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Screening and Studying of Blood miRNAs as Potential Diagnostic Markers for Papillary Thyroid Carcinoma

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Objective: MiRNAs play a pivotal role in tumorigenesis and development by exerting negative regulation on the expression of target genes. In this study, bioinformatics techniques and online database were employed to investigate the specific miRNA-target gene regulatory network in PTC, which was subsequently validated using human blood samples and compared to existing tumor markers.

Methods: The miRNA (GSE50901) and Gene Expression (GSE113629) chip screening data of human PTC tissues were retrieved from GEO database. A comparative analysis was conducted using the GEO2R to identify differentially expressed miRNAs and target genes of the patients with PTC. Prediction of the miRNA-target gene regulatory network, related signal transduction pathways, biological effects and their relationship to prognosis was performed based on GO, KEGG, qRT-PCR detection of human blood samples, analysis of correlation on the existing pathological tumor markers, and ROC.

Results: Compared to the corresponding normal thyroid tissues, a total of 2116 miRNAs were found to be differentially expressed in PTC patients, including 1968 up-regulated and 148 down-regulated genes. The abnormally expressed genes primarily participated in signal pathways associated with tumorigenesis and abnormal gene transcription. By utilizing data from the GEO database, five miRNAs closely linked to PTC prognosis were identified, which were miR-221-3p, miR-222-3p, miR-182-5p, miR-135a-5p, and miR-34a-5p, with elucidating the target genes. Experimental validation, correlation analysis with tumor markers along with bioinformatics analysis revealed a significant increase in expression levels of miR-182-5p in PTC patients which positively correlated with poor prognosis. These molecules could play crucial roles in both initiation and progression of PTC.

Conclusion: This study identified potential novel blood-based miRNA biomarkers for PTC through bioinformatics analysis combined with the detection of human blood samples, thereby offering new possibilities for significant biomarkers associated with diagnosis and prognosis of PTC.

Keywords: papillary thyroid carcinoma, microRNA, differentially expressed genes, bioinformatics, blood samples

Introduction

According to GLOBOCAN's 2022 analysis of cancer morbidity and mortality, the global incidence of thyroid cancer is 4.1 per 100,000 population, with a relatively low mortality rate of 0.5 per 100,000 population. However, thyroid cancer exhibits the fastest increase in cancer incidence, including in China.[1] Among differentiated thyroid cancer (DTC) cases, papillary thyroid carcinoma (PTC) is the most common subtype accounting for about 85% of all.[2] PTC can occur at any age but predominantly affects children and women under the age of 40. It often remains localized within the thyroid gland for several years before spreading to other parts and cervical lymph nodes through intraglandular lymphatic

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vessels.[3] Unfortunately, this nature makes it prone to being overlooked.[4] As PTC incidence continues to rise annually, gaining an in-depth understanding of gene expression characteristics specific to PTC can enhance our comprehension of this disease and facilitate early intervention.

Non-coding microRNAs (miRNAs), a recently discovered class of small, endogenous non-coding RNAs, are important negative regulators of target genes expression.[5,6] MiRNAs are abundant in the genome and affect cell growth, differentiation, apoptosis and adhesion.[7] Dysregulation of miRNA expression is a common feature of many types of human cancer, including PTC.[8] The use of serum as a biological test sample offers the advantages of convenient and non-invasive continuous in vitro testing. Moreover, the exceptional stability of serum microRNAs ensures consistency between actual patient values and experimental test results. So the utilization of microRNAs in whole blood or blood components represents a promising approach for disease diagnosis, including life-threatening conditions. Although several studies have been conducted on the expression and regulatory mechanisms of miRNAs, the identification and exploration of novel miRNA regulatory networks in blood remain unknown, yet imperative for a comprehensive understanding of PTC.

The emergence of bioinformatics research has greatly facilitated the investigation of various types of cancers. Additionally, the bioinformatics analysis of miRNA expression profiles has revealed the potential of miRNA as a biomarker for cancer prognosis.[6,9] A previous study identified 150 differentially expressed miRNAs between PTC and matched normal esophagus tissues, with 5 specific miRNAs closely associated with PTC prognosis.[10] However, the regulatory network involving these differentially expressed miRNAs and their target genes (DEGs), as well as the related signal transduction pathways, remains largely unknown.[11] Therefore, this study employed bioinformatics techniques to analyze differential expression patterns of both miRNAs and genes between PTC and corresponding normal thyroid tissues in order to establish a regulatory relationship between them. Moreover, it aimed to predict their regulatory networks and biological roles by considering associated signal transduction pathways. Furthermore, changes in expressions of key molecules in PTC were investigated and explored for their correlation with prognosis. Simultaneously, blood samples from patients were collected and compared with those from healthy volunteers to verify the association between specific miRNAs and PTC. This approach aims to provide a theoretical foundation for identifying novel markers and therapeutic targets for PTC while shedding light on its pathogenesis.

Materials and Methods

Data Selection

The gene expression profile data related to PTC were retrieved from NCBI Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The selected search terms included “papillary thyroid carcinoma”, “Expression profiling by array” and “Homo sapiens”. GSE113629 was selected for differential miRNA (microRNA) analysis in PTC. The micromatrix data were completed by Agilent-070156 human_miRNA_V21.0_Microarray 046064 (GPL24741). This experiment comprised of 10 human tissue specimens, including 5 patients with PTC and 5 normal thyroid tissues. The differential gene expression in PTC was analyzed with GSE50901 along with Agilent-028004 SurePrint G3 human GE 8x60K Microarray (GPL13607). This experiment included 65 human tissue samples, consisting of 61 patients with PTC and 4 normal thyroid tissues.

