# The combined use of miRNAs and mRNAs as biomarkers for the diagnosis of papillary thyroid carcinoma

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**Abstract.** Thyroid carcinoma (TC) is the most common malignancy of the endocrine system, and papillary thyroid carcinoma (PTC) accounts for the largest proportion of cases with TC. Although histology is considered the gold standard in the diagnosis of PTC, the sensitivity and specificity of this method is low. Therefore, developing novel diagnostic and prognostic biomarkers for PTC is essential. MicroRNAs (miRNAs or miRs) and their target RNAs play critical roles in tumorigenesis and tumor progression. Thus, the characteristic miRNA and mRNA expression profiles may function as diagnostic biomarkers for tumors, making it possible to predict the tumor stage and the prognosis of patients. In the present study, we detected miRNAs and mRNAs which can function as novel biomarkers for the diagnosis of PTC. The sensitivity of the diagnostic tests was evaluated by receiver operating characteristic curve analysis. Pearson's correlation analysis was used to determine the correlation between mRNAs and miRNAs, and cancer types. We found that the area under the curve (AUC) values of 8 miRNAs (miR-106a, miR-15a, miR-30a, miR-30b, miR-20a, miR-20b, miR-30d and miR-30e) and 8 mRNAs [axis inhibition protein 2 (AXIN2), integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor) (ITGA3), tumor protein p53 inducible nuclear protein (TP53INP)1, TP53INP2, B-cell CLL/lymphoma 2 (BCL2), phosphatase and tensin homolog (PTEN), FOS and K(lysine) acetyltransferase 2B (KAT2B)] were >0.90. The combination of miR-15a and AXIN2 significantly improved the diagnostic accuracy. Therefore, our data indicate that the differential expression of miRNAs combined with that of their target mRNAs may serve as a powerful biomarker for distinguishing PTC from benign tissues.

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*Key words:* papillary thyroid carcinoma, biomarker, miRNA, mRNA

## Introduction

Thyroid carcinoma (TC) is the most common malignancy of the endocrine system. An estimated 62,980 new cases of TC were diagnosed, and approximately 1,890 deaths were caused by TC in the United States in 2014 (1). TC is commonly diagnosed at a younger age than the majority of other adult cancers (2). The 4 main histological types of TC are papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC) and anaplastic (undifferentiated) carcinoma (ATC) (3,4). PTC and FTC constitute approximately 90% of total number of TC cases and are treatable and usually curable. However, both PTCs and FTCs may progress to poorly differentiated thyroid carcinomas (PDTCs) or may completely lose differentiation and transform into ATC, a type of poorly differentiated TC, which is aggressive, prone to early metastasis and is associated with a poor prognosis (5). Histology is considered the gold standard for TC diagnosis. However, it is difficult to distinguish between PTC and FTC under a microscope (6). Furthermore, conventional histology fails to provide prognostic and therapeutic information for TC. Some biomarkers, such as thyroglobulin (Tg) (7,8), galectin-3 (9) and HBME-1 (10), have been used in clinical practice for the diagnosis of PTC; however, the sensitivity and specificity of these biomarkers are low, and only a small fraction of these biomarkers can be used as diagnostic or prognostic biomarkers. Therefore, it is essential to develop novel diagnostic and prognostic biomarkers for PTC.

MicroRNAs (miRNAs or miRs), a class of short non-coding RNAs with a length of 19-22 nucleotides, and play important roles in tumorigenesis and cancer progression (11,12). miRNAs regulate gene expression at the post-transcriptional

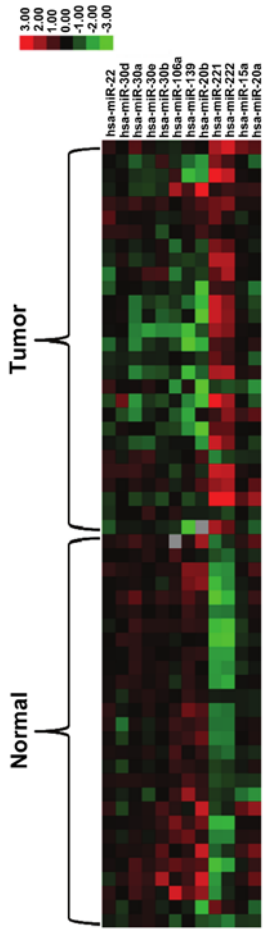


Figure 1. MicroRNA (miRNA) expression analysis using the data of 28 patients with papillary thyroid carcinoma (PTC) obtained from The Cancer Genome Atlas (TCGA). Color gram of miRNA expression profiles of patients with PTC. All miRNAs were divided into 2 groups: upregulated (red) and downregulated (green).

