -      | 686            | KEAP1                |

### Table 2
Table 2. Clinical features of patients in TCGA-THCA Dataset

| Clinical features                  | Training dataset | Validation dataset | Entire TCGA dataset | p value |
|-----------------------------------|------------------|--------------------|---------------------|---------|
| N                                 | 344              | 148                | 492                 |         |
| Follow-up time                    | 1222.17 ± 996.14 | 1237.36 ± 997.68  | 1226.74 ± 995.61    | 0.988   |
| DE-mRNA signature                 | 0.27 ± 0.49      | 0.24 ± 0.54        | 0.26 ± 0.50         | 0.816   |
| Age                               | 46.63 ± 16.08    | 48.72 ± 15.26      | 47.26 ± 15.85       | 0.407   |
| PFI                               |                  |                    |                     | 0.996   |
| Progression-free                  | 310 (90.12%)     | 133 (89.86%)       | 443 (90.04%)        |         |
| Progression                       | 34 (9.88%)       | 15 (10.14%)        | 49 (9.96%)          |         |
| BRAF V600E                        |                  |                    |                     | 0.996   |
| Wildtype                          | 132 (38.37%)     | 55 (37.16%)        | 187 (38.01%)        |         |
| Mutant                            | 201 (58.43%)     | 89 (60.14%)        | 290 (58.94%)        |         |
| NA                                | 11 (3.20%)       | 4 (2.70%)          | 15 (3.05%)          |         |
| RAS mutation                      |                  |                    |                     | 0.995   |
| Wildtype                          | 290 (84.30%)     | 127 (85.81%)       | 417 (84.76%)        |         |
| Mutant                            | 43 (12.50%)      | 17 (11.49%)        | 60 (12.20%)         |         |
| NA                                | 11 (3.20%)       | 4 (2.70%)          | 15 (3.05%)          |         |
| RET mutation                      |                  |                    |                     | 0.997   |
| Wildtype                          | 310 (90.12%)     | 135 (91.22%)       | 445 (90.45%)        |         |
| Mutant                            | 23 (6.69%)       | 9 (6.08%)          | 32 (6.50%)          |         |
| NA                                | 11 (3.20%)       | 4 (2.70%)          | 15 (3.05%)          |         |
| NTRK1 mutation                    |                  |                    |                     | 0.606   |
| Wildtype                          | 327 (95.06%)     | 144 (97.30%)       | 471 (95.73%)        |         |
| Mutant                            | 6 (1.74%)        | 0 (0.00%)          | 6 (1.22%)           |         |
|                     | TERT mutation | Sex | Histological type | T | N |
|---------------------|---------------|-----|-------------------|---|---|
| NA                  | 11 (3.20%)    | 4 (2.70%) | 15 (3.05%) | 0.989 | 0.526 |
| Wildtype            | 330 (95.93%)  | 142 (95.95%) | 472 (95.93%) | 0.998 | 0.989 |
| Mutant              | 3 (0.87%)     | 2 (1.35%) | 5 (1.02%) |     |     |
| NA                  | 11 (3.20%)    | 4 (2.70%) | 15 (3.05%) |     |     |
| Sex                 |               |       |                   | 0.989 | 0.526 |
| Male                | 92 (26.74%)   | 40 (27.03%) | 132 (26.83%) |     |     |
| Female              | 252 (73.26%)  | 108 (72.97%) | 360 (73.17%) |     |     |
| Histological type   |               |       |                   | 0.989 | 0.526 |