Screening and Identification of Differentially Expressed miRNAs and Genes

Using the online analysis software GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/),[12] the differentially expressed miRNAs and genes in human PTC were screened with normal human thyroid tissues as control. The screening conditions were $P = 0.01$ and $|\log FC| \geq 1$, respectively. Subsequently, a protein-protein interaction network was constructed using STRING (https://string-db.org) website).[13] WebGestalt online database (https://www.webgestalt.org/)[14] was employed under the screening condition of $P = 0.01$. Relevant data for cluster analysis were downloaded and combined with the screening results of miRNA and gene expression in GSE113629 and GSE50901 to determine differentially expressed miRNAs and genes in PTC, thus establishing a regulatory network of specific miRNA and relative target genes.

Functional Enrichment of Differentially Expressed Genes

The gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway enrichment analysis were performed using DAVID 6.8 software.[15] The screening criteria were set as P <0.05 and a minimum gene count of ≥2.4. Target genes were predicted and extracted through the intersection of these analyses.

Intersection of Prediction and Extraction of Target Genes

The miRNAs with significant differences in GSE113629 (|log (FC)|≥2) were analyzed using the Functional Enrichment analysis tool (Funrich). Among them, 36 miRNAs were up-regulated and 74 miRNAs were down-regulated. The target genes were predicted separately. The predicted target genes intersected with the differential mRNA and were cross-matched with miRNA, resulting in the identification of regulatory relationships between highly expressed miRNAs and low-expressed mRNAs, as well as between low-expressed miRNAs and high-expressed mRNAs.

The web-based tool GEPIA2 (http://gepia2.cancer-pku.cn/) was utilized for interactive gene expression profiling to analyze differential expression, correlation, and patient survival analysis of tumor and normal samples from TCGA and GTEx datasets. GEPIA2 was employed to further validate the differential expression of 50 genes between normal tissues and cancer tissues. Key genes satisfying both |logFC|>1 and $P < 0.05$ criteria were considered to exhibit significant differences. The differentially expressed miRNA genes were selected, and the regulatory network of miRNA-mRNA was visualized using Cytoscape visualization software.

Human Blood Samples and qRT-PCR Detection

Human blood samples were collected from Shandong Provincial Hospital Affiliated to Shandong First Medical University in Jinan. They were divided into two groups: a control group (n=13) from randomly selected normal volunteers, and an experimental group (n=21) from patients newly diagnosed with PTC who had not undergone any treatment. The gender, age and tumor markers' expression were obtained from the pathological examination reports of the patients. This study has received approval from the Shandong Provincial Hospital Affiliated to Shandong First Medical University.

The total RNA was extracted using Invitrogen TRIzol (Thermo Fisher SCIENTIFIC, Code: 15596018). For miRNA quantification, cDNA synthesis was performed using the Evo M-MLV RT Kit for qPCR (Accurate Biology, Code: AG11707). The qPCR primers for miR-221-3p, miR-222-3p, miR-182-5p, miR-135a-5p and miR-34a-5p were designed and synthesized with the Bulge-Loop miRNA qRT-PCR Primer sets (RIBOBIO, Code: MQPSCM001). Real-time PCR was conducted using the SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biology, Code: AG11701) on a Bio-Rad CFX-96 real-time system. U6 was used as the internal control. All qRT-PCR reactions were performed in triplicates, and relative quantification was calculated by the $2^{-\Delta\Delta Ct}$ method (95% confidence interval) with calibration to the corresponding control. The expression data were analyzed with a Student's t-test. $P < 0.05$ was considered significant difference in statistics.

ROC Analysis

The receiver operating characteristic (ROC) curve for the expression data of key miRNAs was constructed using SPSS 26.0 software, and the AUC value was utilized as a diagnostic index with statistical significance set at $P < 0.05$.

Results

The miRNAs Were Differentially Expressed in PTC

The data from GSE113629 and GSE50901 were analyzed using GEO2R. The results revealed a multitude of aberrant levels of specific miRNA and gene expression in the thyroid gland of patients with PTC compared to normal thyroid tissue. A total of 2116 differentially expressed miRNAs were identified in PTC patients, with 1968 being up-regulated and 148 down-regulated (Figure 1A). Among these, the top 50 significantly up-regulated and down-regulated miRNAs were displayed in Figure S1A. Figure 1B provided a clear representation of selected results. Additionally, screening results demonstrated that there were a total of 536 dysregulated genes in the PTC thyroid tissue compared to normal tissue, with 270 being up-regulated and 266 being down-regulated (Figure 1C). The top 50 significantly up-regulated and down-regulated genes are shown in Figure S1B.