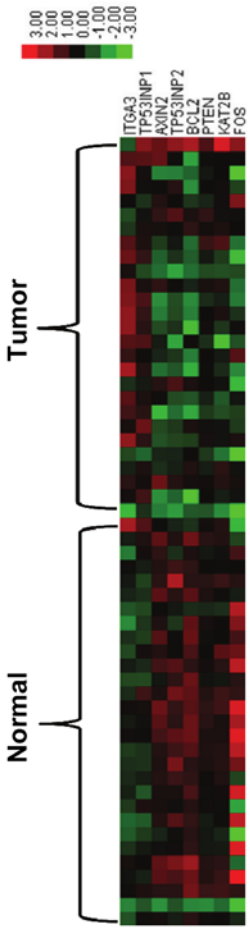


Figure 2. Gene expression analysis using the data of 28 patients with papillary thyroid carcinoma (PTC) obtained from The Cancer Genome Atlas (TCGA). Color gram of mRNA expression profiles of patients with PTC. All mRNAs were divided into 2 groups: upregulated (red) and downregulated (green).

level by binding to the 3'-UTR of their target mRNAs (13). A number of studies have demonstrated that miRNA expression is associated with cell proliferation, metastasis, invasion and and response to therapy (14-20). miRNA expression differs between cancer tissues and adjacent normal tissues in patients (21-23). These data indicate that miRNAs may be used as potential biomarkers for the diagnosis and prognosis of patients with cancer.

In the present study, we examined the expression profiles of miRNAs and mRNAs in patients with PTC and evaluated their potential for use as biomarkers for PTC diagnosis. The differential expression of miRNAs, combined with that of their target mRNAs, may serve as a powerful biomarker for distinguishing PTC from benign tissues.

## Materials and methods

*Data sources.* miRNA expression data, transcription sequencing (RNA-Seq) data and the corresponding clinical information for 28 patients with PTC were obtained from The Cancer Genome Atlas (TCGA) data portal (http://cancergenome.nih.gov). This database is freely available for non-commercial and academic use. The TCGA data, as well as the cBioPortal for Cancer Genomics (http://www.cbioportal.org) and Oncomine (http://www.oncomine.org) data were in the form of RNA sequencing data on an array platform. The sequencing data from TCGA were available in the form of 'reads per million (level 3)' for each miRNA. As regards RNA-Seq gene expression, only data from patients with matched tumor and normal samples were used. cBioPortal and Oncomine were also used to examine the expression of miRNAs and RNAs from the TCGA data portal. miRNA expression analysis of the 28 patients with PTC in the TCGA data portal was carried out using the software package TreeView version 1.1.

*Receiver operating characteristic (ROC) curve analysis.* To evaluate the sensitivity of the diagnostic tests, ROC curve analyses were performed using MedCalc® statistical software (11.4.2.0; MedCalc statistical software, Mariakerke, Belgium). The area under the ROC curve (AUC), which has been described as a simple and convenient overall measure of diagnostic test accuracy, represents the probability and correspondence between the ROC curve and the tested factors.

*miR-15a*/axis inhibition protein 2 (*AXIN2) expression in other types of cancer.* Pearson's correlation analysis was used to determine the correlation between mRNAs and miRNAs, and cancer types, including p-values and the false discovery rate (FDR).