| Thyroid Papillary Carcinoma - Classical/usual | 247 (71.80%) | 103 (69.59%) | 350 (71.14%) |     |     |
| Thyroid Papillary Carcinoma - Follicular (>= 99% follicular patterned) | 67 (19.48%) | 34 (22.97%) | 101 (20.53%) |     |     |
| Thyroid Papillary Carcinoma - Tall Cell (>= 50% tall cell features) | 25 (7.27%) | 9 (6.08%) | 34 (6.91%) |     |     |
| Others              | 5 (1.45%)     | 2 (1.35%) | 7 (1.42%) |     |     |
| T                   |               |       |                   | 0.989 |     |
| T1                  | 99 (28.78%)   | 43 (29.05%) | 142 (28.86%) |     |     |
| T2                  | 117 (34.01%)  | 44 (29.73%) | 161 (32.72%) |     |     |
| T3                  | 112 (32.56%)  | 55 (37.16%) | 167 (33.94%) |     |     |
| T4                  | 15 (4.36%)    | 6 (4.05%) | 21 (4.27%) |     |     |
| NA                  | 1 (0.29%)     | 0 (0.00%) | 1 (0.20%) |     |     |
| N                   |               |       |                   | 0.526 |     |
| N0                  | 161 (46.80%)  | 63 (42.57%) | 224 (45.53%) |     |     |
| N1                  | 45 (13.08%)   | 13 (8.78%) | 58 (11.79%) |     |     |
| N1a                 | 55 (15.99%)   | 33 (22.30%) | 88 (17.89%) |     |     |
|                | Count (Percentage) | Count (Percentage) | Count (Percentage) |
|----------------|--------------------|--------------------|--------------------|
| **N1b**        | 45 (13.08%)        | 27 (18.24%)        | 72 (14.63%)        |
| **NA**         | 38 (11.05%)        | 12 (8.11%)         | 50 (10.16%)        |
| **M**          | 0.573              |                    |                    |
| **M0&Mx**      | 199 (57.85%)       | 78 (52.70%)        | 277 (56.30%)       |
| **M1**         | 145 (42.15%)       | 70 (47.30%)        | 215 (43.70%)       |
| **AJCC stage** | 0.519              |                    |                    |
| **Stage I**    | 207 (60.17%)       | 72 (48.65%)        | 279 (56.71%)       |
| **Stage II**   | 35 (10.17%)        | 15 (10.14%)        | 50 (10.16%)        |
| **Stage III**  | 66 (19.19%)        | 42 (28.38%)        | 108 (21.95%)       |
| **Stage IV**   | 35 (10.17%)        | 18 (12.16%)        | 53 (10.77%)        |
| **NA**         | 1 (0.29%)          | 1 (0.68%)          | 2 (0.41%)          |
| **Residual tumor** | 0.922          |                    |                    |
| **R0**         | 258 (75.00%)       | 117 (79.05%)       | 375 (76.22%)       |
| **Rx**         | 48 (13.95%)        | 14 (9.46%)         | 62 (12.60%)        |
| **R1**         | 35 (10.17%)        | 16 (10.81%)        | 51 (10.37%)        |
| **R2**         | 3 (0.87%)          | 1 (0.68%)          | 4 (0.81%)          |
| **Extrathyroid extension** | 0.997          |                    |                    |
| **None**       | 230 (66.86%)       | 95 (64.19%)        | 325 (66.06%)       |
| **Minimal (T3)** | 90 (26.16%)     | 42 (28.38%)        | 132 (26.83%)       |
| **Moderate or Advanced (T4)** | 11 (3.20%)     | 6 (4.05%)          | 17 (3.46%)         |
| **NA**         | 13 (3.78%)         | 5 (3.38%)          | 18 (3.66%)         |
| **Multifocality** | 0.994          |                    |                    |
| **Unifocal**   | 178 (51.74%)       | 80 (54.05%)        | 258 (52.44%)       |
| Anatomic site | Multifocal | Unilateral | Bilateral | NA |
|---------------|-----------|------------|-----------|----|
|               | 159 (46.22%) | 267 (77.62%) | 61 (17.73%) | 2 (0.58%) |
|               | 65 (43.92%) | 113 (76.35%) | 24 (16.22%) | 3 (2.03%) |
|               | 224 (45.53%) | 380 (77.24%) | 85 (17.28%) | 5 (1.02%) |