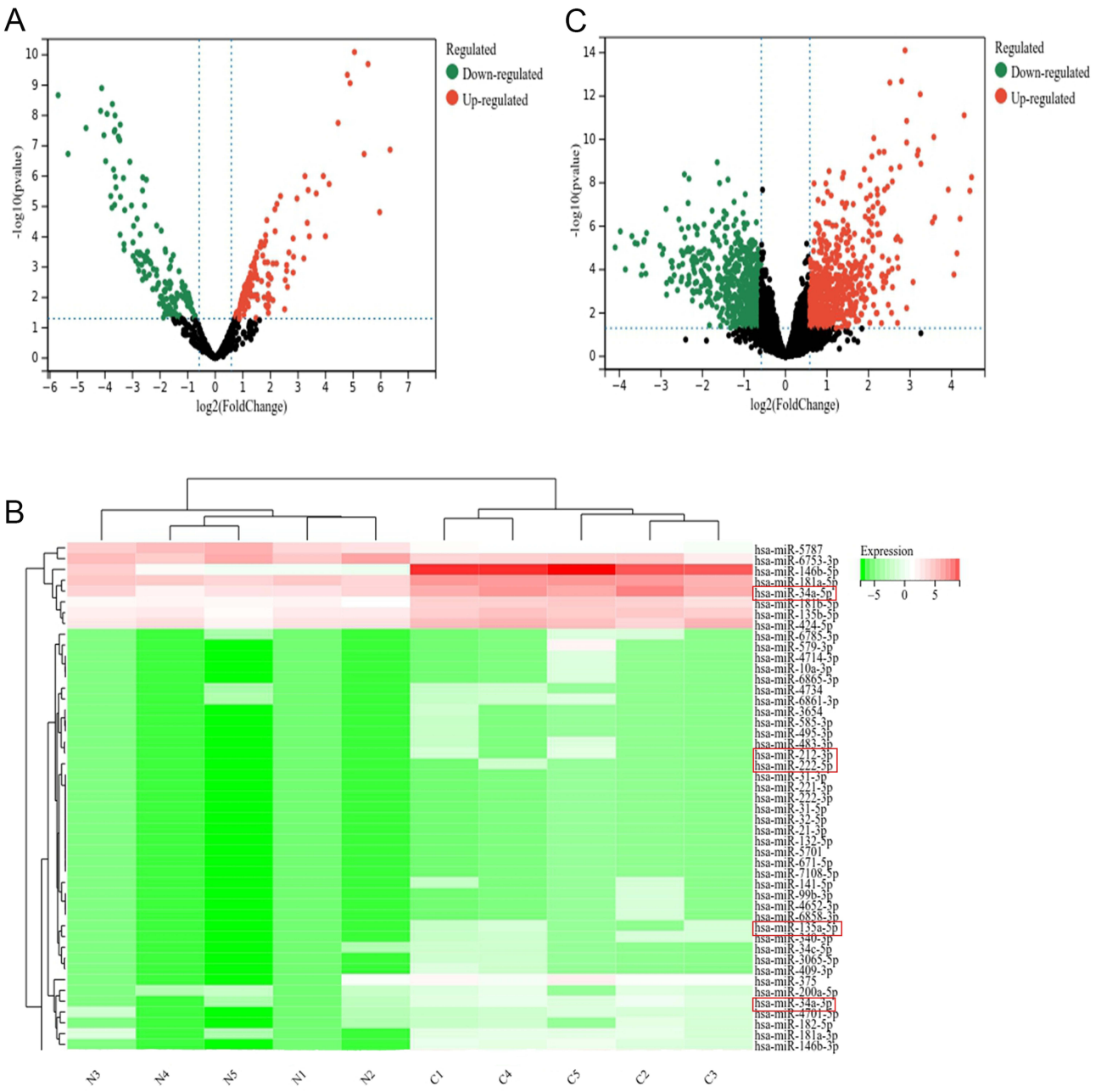

Figure 1 The miRNAs differentially expressed in PTC. (**A**) The miRNAs that are differentially expressed in the samples of PTC patients compared to normal controls from dataset GSE113629. (**B**)The key up-regulated and down-regulated miRNAs identified in GSE113629. (**C**) The genes that are differentially expressed in the samples of PTC patients compared to normal controls from dataset GSE50901. (Cancer vs normal, green indicates up-regulation, red indicates down-regulation).

The Proteins Net Encoded by the Differentially Expressed miRNAs - Targeted Genes in PTC

The functional correlation of the proteins encoded by differentially expressed genes in PTC was analyzed by STRING12.0. It was found that in the network of overexpressed genes in PTC, the number of nodes was 225,359 sides, the average node degree was 3.19, and the average local clustering coefficient was 0.389. Interaction network analysis of proteins encoded by differential genes (PPI) enriched *P* values less than 1.0e-16 (Figure 2A) At the same time, in the network of proteins encoded by low expression genes in PTC, the number of nodes reached 185 and the number of edges reached 219. The average node degree was 2.37 and the average local clustering coefficient was 0.39.