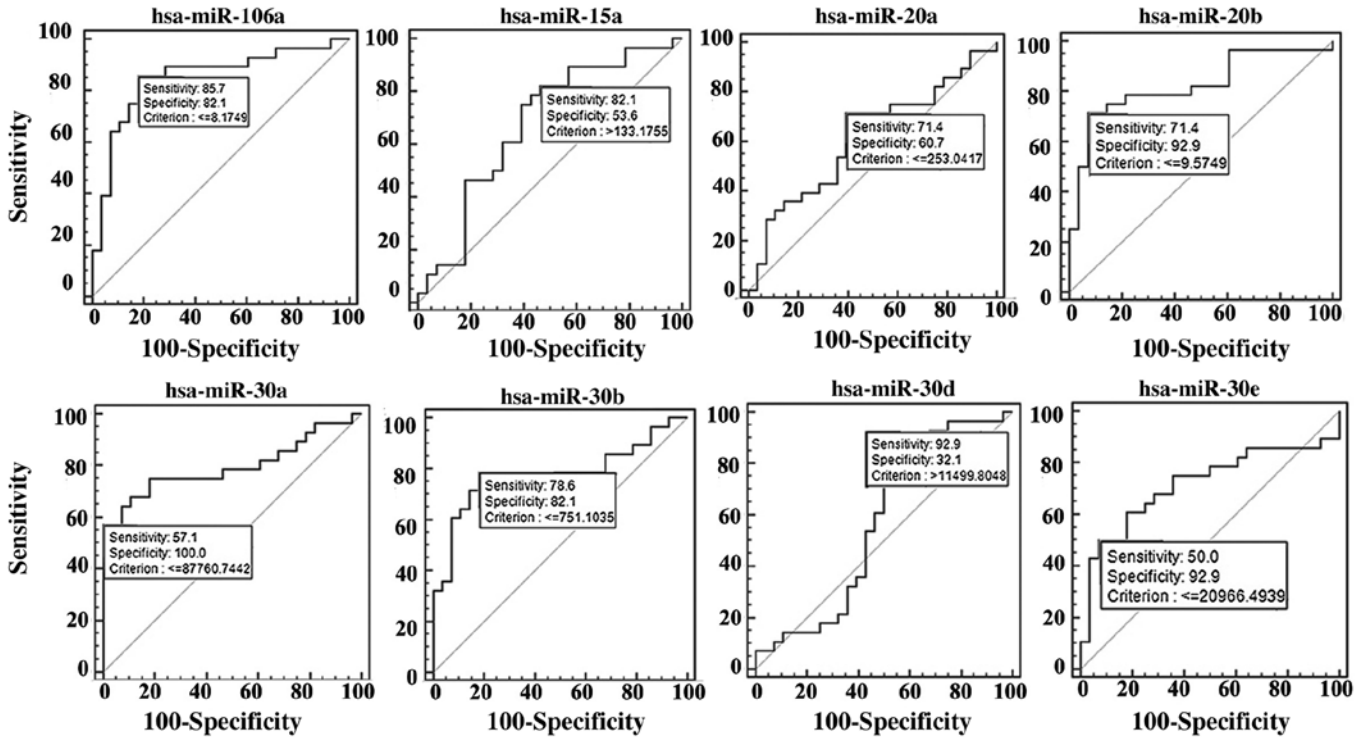


Figure 3. Receiver operating characteristic (ROC) curves of the 8-miRNA signature in patients with papillary thyroid carcinoma (PTC).ROC curve analysis of miR-106a, miR-15a, miR-20a, miR-20b, miR-30a, miR-30b, miR-30d and miR-30e in tumors and normal tissues.

*Statistical analysis*. All statistical analyses were carried out using SPSS for Windows, version 19.0 (SPSS, Inc., Chicago, IL, USA). Combined predictors were established using the logistic regression method. ROC curves were established to evaluate the diagnostic effects of miRNAs. The results are expressed as the means ± SD. P-values <0.05 were considered to indicate statistically significant differences.

## Results

*Screening of differentially expressed miRNAs*. Both tumor tissues and matched normal tissues from the same patient were used for miRNA expression profile analysis. The data of 28 patients with PTC, from TCGA, were included in the present study. miRNA expression was calculated from 'reads per million' values of the tumor and matched normal samples. We found that 12 miRNAs (miR-20a, miR-15a, miR222, miR-221, miR-20b, miR-139, miR-106a, miR-30b, miR-30e, miR-30a, miR-30d and miR-22) demonstrated a >2-fold difference in expression between the tumor tissues and normal tissues in 70% of the patients. The upregulated and downregulated miRNAs are presented in Fig. 1.

*Screening of differentially expressed genes*. We further examined differentially expressed genes in the tumor tissues and matched normal tissues in the 28 patients with PTC. A total of 8 genes [integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor) (ITGA3), tumor protein p53 inducible nuclear protein (TP53INP)1, AXIN2, TP53INP2, B-cell CLL/lymphoma 2 (BCL2), phosphatase and tensin homolog (PTEN), K(lysine) acetyltransferase 2B (KAT2B) and FOS] were identified as differentially expressed between the PTC tissues and the matched normal thyroid tissues. The upregulated and downregulated genes are presented in Fig. 2.