Table 3
Table 3. Baseline characteristics of patients included for the evaluation of prognostic factors and establishment of nomogram

| Clinical features     | Low-risk | High-risk | p value |
|----------------------|----------|-----------|---------|
|                      | Mean ± SD|           |         |
| Follow-up time (day) | 1224.96 ± 968.27 | 1297.97 ± 925.63 | 0.570   |
| DE-mRNA signature    | 0.18 ± 0.37 | 0.97 ± 0.27 | <0.001  |
| Age                  | 46.54 ± 14.69 | 49.93 ± 18.93 | 0.102   |
| N (%)                |           |           | <0.001  |
| PFI                  |           |           |         |
| Progression-free     | 300 (93.46%) | 49 (72.06%) |         |
| Progression          | 21 (6.54%) | 19 (27.94%) |         |
| BRAF V600E           |           |           | 0.026   |
| Wildtype             | 121 (37.69%) | 16 (23.53%) |         |
| Mutant               | 200 (62.31%) | 52 (76.47%) |         |
| RAS mutation         |           |           | 0.364   |
| Wildtype             | 280 (87.23%) | 62 (91.18%) |         |
| Mutant               | 41 (12.77%) | 6 (8.82%) |         |
| RET mutation         |           |           | 0.293   |
| Wildtype             | 295 (91.90%) | 65 (95.59%) |         |
| Mutant               | 26 (8.10%) | 3 (4.41%) |         |
| NTRK1 mutation       |           |           | 0.256   |
| Wildtype             | 315 (98.13%) | 68 (100.00%) |         |
| Mutant               | 6 (1.87%) | 0 (0.00%) |         |
| TERT mutation        |           |           | 0.182   |
| Wildtype             | 318 (99.07%) | 66 (97.06%) |         |
| Mutant               | 3 (0.93%) | 2 (2.94%) |         |
| Sex                  |           |           | 0.180   |
| Male                 | 83 (25.86%) | 23 (33.82%) |         |
| Female               | 238 (74.14%) | 45 (66.18%) |         |
| Histological type                                      | 0.303               |
|------------------------------------------------------|---------------------|
| Thyroid Papillary Carcinoma - Classical/usual        | 226 (70.40%) 52 (76.47%) |
| Thyroid Papillary Carcinoma - Follicular (>= 99% follicular patterned) | 67 (20.87%) 8 (11.76%) |
| Thyroid Papillary Carcinoma - Tall Cell (>= 50% tall cell features) | 22 (6.85%) 7 (10.29%) |
| Others                                               | 6 (1.87%) 1 (1.47%) |
| **T**                                                | <0.001              |
| T1                                                   | 107 (33.33%) 6 (8.82%) |
| T2                                                   | 104 (32.40%) 20 (29.41%) |
| T3                                                   | 103 (32.09%) 32 (47.06%) |
| T4                                                   | 7 (2.18%) 10 (14.71%) |
| **N**                                                | <0.001              |
| N0                                                   | 175 (54.52%) 18 (26.47%) |
| N1                                                   | 39 (12.15%) 16 (23.53%) |
| N1a                                                  | 63 (19.63%) 16 (23.53%) |
| N1b                                                  | 44 (13.71%) 18 (26.47%) |
| **M**                                                | 0.040               |
| M0&Mx                                                | 199 (61.99%) 33 (48.53%) |
| M1                                                   | 122 (38.01%) 35 (51.47%) |
| **AJCC_STAGE**                                       | <0.001              |
| Stage I                                              | 188 (58.57%) 28 (41.18%) |
| Stage II                                             | 37 (11.53%) 2 (2.94%) |
| Stage III                                            | 72 (22.43%) 18 (26.47%) |
| Stage IV                                             | 24 (7.48%) 20 (29.41%) |
| **Residual tumor**                                   | 0.073               |
| R0                                                   | 265 (82.55%) 48 (70.59%) |
| Rx                                                   | 23 (7.17%) 10 (14.71%) |
| R1                                                   | 30 (9.35%) 10 (14.71%) |
| R2                                                   | 3 (0.93%) 0 (0.00%)  |
| Extrathyroid extension                  |        |        |
|---------------------------------------|--------|--------|
| None                                  | 228    | 35     |
| Minimal (T3)                          | 87     | 25     |
| Moderate or Advanced (T4)             | 6      | 8      |
| Multifocality                         |        | 0.006  |
| Unifocal                              | 163    | 47     |
| Multifocal                            | 158    | 21     |
| Anatomic site                         |        | 0.585  |
| Unitateral                            | 248    | 56     |
| Isthmus                               | 17     | 2      |
| Bilateral                             | 56     | 10     |

Table 4
### Table 4. Unadjusted univariate Cox analysis

| Clinical features | Statistics       | PFI          |
|-------------------|------------------|--------------|
| DE-mRNA signature | 0.32 ± 0.46      | 6.90 (3.45, 13.79) <0.0001 |

**BRAF V600E**

|                     | Statistics       | PFI          |
|---------------------|------------------|--------------|
| Wildtype            | 137 (35.22%)     | 1.0          |
| Mutant              | 252 (64.78%)     | 1.06 (0.55, 2.06) 0.860 |

**RAS mutation**

|                     | Statistics       | PFI          |
|---------------------|------------------|--------------|
| Wildtype            | 342 (87.92%)     | 1.0          |
| Mutant              | 47 (12.08%)      | 1.72 (0.76, 3.89) 0.194 |

**TERT mutation**

|                     | Statistics       | PFI          |
|---------------------|------------------|--------------|
| Wildtype            | 384 (98.71%)     | 1.0          |
| Mutant              | 5 (1.29%)        | 0.00 (0.00, Inf) 0.997 |

**RET mutation**

|                     | Statistics       | PFI          |
|---------------------|------------------|--------------|
| Wildtype            | 360 (92.54%)     | 1.0          |
| Mutant              | 29 (7.46%)       | 0.72 (0.17, 3.00) 0.657 |

**NTRK1 mutation**

|                     | Statistics       | PFI          |
|---------------------|------------------|--------------|
| Wildtype            | 383 (98.46%)     | 1.0          |
| Mutant              | 6 (1.54%)        | 0.00 (0.00, Inf) 0.996 |

**Sex**

|                     | Statistics       | PFI          |
|---------------------|------------------|--------------|
| Male                | 106 (27.25%)     | 1.0          |
| Female              | 283 (72.75%)     | 0.76 (0.39, 1.47) 0.411 |