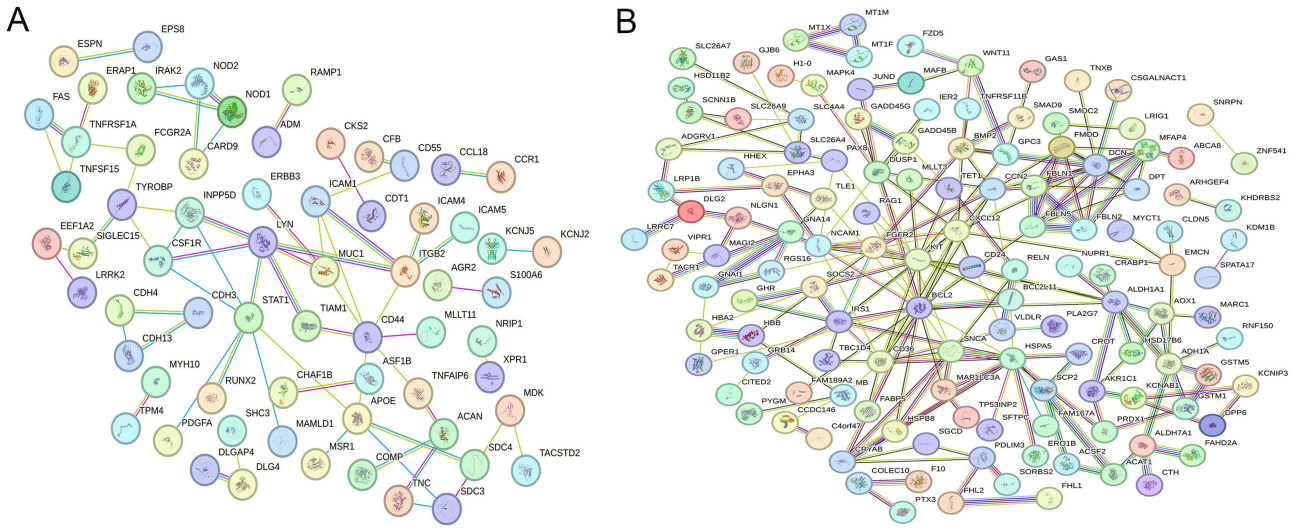

Figure 2 The encoding protein network of differentially expressed genes. (**A**) The PPI of the up-regulated genes-encoded proteins. (**B**) The PPI of the down-regulated genes-encoded proteins.

The *P* value enriched by differential gene coding protein interaction network analysis (PPI) was 1.11e-16 (Figure 2B). The above findings suggested a strong functional correlation between the proteins encoded by differentially expressed genes in PTC, which could play a crucial regulatory role in the initiation and progression of this disease.

The Functional Roles and Associated Signal Transduction Pathways of Differentially Expressed Genes Targeted by the Dysregulated miRNAs in PTC

Through GO and KEGG functional enrichment analysis, it could be obtained the molecular function, cellular components and biological processes of differentially expressed genes in PTC compared with normal thyroid tissues. The results revealed that highly expressed gene coding proteins in PTC were primarily involved in cell adhesion pathway and cytokine signal transduction pathway (Figure 3A), while the main biological processes included signal transduction and cell adhesion. The associated cellular components were plasma membrane, extracellular region and exocrine. The predominant molecular functions comprised protein binding, protein homodimerization activity, protein kinase binding, among others (Figure 3B). Conversely, low-expression genes in PTC encoded tumor-related transduction pathways and cytokine transduction pathways (Figure 3C). The primary biological processes encompassed cytoplasm, plasma membrane, extracellular region as well as extracellular exocrine body. The related cellular components consisted of protein binding, extracellular space along with cell surface. The key molecular functions included bicarbonate transport, negative regulation of cell migration, cell adhesion, and thymus development (Figure 3D).

Convergence of Gene Prediction and Target Gene Extraction

The Funrich software was utilized to identify significantly different miRNAs (|log(FC)|≥2) in GSE113629. Among these, 36 miRNAs were found to be up-regulated while 74 miRNAs were down-regulated, and their respective target genes were predicted. Subsequently, a total of 2808 low expression genes and 862 high expression genes were obtained. By intersecting with the corresponding genes in GSE50901, 39 low expression genes (Figure 4A) and 11 high expression genes (Figure 4B) were obtained. To analyze the differential expression of these selected 50 genes between normal and cancer tissues, the GEPIA2 database was employed. As a result, it was observed that the expression levels of five genes in cancer tissues were significantly higher than those in normal tissues, whereas the expression levels of twenty-nine other genes in cancer tissues were significantly lower compared to normal tissues. However, no significant differences in gene expression were detected for the remaining sixteen genes when comparing cancer tissue samples with normal tissue samples. Finally, using the visual Cytoscape visualization software, draw the regulatory network diagram depicting miRNA-mRNA interactions was constructed, which was shown in