*ROC curve analysis of the differentially expressed miRNAs*. The differentially expressed miRNAs in the PTC tissue samples were selected for further analysis. ROC curve analysis was performed on 28 tumor and 28 normal tissues to determine whether these miRNAs are related to the PTC histological status. The miRNAs, miR-106a, miR-15a, miR-20a, miR-20b, miR-30a, miR-30b, miR-30d and miR-30e, were found to be associated with PTC (Fig. 3). All of their AUC values were >0.90, and thus, this indicates that these miRNAs can be used as effective biomarkers for the diagnosis of PTC.

*ROC curve analysis of the differentially expressed genes*. ROC curve analysis was then carried out on the basis of the results from obtained using the PTC tissues, as compared with those obtained using the normal tissues. The expression of the target genes, AXIN2, ITGA3, TP53INP1, TP53INP2, BCL2, PTEN, FOS and KAT2N, was found to be associated with PTC (Fig. 4). All of these genes exhibited high sensitivity (60.7, 71.4, 64.3, 82.1, 89.3, 85.7, 89.3 and 85.7%, respectively) and specificity (92.9, 96.4, 85.7, 75.0, 92.9, 46.4, 63.9 and 67.3%, respectively).

*The potential value of combined biomarkers*. We further examined the potential for using miRNAs combined with their target mRNAs in the diagnosis of PTC. ROC curve analysis revealed that when miR-15a was combined with its