**Age**

|                     | Statistics       | PFI          |
|---------------------|------------------|--------------|
| ≥55 years           |                  |              |
| ≤55 years           | 47.13 ± 15.54    | 1.02 (1.00, 1.04) 0.042 |

Age
| Table | Value | p-value |
|------|-------|---------|
| >55 years | 114 (29.31%) | 2.36 (1.27, 4.41) 0.007 |
| Histological type | | |
| Thyroid Papillary Carcinoma - Classical/usual | 278 (71.47%) | 1.0 |
| Thyroid Papillary Carcinoma - Follicular (>= 99% follicular patterned) | 75 (19.28%) | 0.72 (0.28, 1.87) 0.501 |
| Thyroid Papillary Carcinoma - Tall Cell (>= 50% tall cell features) | 29 (7.46%) | 2.52 (1.04, 6.08) 0.041 |
| Others | 7 (1.80%) | 0.00 (0.00, Inf) 0.997 |
| Aggressive subtype | | |
| No | 359 (92.29%) | 1.0 |
| Yes | 30 (7.71%) | 2.70 (1.13, 6.46) 0.025 |
| T | | |
| T1 | 113 (29.05%) | 1.0 |
| T2 | 124 (31.88%) | 2.63 (0.85, 8.16) 0.094 |
| T3 | 135 (34.70%) | 4.22 (1.44, 12.36) 0.009 |
| T4 | 17 (4.37%) | 5.73 (1.43, 22.98) 0.014 |
| N | | |
| N0 | 193 (49.61%) | 1.0 |
| N1 | 196 (50.39%) | 2.11 (1.09, 4.08) 0.027 |
| M | | |
| M0&Mx | 232 (59.64%) | 1.0 |
| M1 | 157 (40.36%) | 1.65 (0.89, 3.08) 0.112 |
| AJCC stage | | |
| Stage I | 216 (55.53%) | 1.0 |
| Stage    | Count (Percentage) | Hazard Ratio (95% CI) | p-Value |
|----------|--------------------|-----------------------|---------|
| Stage II | 39 (10.03%)        | 1.13 (0.32, 3.93)     | 0.849   |
| Stage III| 90 (23.14%)        | 2.67 (1.27, 5.60)     | 0.010   |
| Stage IV | 44 (11.31%)        | 3.89 (1.68, 9.02)     | 0.002   |

Residual tumor

| Type | Count (Percentage) | Hazard Ratio (95% CI) | p-Value |
|------|--------------------|-----------------------|---------|
| R0   | 313 (80.46%)       | 1.0                   |         |
| Rx   | 33 (8.48%)         | 1.76 (0.61, 5.01)     | 0.293   |
| R1   | 40 (10.28%)        | 1.74 (0.72, 4.18)     | 0.219   |
| R2   | 3 (0.77%)          | 3.23 (0.44, 23.91)    | 0.251   |

Extrathyroid extension

| Type              | Count (Percentage) | Hazard Ratio (95% CI) | p-Value |
|-------------------|--------------------|-----------------------|---------|
| None              | 263 (67.61%)       | 1.0                   |         |
| Minimal (T3)      | 112 (28.79%)       | 1.85 (0.97, 3.50)     | 0.060   |
| Moderate or Advanced (T4) | 14 (3.60%) | 1.77 (0.42, 7.58) | 0.439 |

Neoplasm largest dimension (cm)

| Dimension | Count (Percentage) | Hazard Ratio (95% CI) | p-Value |
|-----------|--------------------|-----------------------|---------|
| ≤2cm      | 153 (39.33%)       | 1.24 (1.04, 1.46)     | 0.014   |
| >2cm      | 236 (60.67%)       | 3.61 (1.51, 8.61)     | 0.004   |

Multifocality

| Type   | Count (Percentage) | Hazard Ratio (95% CI) | p-Value |
|--------|--------------------|-----------------------|---------|
| Unifocal | 210 (53.98%)     | 1.0                   |         |
| Multifocal | 179 (46.02%) | 0.82 (0.43, 1.57) | 0.553   |

Anatomic site

| Type | Count (Percentage) | Hazard Ratio (95% CI) | p-Value |
|------|--------------------|-----------------------|---------|
| Unilateral | 304 (78.15%)   | 1.0                   |         |
| Isthmus | 19 (4.88%)        | 0.45 (0.06, 3.27)     | 0.429   |
| Bilateral | 66 (15.97%)  | 0.94 (0.39, 2.26)     | 0.898   |
Table 5
Table 5. Multivariate Cox regression analysis