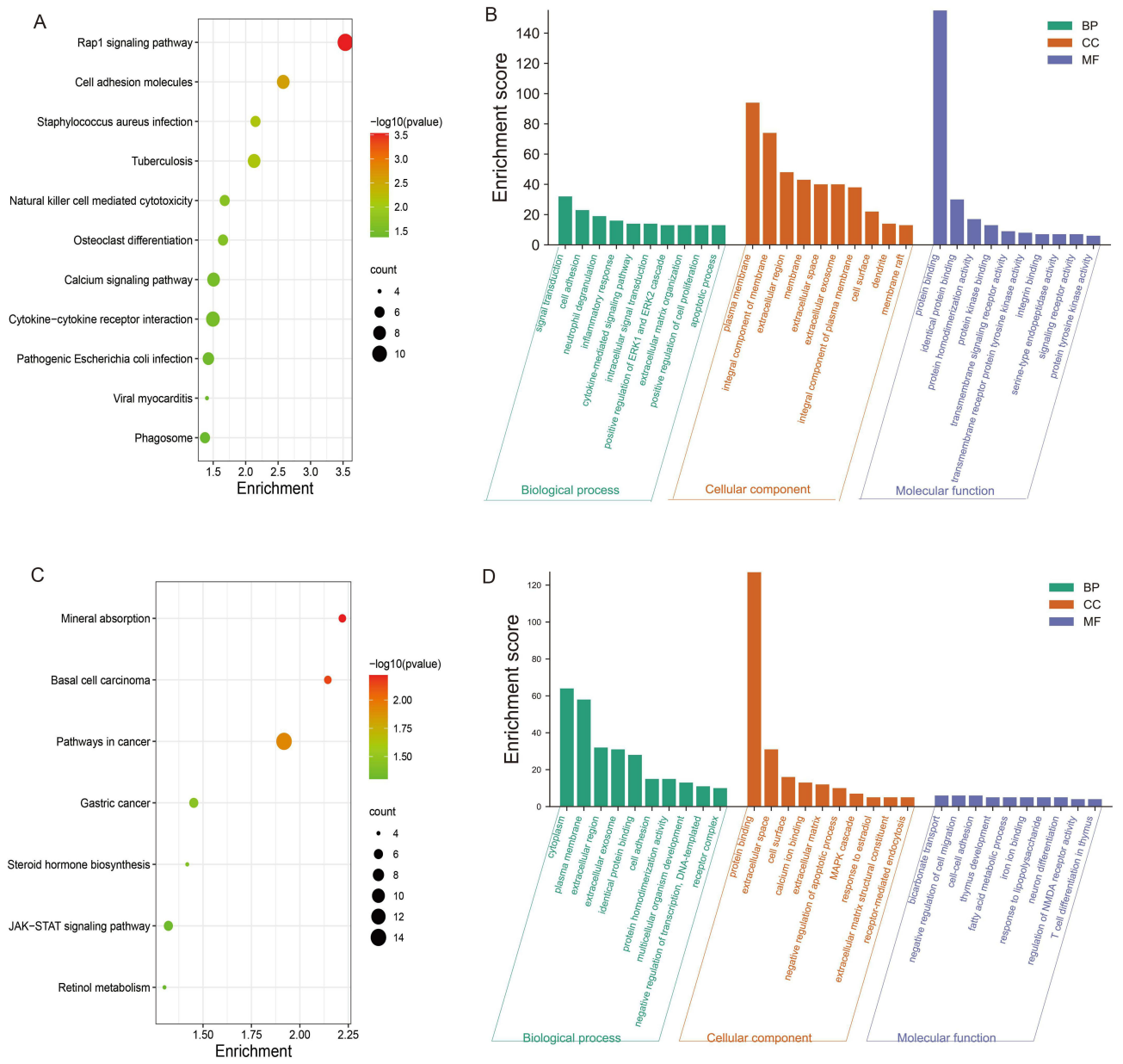

Figure 3 The enrichment results of the key genes obtained through GO and KEGG analysis. (**A**) The results of KEGG analysis of up-regulated genes. (**B**) The results of GO analysis of up-regulated genes. (**C**) The results of KEGG analysis of down-regulated genes. (**D**) The results of GO analysis of down-regulated genes.

Figure 4C. Notably, this diagram revealed that one target gene could be regulated by multiple miRNA while one miRNA could regulate multiple target genes.

The miRNAs were arranged in descending order of differential expression as follows: hsa-miR-221-3p, hsa-miR-222-3p, hsa-miR-182-5p, hsa-miR-135a-5p, hsa-miR-34a-5p, hsa-miR-212-3p, hsa-miR-503-5p, hsa-miR-144-3p, hsa-miR-204-5p, hsa-miR-32-5p, hsa-miR-181b-5p, hsa-miR-181a-5p, hsa-miR-424-5p, hsa-miR-135b-5p. The top five miRNAs exhibiting the most significant differential expression were selected as the subjects for subsequent experimental investigations, namely hsa-miR-221-3p, hsa-miR-222-3p, hsa-miR-182-5p, hsa-miR-135a-5p and hsa-miR-34a-5p. Their corresponding target genes could be found in Table S1.