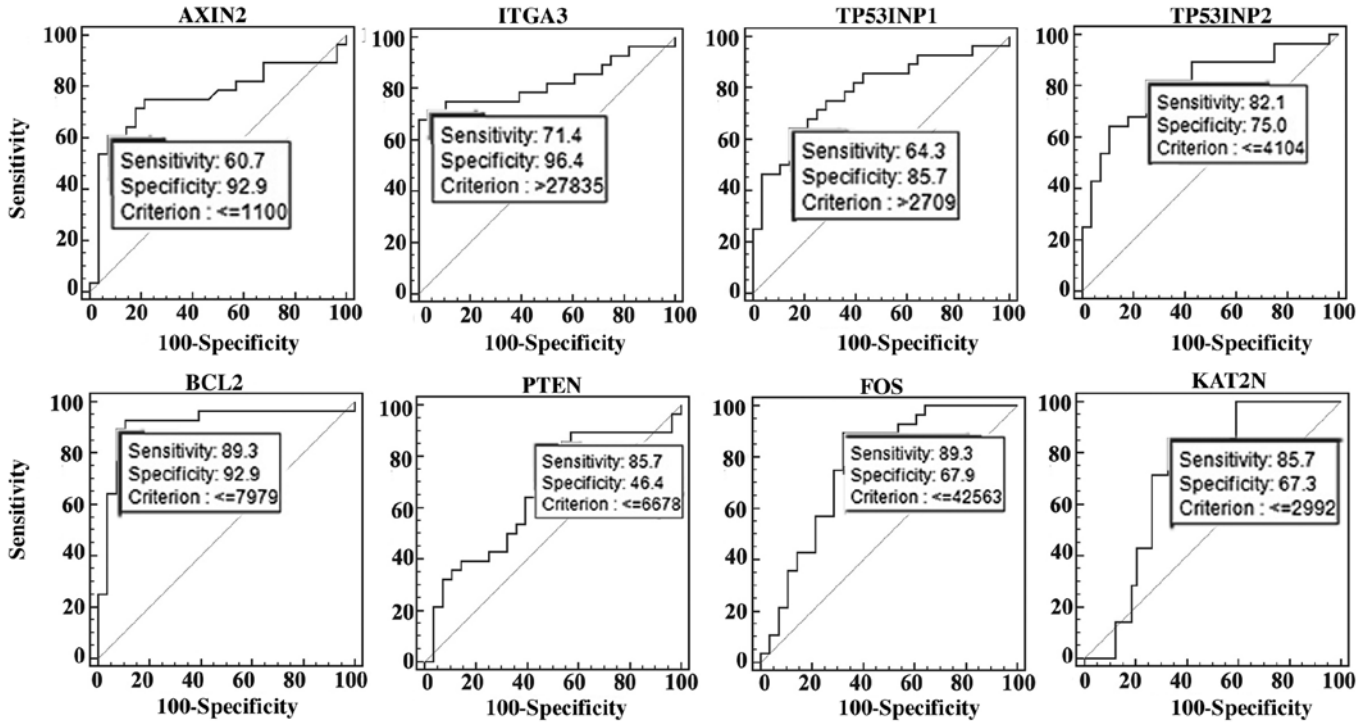


Figure 4. Receiver operating characteristic (ROC) curves of the 8-mRNA signature in patients with papillary thyroid carcinoma (PTC). ROC curve analysis of AXIN2, ITGA3, TP53INP1, TP53INP2, BCL2, PTEN, FOS and KAT2N in tumors and normal tissues.

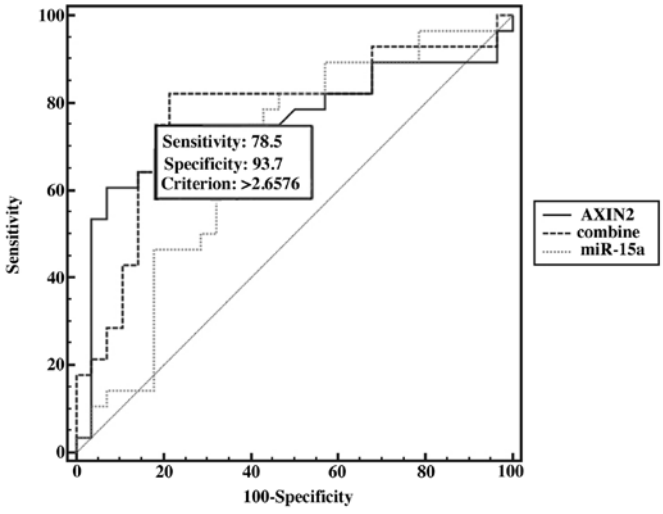


Figure 5. Receiver operating characteristic (ROC) curves of miR-15a combined with AXIN2 as a biomarker for the diagnosis of patients with papillary thyroid carcinoma (PTC).

target gene, AXIN2, the AUC values increased, and miR-15a combined with AXIN2 improved the sensitivity (78.5%) and specificity (93.7%) (Fig. 5). Moreover, we found that miR-15a and AXIN2 expression were changed coordinately in 8 types of cancer, as shown in Table I. We also analyzed the expression of the other miRNAs and their target genes in different types of cancer (Table I). Our results suggest that these miRNAs and mRNAs may be used as potential biomarkers for the diagnosis of PTC.

## Discussion

The current clinical approaches for the diagnosis of PTC include researching patient history, physical examination, imaging, fine-needle aspiration (FNA) and surgical pathology. FNA and surgical pathology are the gold standard for the diagnosis of PTC. However, both methods are invasive and their predictive value is limited. Therefore, as has been described in a previous study, it is essential to identify novel biomarkers to predict the diagnosis and prognosis of patients with PTC (24). Previous studies have reported that certain miRNAs may be used as biomarkers for the diagnosis and prognosis of breast cancer and various diseases (25,26). As compared with conventional protein-based biomarkers, certain miRNAs have several potential advantages, including easy detection by PCR, relative homogeneity and highly specific expression profiles (25).

Several analyses of miRNA and mRNA expression profiles have demonstrated that the study of the differential expression of miRNAs and mRNAs has potential value for tumor diagnosis and prognosis in patients with TC (27-30). Recent studies have demonstrated that some miRNAs have the potential to be used as diagnostic or prognostic markers for PTC (24,31). Combining two or three markers constitutes a more accurate approach to differentiating malignant tumors from their benign counterparts when compared with using a single biomarker (32-34). However, to the best of our knowledge, no studies to date have examined the combined use of miRNAs and mRNAs as biomarkers for the diagnosis of TC. Previous studies have reported a series of differentially expressedmiRNAs and mRNAs in PTC (35-37). The differ-

Table I. Expression of miRNAs and their target genes in different types of cancer.