| Clinical features | Non-adjusted | Adjust I | Adjust II | Adjust III |
|-------------------|--------------|----------|-----------|------------|
| DE-mRNA signature | 6.90 (3.45, 13.79) <0.0001 | 5.60 (2.73, 11.49) <0.0001 | 5.60 (2.73, 11.49) <0.0001 | 5.39 (2.45, 11.85) <0.0001 |
| BRAF V600E        |              |          |           |            |
| Wildtype          | 1.0          | 1.0      | 1.0       | NA         |
| Mutant            | 1.06 (0.55, 2.06) 0.860 | 0.97 (0.50, 1.90) 0.936 | 0.63 (0.32, 1.25) 0.183 | NA |
| RAS mutation      |              |          |           |            |
| Wildtype          | 1.0          | 1.0      | 1.0       | 1.0        |
| Mutant            | 1.72 (0.76, 3.89) 0.194 | 2.00 (0.87, 4.57) 0.100 | 2.42 (1.05, 5.57) 0.037 | 5.99 (1.88, 19.05) 0.002 |
| TERT mutation     |              |          |           |            |
| Wildtype          | 1.0          | 1.0      | 1.0       | NA         |
| Mutant            | 0.00 (0.00, Inf) 0.997 | 0.00 (0.00, Inf) 0.997 | 0.00 (0.00, Inf) 0.997 | NA |
| RET mutation      |              |          |           |            |
| Wildtype          | 1.0          | 1.0      | 1.0       | NA         |
| Mutant            | 0.72 (0.17, 3.00) 0.657 | 0.69 (0.16, 2.91) 0.611 | 1.05 (0.24, 4.53) 0.946 | NA |
| NTRK1 mutation    |              |          |           |            |
| Wildtype          | 1.0          | 1.0      | 1.0       | NA         |
| Mutant            | 0.00 (0.00, Inf) 0.996 | 0.00 (0.00, Inf) 0.996 | 0.00 (0.00, Inf) 0.997 | NA |
| Sex               |              |          |           |            |
| Male              | 1.0          | 1.0      | 1.0       | NA         |
| Female            | 0.76 (0.39, 1.47) 0.411 | 0.91 (0.46, 1.81) 0.789 | 1.00 (0.50, 1.99) 0.998 | NA |
| Age               | 1.02 (1.00, 1.04) 0.042 | 1.00 (0.97, 1.03) 0.847 | 0.99 (0.97, 1.02) 0.618 | 1.00 (0.97, 1.03) 0.957 |
| Age ≤55 years     | 1.0          | 1.0      | 1.0       | 1.0        |
|                | >55 years | Histological type | Aggressive subtype | T         | N         | M         | AJCC Stage |
|----------------|-----------|-------------------|-------------------|-----------|-----------|-----------|------------|
|                | 2.36 (1.27, 4.41) 0.007 | Thyroid Papillary Carcinoma - Classical/usual | 1.0 | 1.0 | 1.0 | Stage I |
|                | 1.46 (0.68, 3.14) 0.331 | Thyroid Papillary Carcinoma - Follicular (>= 99% follicular patterned) | 0.72 (0.28, 1.87) 0.501 | 0.79 (0.30, 2.08) 0.634 | 0.91 (0.34, 2.41) 0.846 | 0.53 (0.15, 1.82) 0.310 | Stage II |
|                | 1.14 (0.49, 2.64) 0.758 | Thyroid Papillary Carcinoma - Tall Cell (>= 50% tall cell features) | 2.52 (1.04, 6.08) 0.041 | 1.91 (0.76, 4.81) 0.172 | 1.48 (0.58, 3.79) 0.418 | 0.04 (0.00, Inf) 0.999 |          |
|                | 1.43 (0.53, 3.81) 0.479 | Others | 0.00 (0.00, Inf) 0.997 | 0.00 (0.00, Inf) 0.997 | 0.00 (0.00, Inf) 0.997 | 0.00 (0.00, Inf) 0.996 |          |
| Aggressive subtype | No | 1.0 | 1.0 | 1.0 | 1.0 |          |
|                | Yes | 2.70 (1.13, 6.46) 0.025 | 2.00 (0.80, 4.99) 0.139 | 1.50 (0.59, 3.83) 0.393 | 49.47 (0.00, Inf) 0.999 |          |
| T              | No | 1.0 | 1.0 | 1.0 | 1.0 |          |
|                | Yes | 2.63 (0.85, 8.16) 0.094 | 2.65 (0.81, 8.67) 0.107 | 2.08 (0.64, 6.78) 0.226 | 2.29 (0.67, 7.74) 0.184 |          |
| N              | No | 1.0 | 1.0 | 1.0 | 1.0 |          |
|                | Yes | 4.22 (1.44, 12.36) 0.009 | 3.01 (0.95, 9.56) 0.062 | 2.41 (0.77, 7.59) 0.132 | 3.44 (0.77, 15.38) 0.107 |          |
| M              | No | 1.0 | 1.0 | 1.0 | 1.0 |          |
|                | Yes | 5.73 (1.43, 22.98) 0.014 | 2.47 (0.51, 12.06) 0.263 | 1.33 (0.27, 6.61) 0.725 | 1.35 (0.15, 12.00) 0.787 | Stage II |
| AJCC Stage     | Yes | 1.13 (0.32, 3.93) 0.849 | 1.19 (0.31, 4.59) 0.800 | 1.61 (0.41, 6.31) 0.492 | 1.15 (0.19, 7.01) 0.876 |          |
|                      | Stage III | Stage IV | Residual tumor | Extrathyroid extension | Neoplasm largest dimension | Neoplasm largest dimension | Multifocality | Anatomic site |
|----------------------|-----------|----------|----------------|------------------------|---------------------------|---------------------------|---------------|---------------|