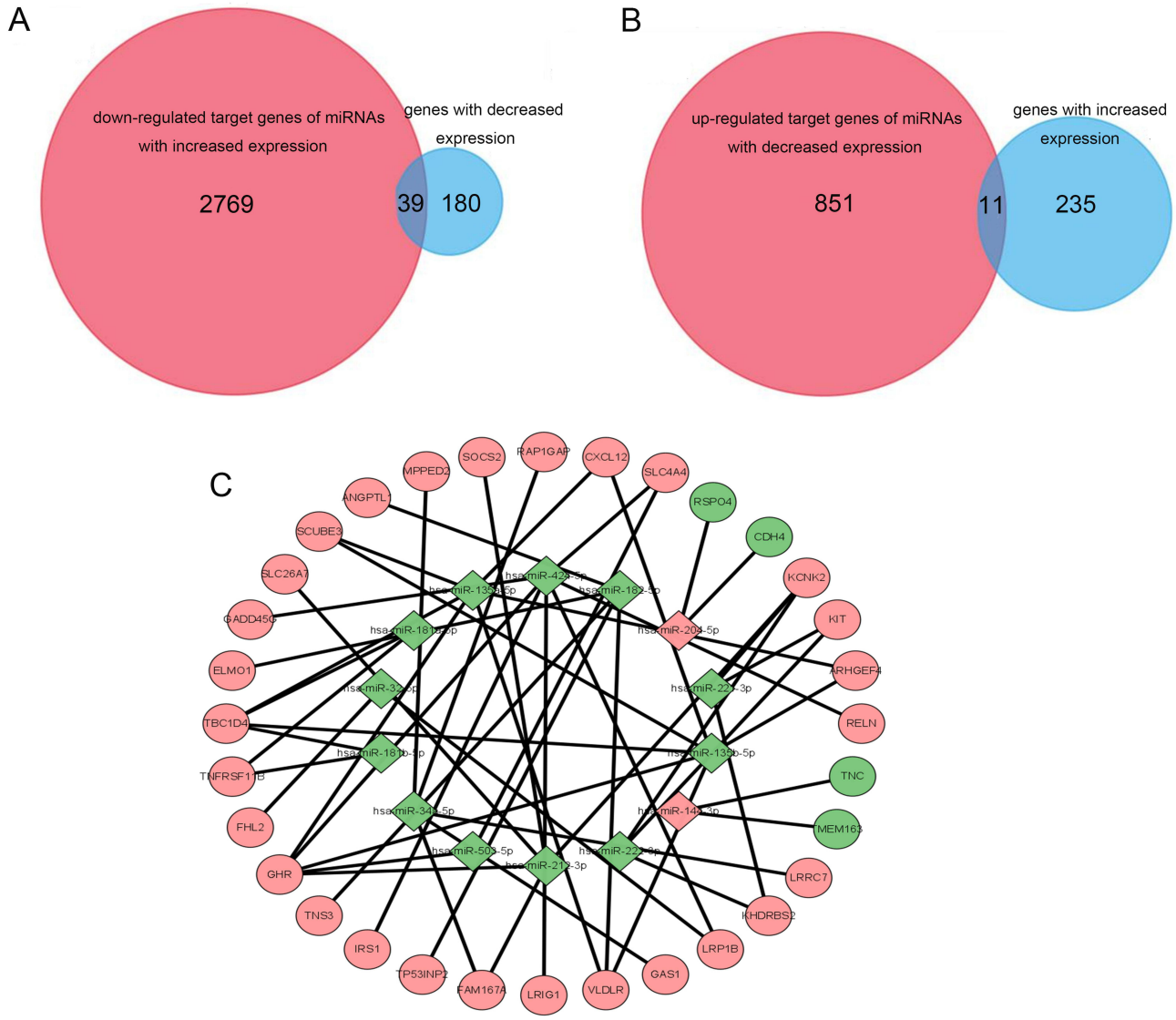

Figure 4 The intersection of the key miRNAs and their differentially expressed target genes in GES50901 database. (**A**) The cross-comparison result of down-expression genes between prediction from targets of up-regulated miRNAs and GES50901 database. (**B**) The cross-comparison result of up-expression genes between prediction from targets of down-regulated miRNAs and GES50901 database. (**C**) The key miRNA-mRNA network (green color represents up-expression while pink color represents down-expression).

The Key Blood miRNAs Were Identified by Detecting Their Expression Levels in Human Blood Samples

The demographic characteristics (including age and gender) of the patients who provided blood samples, along with their correlation to the expression of key miRNAs was presented in Table 1.

The qRT-PCR results, which assessed the expression levels of selected key miRNAs in human blood samples, were presented in Figure 5A–E. Initial observations from these findings suggested that hsa-miR-222-3p, hsa-miR-182-5p, and hsa-miR-135a-5p exhibited significantly higher expression levels in blood samples from PTC patients compared to normal blood samples. Conversely, there were no significant changes in the expression levels of hsa-miR-221-3p and hsa-miR-34a-5p between blood samples from PTC patients and normal individuals. Among the three miRNAs over-expressed in PTC blood samples, the expression levels of hsa-miR-222-3p and hsa-miR-182-5p aligned with predictions made by The Cancer Genome Atlas (TCGA) online database. However, the expression level of hsa-miR-135a-5p contradicted TCGA's prediction (Figure S2A–E). Survival curve analysis using the Kaplan-Meier Plotter database demonstrated that only high expression of hsa-miR-182-5p was associated with shorter overall survival among patients

Table 1 Correlation of Key miRNAs' Expression with Age and Gender in PTC Patients

Category		Age		*P*	Gender		*P*
		≥55 Years	<55 Years		Male	Female	
miR-221-3p	No. of patients	5	16	>0.05	4	17	>0.05
	High	2	6		2	6	
	Low	3	10		2	11	
miR-222-3p	No. of patients	6	15	>0.05	5	16	>0.05
	High	2	8		2	8	
	Low	4	7		3	8	
miR-182-5p	No. of patients	5	16	>0.05	4	17	>0.05
	High	2	8		2	8	
	Low	3	8		2	9	
miR-135a-5p	No. of patients	6	15	>0.05	5	16	>0.05
	High	4	9		2	11	
	Low	2	6		3	5	
miR-34a-5p	No. of patients	5	16	>0.05	5	16	>0.05
	High	1	7		1	7	
	Low	4	9		4	9	

with PTC (Figure S2F–J). The positive correlation between the expression levels of the five key miRNAs and the tumor markers thyroid peroxidase (TPO), CD56, CK19 and Galectin-3 was presented in Figure 5F. The results demonstrating a negative correlation between the expression levels of these five key miRNAs and tumor markers Thyroglobulin