| miRNA | Target gene | Cancer type | Sample no. | r | Rank | P-value | FDR |
|---|---|---|---|---|---|---|---|
| hsa-miR-15a | AXIN2 | Bladder urothelial cancer (BLCA) | 229 | -0.29099 | 32222 | 7.58506E-06 | 9.23922E-05 |
| | | Breast cancer (BRCA) | 748 | -0.21574 | 53605 | 2.50939E-09 | 1.84287E-08 |
| | | Head and neck squamous cell carcinoma (HNSC) | 428 | -0.34684 | 7364 | 1.52465E-13 | 8.20853E-12 |
| | | Kidney renal clear cell carcinoma (KIRC) | 300 | -0.44051 | 9438 | 1.13431E-15 | 4.65868E-14 |
| | | Lung adenocarcinoma (LUAD) | 441 | -0.12922 | 102516 | 0.00657988 | 0.0251174 |
| | | Lung squamous cell carcinoma (LUSC) | 362 | -0.16374 | 101085 | 0.0017737 | 0.00692297 |
| | | Papillary thyroid carcinoma (PTC) | 557 | -0.10371 | 164644 | 0.0143314 | 0.0344256 |
| | | Uterine corpus endometrial carcinoma (UCEC) | 161 | -0.22901 | 59904 | 0.0034772 | 0.0227855 |
| hsa-miR-20b | TP53INP1 | Kidney chromophobe (KICH) | 91 | -0.37705 | 57043 | 0.000229526 | 0.00156243 |
| | | Kidney renal clear cell carcinoma (KIRC) | 300 | -0.23217 | 71929 | 4.90559E-05 | 0.000264361 |
| | | Lung adenocarcinoma (LUAD) | 441 | -0.11523 | 121627 | 0.015477 | 0.0497973 |
| | | Papillary thyroid carcinoma (PTC) | 557 | -0.19305 | 69177 | 4.4499E-06 | 2.54406E-05 |
| hsa-miR-106a | TP53INP1 | Breast cancer (BRCA) | 748 | -0.13769 | 120249 | 0.000158461 | 0.000518767 |
| | | Colorectal cancer (CRC) | 299 | -0.33271 | 9916 | 3.68032E-09 | 1.4467E-07 |
| | | Kidney chromophobe (KICH) | 91 | -0.36623 | 61281 | 0.000356682 | 0.00226009 |
| | | Kidney renal clear cell carcinoma (KIRC) | 300 | -0.22882 | 73995 | 0.000063344 | 0.000331829 |
| | | Lung squamous cell carcinoma (LUSC) | 362 | -0.19645 | 74972 | 0.000168949 | 0.00088911 |
| | | Papillary thyroid carcinoma (PTC) | 557 | -0.13739 | 120320 | 0.00115165 | 0.00378548 |
| hsa-miR-20a | TP53INP2 | Bladder urothelial cancer (BLCA) | 229 | -0.49808 | 2457 | 9.24247E-16 | 1.47643E-13 |
| | | Breast cancer (BRCA) | 748 | -0.33902 | 11844 | 1.42082E-21 | 4.7225E-20 |
| | | Colorectal cancer (CRC) | 299 | -0.17681 | 63670 | 0.00214911 | 0.0131569 |
| | | Head and neck squamous cell carcinoma (HNSC) | 428 | -0.26834 | 24475 | 1.71039E-08 | 2.77065E-07 |
| | | Kidney chromophobe (KICH) | 91 | -0.32027 | 82252 | 0.00196898 | 0.00929535 |
| | | Kidney renal clear cell carcinoma (KIRC) | 300 | -0.17722 | 113053 | 0.00206243 | 0.00707144 |
| | | Lung squamous cell carcinoma (LUSC) | 362 | -0.31744 | 23413 | 6.43229E-10 | 1.08395E-08 |
| | | Skin cutaneous melanoma (SKCM) | 342 | -0.15318 | 71854 | 0.00452332 | 0.0247739 |
| | | Papillary thyroid carcinoma (PTC) | 557 | -0.14565 | 111172 | 0.000564255 | 0.00200733 |
| | | Uterine corpus endometrial carcinoma (UCEC) | 161 | -0.3447 | 18090 | 7.54732E-06 | 0.000163772 |
| hsa-miR-15a | BCL2 | Bladder urothelial cancer (BLCA) | 229 | -0.40527 | 8501 | 1.83006E-10 | 8.44936E-09 |
| | | Breast cancer (BRCA) | 748 | -0.21583 | 53550 | 2.46948E-09 | 1.81542E-08 |
| | | Colorectal cancer (CRC) | 299 | -0.1683 | 70445 | 0.00351403 | 0.019444 |
| | | Head and neck squamous cell carcinoma (HNSC) | 428 | -0.13371 | 124582 | 0.00559585 | 0.0178082 |
| | | Lung adenocarcinoma (LUAD) | 441 | -0.12502 | 108048 | 0.00858301 | 0.0310865 |
| | | Papillary thyroid carcinoma (PTC) | 557 | -0.26928 | 30480 | 1.04246E-10 | 1.35264E-09 |
| | | Uterine corpus endometrial carcinoma (UCEC) | 161 | -0.32028 | 23449 | 3.44386E-05 | 0.000576509 |
| hsa-miR-20a | KAT2B | Bladder urothelial cancer (BLCA) | 229 | -0.31374 | 25136 | 1.27E-06 | 1.98E-05 |
| | | Breast cancer (BRCA) | 748 | -0.27776 | 25792 | 1.02E-14 | 1.56E-13 |
| | | Colorectal cancer (CRC) | 299 | -0.37313 | 6006 | 2.61E-11 | 1.69E-09 |
| | | Head and neck squamous cell carcinoma (HNSC) | 428 | -0.37751 | 4310 | 6.05E-16 | 5.56E-14 |
| | | Acute myeloid leukemia (LAML) | 172 | -0.2645 | 28945 | 0.000454575 | 0.00572662 |
| | | Lung adenocarcinoma (LUAD) | 441 | -0.30742 | 8650 | 4.17E-11 | 1.89E-09 |
| | | Lung squamous cell carcinoma (LUSC) | 362 | -0.31728 | 23452 | 6.57E-10 | 1.11E-08 |
| | | Ovarian serous cystadenocarcinoma (OV) | 265 | -0.12206 | 86631 | 0.0471435 | 0.208096 |
| | | Papillary thyroid carcinoma (PTC) | 557 | -0.12636 | 133650 | 0.00281234 | 0.00832219 |
| | | Uterine corpus endometrial carcinoma (UCEC) | 161 | -0.21163 | 70910 | 0.00703863 | 0.0389642 |