|                      | 2.67 (1.27, 5.60) 0.010 | 2.84 (1.06, 7.57) 0.038 | 2.48 (0.85, 7.20) 0.096 | 1.39 (0.42, 4.62) 0.595 | 2.39 (1.68, 9.02) 0.002 | 4.05 (1.38, 11.91) 0.011 | 3.34 (1.06, 10.50) 0.039 | 2.07 (0.53, 8.07) 0.293 |
| Residual tumor       |           |          |                |                        |                           |                           |               |               |
| R0                   | 1.0       | 1.0      | 1.0            | 1.0                    |                           |                           |               |               |
| Rx                   | 1.76 (0.61, 5.01) 0.293 | 1.35 (0.47, 3.93) 0.579 | 1.25 (0.43, 3.64) 0.684 | 1.56 (0.48, 5.06) 0.462 | 1.0                       | 1.0                       | 1.0           |               |
| R1                   | 1.74 (0.72, 4.18) 0.219 | 1.20 (0.49, 2.96) 0.691 | 1.14 (0.46, 2.84) 0.776 | 1.17 (0.44, 3.12) 0.748 | 1.0                       | 1.0                       | 1.0           |               |
| R2                   | 3.23 (0.44, 23.91) 0.251 | 5.58 (0.47, 66.80) 0.174 | 4.15 (0.33, 52.73) 0.272 | 11.00 (0.78, 154.44) 0.075 |                           |                           |               |               |
| Extrathyroid extension|           |          |                |                        |                           |                           |               |               |
| None                 | 1.0       | 1.0      | 1.0            | 1.0                    |                           |                           |               |               |
| Minimal (T3)         | 1.85 (0.97, 3.50) 0.060 | 1.30 (0.64, 2.61) 0.465 | 1.23 (0.62, 2.47) 0.553 | 0.71 (0.24, 2.10) 0.533 |                           |                           |               |               |
| Moderate or Advanced (T4) | 1.77 (0.42, 7.58) 0.439 | 0.68 (0.14, 3.30) 0.632 | 0.46 (0.09, 2.23) 0.332 | 0.83 (0.09, 7.48) 0.865 |                           |                           |               |               |
| Neoplasm largest dimension | 1.24 (1.04, 1.46) 0.014 | 1.14 (0.96, 1.36) 0.140 | 1.04 (0.86, 1.25) 0.686 | 0.95 (0.76, 1.20) 0.691 |                           |                           |               |               |
| Neoplasm largest dimension ≤2cm | 1.0 | 1.0 | 1.0 | 1.0 | 3.61 (1.51, 8.61) 0.004 | 3.55 (1.48, 8.52) 0.005 | 2.69 (1.10, 6.55) 0.030 | 2.50 (0.76, 8.20) 0.131 |
| >2cm                 | 0.82 (0.43, 1.57) 0.553 | 0.72 (0.37, 1.39) 0.322 | 0.92 (0.47, 1.77) 0.792 |                           |                           |                           |               |               |
| Multifocality        | 1.0       | 1.0      | 1.0            | NA                     |                           |                           |               |               |
| Unifocal             | 1.0       | 1.0      | 1.0            | NA                     |                           |                           |               |               |
| Multifocal           | 0.82 (0.43, 1.57) 0.553 | 0.72 (0.37, 1.39) 0.322 | 0.92 (0.47, 1.77) 0.792 |                           |                           |                           |               |               |
| Anatomic site        |           |          |                |                        |                           |                           |               |               |
| Unilateral           | 1.0       | 1.0      | 1.0            | NA                     |                           |                           |               |               |
| Isthmus              | 0.45 (0.06, 3.27) 0.429 | 0.48 (0.06, 3.54) 0.472 | 0.41 (0.06, 3.00) 0.377 |                           |                           |                           |               |               |
| Bilateral            | 0.94 (0.39, 2.26) 0.898 | 0.73 (0.30, 1.77) 0.486 | 0.88 (0.36, 2.14) 0.782 |                           |                           |                           |               |               |
| Adjust I model adjust for: Age, Sex, and AJCC Stage. |
Adjust II model adjust for: Age, Sex, AJCC Stage, and DE-mRNA signature.