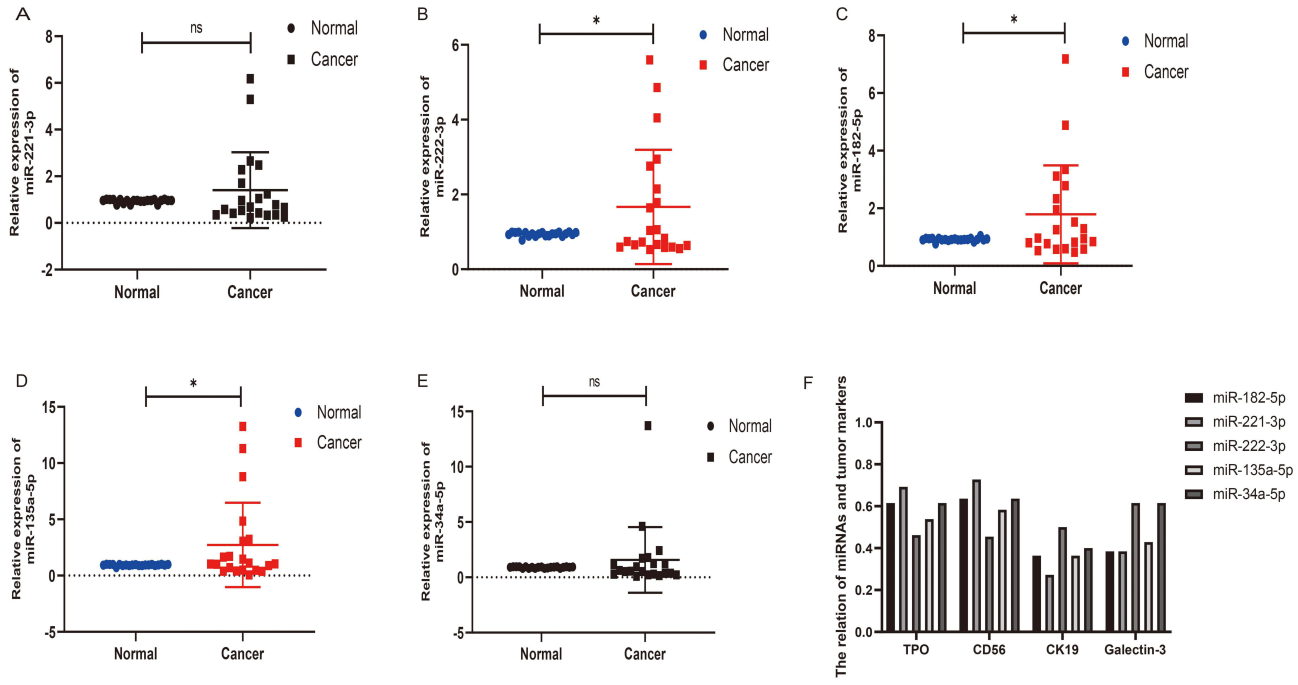

Figure 5 The expression levels of five key miRNAs in the blood samples from PTC patients compared to the normal and the correlation with the tumor markers. (**A**)The expression level of hsa-miR-221-3p increased in the blood samples of PTC patients with no significant difference (ns). (**B**) The expression level of hsa-miR-222-3p increased significantly in the blood samples of PTC patients (**P* <0.05 vs normal). (**C**) The expression level of hsa-miR-182-5p increased significantly in the blood samples of PTC patients (**P* <0.05 vs normal). (**D**) The expression of hsa-miR-135a-5p increased significantly in the blood samples of PTC patients (**P* <0.05 vs normal). (**E**) The expression of hsa-miR-34a-5p increased in the blood samples of PTC patients but no significant difference (ns). (**F**) The positive correlation of five key miRNAs with tumor markers in PTC. *P* >0.05 vs normal.
Abbreviation: ns, no statistical significance.

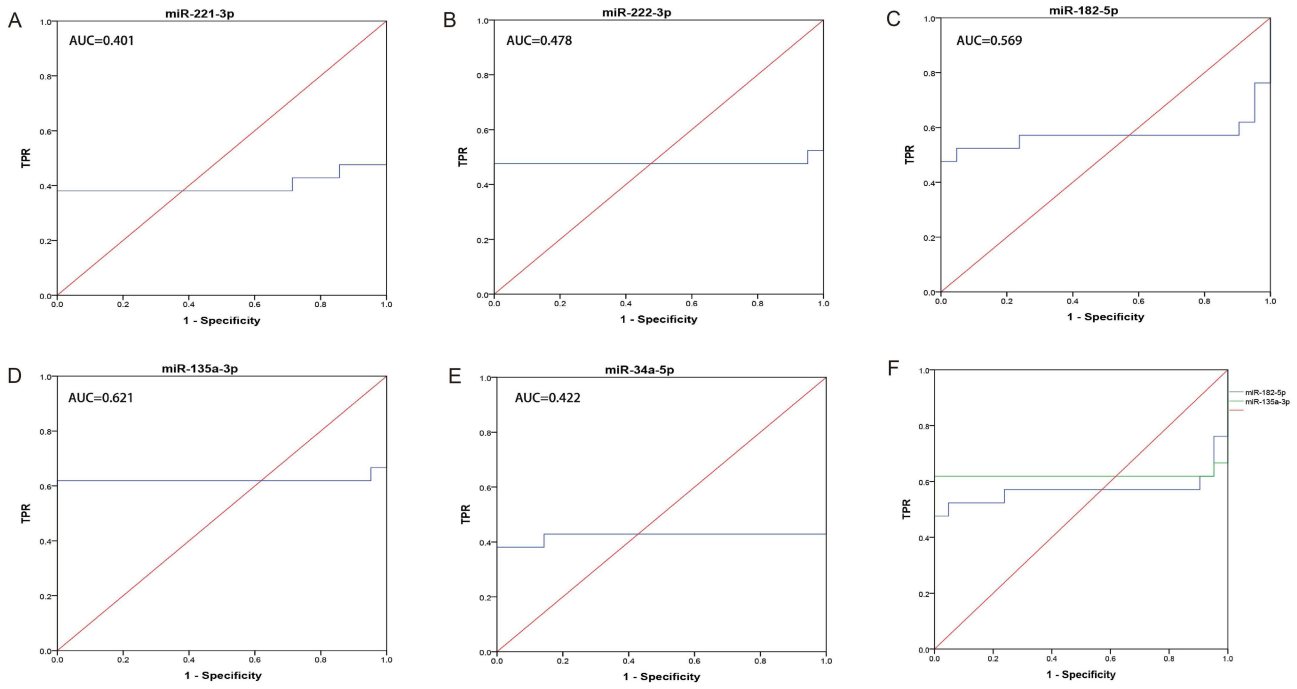

Figure 6 The key miRNAs could have diagnostic value in PTC determined by ROC analysis. (**A**) The value of hsa-miR-221-3p AUC was 0.401. (**B**) The value of hsa-miR-222-3p AUC was 0.478. (**C**) The value of hsa-miR-182-5p AUC was 0.569. (**D**) The value of hsa-miR-135a-3p AUC was 0.621. (**E**) The value of hsa-miR-34a-5p AUC was 0.422. (**F**) The combined ROC curves of miR-182-5p and miR-135a-5p.

antibody (TgAb), calcitonin (Ctn), carcinoembryonic antigen (CEA) and thyroid-stimulating hormone (TSH) are presented in Table S2.