FDR, false discovery rate.

ential expression profiles were analyzed, and ROC curve analyses were performed to assess the predictive power of these miRNAs and mRNAs. We found that 8 miRNAs (miR-106a, miR-15a, miR-30a, miR-30b, miR-20a, miR-20b, miR-30d and miR-30e) and 8 mRNAs (AXIN2, ITGA3, TP53INP1, TP53INP2, BCL2, PTEN, FOS and KAT2B) had higher predictive powers, and the AUC values were >0.90. These results indicated that these miRNAs and mRNAs are good biomarker candidates for the clinical diagnosis of PTC.

Currently, FNA is the most accurate diagnostic method used for detecting TC (38); however, up to 30% of fine-needle aspiration biopsy cytological samples are reported as 'suspicious' or 'indeterminate' (39). Therefore, additional methods to increase the sensitivity and specificity of diagnosis are highly desirable. Molecular markers, such as B-raf proto-oncogene, serine/threonine kinase (BRAF), RAS, RET/PTC, paired box 8 (PAX8)/peroxisome proliferator-activated receptor c (PPARc) or galectin-3 may be considered for determining cytology, according to the American Thyroid Association guidelines (40). In a previous study of ours, TP53INP1, TP53INP2, AXIN2 and ITGA3 were found to be differentially expressed in PTC tissues when compared with the normal tissues (34). In the present study, we revealed that aside from those 4 genes, BCL2, PTEN, FOS and KAT2B also have potential value for the diagnosis of PTC. Usually, an AUC value >0.5 is considered suitable for clinical diagnosis. To increase the sensitivity and specificity and the AUC, we combined the mRNA expression of AXIN2 and miR-15a in 28 patients using logistic regression analysis. The results revealed that the combination of AXIN2 and miR-15a increased diagnostic accuracy, as compared with the use of a single molecule (the sensitivity was 78.5% and the specificity was 93.7%). These results suggest that the combination of AXIN2 and miR-15a is a strong and independent predictor for the diagnosis of PTC.

In conclusion, our data demonstrate that the combined use of miRNAs and their target mRNAs may provide a novel predicting tool for the diagnosis of PTC. The combination of miRNAs and mRNAs significantly improved the diagnostic accuracy. The data of the present study may serve as the basis for further studies on PTC diagnosis. Further studies are required to examine the mechanisms of action of different miRNAs and mRNAs in PTC.

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