Adjust III model adjust for parameters with $p < 0.25$ based on univariate analysis.

**Figures**

A

![Diagram showing the process from DE-circRNAs to DE-miRNAs and DE-mRNAs, followed by correlation analysis and centroid regression analysis.]

B

![Dot plot for DE-circRNAs showing fold change and log2 fold change with Venn diagram illustrating the overlap of down and upregulated circRNAs.]

C

![Dot plot for DE-miRNAs showing fold change and log2 fold change with Venn diagram illustrating the overlap of down and upregulated miRNAs.]

D

![Dot plot for DE-mRNAs showing fold change and log2 fold change with Venn diagram illustrating the overlap of down and upregulated mRNAs.]

**Figure 1**
Differential expression analysis and prediction of potential circRNA associated competing endogenous RNA (ceRNA) regulatory relationships. (A) shows the workflow of constructing a circRNA associated ceRNA network and the establishment of novel prognostic DE-mRNA signature and nomogram to predict risk of progression in post-surgical papillary thyroid carcinoma (PTC). (B) The volcano plot and the venn diagram show that 138 differentially expressed circRNAs (DE-circRNAs) are identified in PTC (117 upregulated and 21 downregulated). Of which, 54 DE-circRNAs are associated with potential ceRNA regulatory relationships. (C) The volcano plot and the venn diagram show that 113 differentially expressed miRNAs (DE-miRNAs) are identified in PTC (32 upregulated and 81 downregulated). Of which, 14 DE-miRNAs are associated with potential ceRNA regulatory relationships. (D) The volcano plot and the venn diagram show that 2252 differentially expressed mRNAs (DE-mRNAs) were identified in PTC (905 upregulated and 1347 downregulated). Of which, 968 DE-mRNAs are associated with potential ceRNA regulatory relationships.
Figure 2

Functional enrichment analysis of the 698 DE-mRNAs with potential circRNA associated ceRNA regulatory relationships. (A) Top 20 most enriched biological processes of the DE-mRNAs. (B) Top 20 most enriched cellular components of the DE-mRNAs. (C) Top 20 most enriched molecular functions of the DE-mRNAs. (D) Top 20 most enriched pathways of the DE-mRNAs.
**Figure 3**

Construction of a circRNA-miRNA-mRNA ceRNA network in the PTC. DE-circRNAs are presented in the diamond nodes, DE-miRNAs are presented in the rounded square nodes and the DE-mRNAs are presented in the elliptical nodes. Nodes shown in red indicate upregulation in PTC while nodes shown in green indicate downregulation. The circRNA-miRNA-mRNA ceRNA network in PTC includes 25 DE-circRNAs (hsa_circ_0003645, hsa_circ_0089153, hsa_circ_0005699, hsa_circ_0062389, hsa_circ_0028198, hsa_circ_0092275, hsa_circ_0074854, hsa_circ_0028196, hsa_circ_0001658, hsa_circ_0001806, hsa_circ_0007146, hsa_circ_0005785, hsa_circ_0061137, hsa_circ_0009172, hsa_circ_0004599, hsa_circ_0064557, hsa_circ_0038718, hsa_circ_0008784, hsa_circ_0000228, hsa_circ_0000965, hsa_circ_0084443, hsa_circ_0000282, hsa_circ_0001917, hsa_circ_0008354 and hsa_circ_0049271), 6 DE-miRNAs (hsa-miR-146b-3p, hsa-miR-337-3p, hsa-miR-577, hsa-miR-1179, hsa-miR-139-3p and hsa-miR-139-5p) and 150 DE-mRNAs.
Figure 4

Construction of a ceRNA regulatory subnetwork associated with progression-free interval (PFI) in PTC. (A) The Sankey diagram presents the regulatory relationship within the ceRNA regulatory subnetwork associated with PFI in PTC (left). Forest plot of hazard ratio (HR) presenting the prognostic value of the PFI related DE-mRNAs within the ceRNA subnetwork and the corresponding logFC values for differential expression (PTC versus normal) (right). (B) Basic structure of the eleven DE-circRNAs within the ceRNA
The structural patterns of hsa_circ_0003645, hsa_circ_0089153, hsa_circ_0005699, hsa_circ_0007146, hsa_circ_0038718, hsa_circ_0001658, hsa_circ_0008784, hsa_circ_0000965, hsa_circ_0001917, hsa_circ_0008354, and hsa_circ_0049271 provided by Cancer Specific CircRNA Database (CSCD) are shown. The microRNA response element (MRE) is presented in red. The RNA binding protein (RBP) is presented in blue and the open reading frame (ORF) is presented in green.

Figure 5
Identification of hub PFI related DE-mRNAs regulated by ceRNA mechanism and establishment of DE-mRNA signature. (A) LASSO coefficient profiles of the 27 prognostic DE-mRNAs within the subnetwork. (B) LASSO deviance profiles of the 27 prognostic DE-mRNAs within the subnetwork. (C) The Sankey diagram presents the regulatory relationship between the hub PFI related DE-mRNAs and the corresponding DE-miRNAs and DE-circRNAs. (D) to (I) show Kaplan–Meier curves for the hub DE-mRNAs related to PFI based on the optimal cutoff values in PTC. The horizontal axis shows the follow-up time in months and the vertical axis shows the probability of progression-free survival.
Validation of the predicting performance of the DE-mRNA signature. (A) to (C) show the time-dependent receiver operating characteristic (ROC) curves for one-year, two-year, three-year, four-year and five-year PFI predicted by the DE-mRNA signature in the training dataset, the validation dataset and the entire TCGA-THCA dataset, respectively. (D) to (F) show the Kaplan–Meier curves for the DE-mRNA signature based on the optimal cutoff value in the training dataset, the validation dataset and the entire TCGA-THCA dataset, respectively. The horizontal axis shows the follow-up time in months and the vertical axis shows the probability of progression-free survival. (G) to (I) show the trends in expression profiles of the six hub DE-mRNAs (bottom) and the deterioration of prognosis (middle) along with the incremental risk of progression based on the DE-mRNA signature (upper) in the training dataset, the validation dataset and the entire TCGA-THCA dataset, respectively.
Figure 7

The molecular, clinical and mutational relevance of the DE-mRNA signature. (A) to (E) show top functional gene sets enriched in the high-risk group predicted by the DE-mRNA signature based on the optimal cutoff values in PTC. (F) shows the distribution of the risk score calculated by the DE-mRNA signature in different groups of T stages in the TCGA-THCA dataset. (G) shows the distribution of the risk score calculated by the DE-mRNA signature in different groups of N stages in the TCGA-THCA dataset. (H)
shows the distribution of the risk score calculated by the DE-mRNA signature in different groups of M stages in the TCGA-THCA dataset. (I) shows the distribution of the risk score calculated by the DE-mRNA signature in different groups of AJCC stages in the TCGA-THCA dataset. (J) shows the distribution of the risk score calculated by the DE-mRNA signature in different groups of residual tumor statuses in the TCGA-THCA dataset. (K) shows the distribution of the risk score calculated by the DE-mRNA signature in groups of aggressive and non-aggressive subtypes in the TCGA-THCA dataset. (L) shows the distribution of the risk score calculated by the DE-mRNA signature in different groups of BRAF V600E mutation status in the TCGA-THCA dataset. (M) shows the distribution of the risk score calculated by the DE-mRNA signature in different groups of RAS mutation status in the TCGA-THCA dataset. (N) shows the relationship among the DE-mRNA signature, the expression profiles of the six hub DE-mRNAs (CLCNKB, FXYD6, FBXO27, RIMS2, SPC24 and CDKN2A) and corresponding DE-miRNAs (hsa-miR-146b-3p, hsa-miR-139-3p and hsa-miR-139-5p) and the mutational profiles (BRAF, RAS, RET, NTRK1 and TERT) of PTC. Data of genetic alteration were obtained from the cBioPortal for Cancer Genomics (https://www.cbioportal.org). *P < 0.05, **P < 0.01. *** P < 0.001. **** P < 0.0001.
Building and validation of a DE-mRNA signature based prognostic nomogram predicting PFI in PTC. (A) shows a DE-mRNA signature based prognostic nomogram predicting one-year, two-year, three-year, four-year and five-year PFI in PTC. (B) shows the Kaplan–Meier curves for the nomogram based on the optimal cutoff value in the entire TCGA-THCA dataset. The horizontal axis shows the PFI time in months and the vertical axis shows the probability of progression-free survival. (C) The calibration plot for...
internal validation of the nomogram. The X axis represents the predicted risk of progression while the Y axis represents the observed risk of progression. (D) shows the time-dependent ROC curves for one-year PFI predicted by the nomogram in compare with the ATA risk stratification system, the MACIS score and the AJCC staging system. (E) shows the time-dependent ROC curves for two-year PFI predicted by the nomogram in compare with the ATA risk stratification system, the MACIS score and the AJCC staging system. (F) shows the time-dependent ROC curves for three-year PFI predicted by the nomogram in compare with the ATA risk stratification system, the MACIS score and the AJCC staging system. (G) shows the time-dependent ROC curves for four-year PFI predicted by the nomogram in compare with the ATA risk stratification system, the MACIS score and the AJCC staging system. (H) shows the time-dependent ROC curves for five-year PFI predicted by the nomogram in compare with the ATA risk stratification system, the MACIS score and the AJCC staging system. AUCs were compared with the method described by Paul Blanche et al [20].

Supplementary Files

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- FigureS1.tif
- TableS2.xlsx
- TableS1.xlsx