The Diagnostic Value of Five Key miRNAs in PTC Patients

To further elucidate the diagnostic value of five key miRNAs in PTC, ROC analysis was performed based on their expression levels. The results indicated that hsa-miR-182-5p and hsa-miR-135a-5p exhibited significant diagnostic potential, with areas under the curve (AUC) values of 0.569 and 0.621, respectively. Conversely, all the AUC values for hsa-miR-221-3p, hsa-miR-222-3p, and hsa-miR-34a-5p were less than 0.5, suggesting limited diagnostic significance (Figure 6A–E). Combining hsa-miR-182-5p and hsa-miR-135a-5p enhances the diagnostic potential even further (Figure 6F).

Discussion

In this study, the regulatory network of miRNAs-target genes in PTC was investigated using bioinformatics techniques and online databases, with validation conducted using human blood samples. The study identified five differentially expressed miRNAs, namely miR-221, miR-222, miR-182, miR-135a, and miR-34a, which are closely associated with the prognosis of PTC. Furthermore, their target genes were identified. Through comprehensive bioinformatics analysis and experimental identification, the significant roles of these miRNAs in the carcinogenesis and progression of PTC were revealed, providing a solid theoretical foundation for the prevention, diagnosis, and treatment of related diseases.

Due to its low malignancy and recurrence rate, as well as high survival rate, PTC is often considered an "inert tumor".[16] However, approximately 10% of PTC patients are at risk of relapse, which can lead to invasive metastasis and even fatal consequences.[17] While cancer patients with malignant recurrence typically exhibit tumor invasion and lymph node metastasis in the early stage of tumor development,[18,19] there remains a dearth of effective methodologies for identifying high-risk patients with minor trauma, such as the assessment of specific biomarkers in peripheral blood. With the advancement of the research on epigenetics and its role in tumorigenesis, miRNAs, a class of important non-coding RNAs that regulate gene expression at the post-transcriptional level, have emerged as crucial targets for

investigation.[20,21] MiRNAs are small RNA molecules approximately 20–24 nucleotides long that are widely present in eukaryotes.[6] They exert their regulatory function by binding to the 3' -untranslated region of target mRNA molecules, thereby playing pivotal roles in gene expression regulation, cell cycle control, and organism development timing.[22,23] In tumorigenesis processes, special miRNAs act as endogenous regulators modulating tumor suppressor genes or proto-oncogenes, thus highlighting their significance in early clinical diagnosis and treatment.[24,25] Increasing evidence suggests that miRNAs can serve as valuable diagnostic and therapeutic indicators for various types of tumors.[26–28]

The study revealed significant dysregulation of miRNAs and genes expression in thyroid tissues of PTC patients compared to normal tissues, with aberrantly expressed genes primarily involved in tumorigenesis-related processes such as signaling pathways[29,30] and transcriptional regulation.[31] Functional enrichment analysis further highlighted the crucial regulatory roles played by these differentially expressed genes in PTC occurrence and development, particularly through cell adhesion and signal transduction processes.[32–34] Bioinformatics analysis also predicted the signaling pathways and biological effects associated with these miRNAs and genes, identifying their correlation with PTC patient prognosis.

MiRNAs have been demonstrated to play a pivotal role in the pathogenesis and progression of thyroid papillary carcinoma.[35] For instance, miR-146a and miR-146b are extensively investigated dysregulated miRNAs in thyroid tumors.[36] They exhibit upregulation in thyroid specimens compared to normal tissues and benign thyroid lesions.[37] The activation of the HMGB1/RAGE pathway not only contributes to chronic inflammation and suppresses phosphatase and tensin homologue (PTEN), a cellular regulator, but also induces the expression of miR-221 and −222, thereby promoting the development of PTC.[38,39] A study has revealed that downregulation of miR-129 is observed in PTC through MAL2 gene regulation.[40] Several studies have reported conflicting roles for miR-218 in thyroid tumor cells; while it can promote proliferation, invasion, and metastasis, it can also inhibit tumorigenesis by targeting Runx2 specifically within PTC context.[41] Additionally, miR-485-5p inhibits PTC development by regulating KLK7 expression levels.[42] However, limited studies have been conducted on the role of miR-182-5p and miR-34a-5p in PTC, thus highlighting their potential as novel targets for early diagnosis and treatment of invasive PTC. It is predicted that miR-182-5p may regulate TNS3 and TP53INP2 genes, while miR-34a-5p may target FAM167A and GAS1 genes. Notably, structural defects have been confirmed in ABD II (in TNS1) and C1 (in TNS2) domains of TNS3.[43] Moreover, it has been established that TNS3 plays a crucial role as a junction protein during podocyte formation by interacting with Dock5.[44] Overexpression of TNS3 in cancer cells leads to enhanced invasiveness. ZSCAN18 can bind to the promoter region of tumor protein 53-induced nuclear protein 2 (TP53INP2) gene, thereby promoting autophagy. Transcriptional regulation of TP53INP2 induces autophagy while inhibiting gastric cancer proliferation.[45] FAM167A activates the non-classical NF-kappa B pathway through its interaction with cell adhesion protein DSG1, consequently up-regulating NF-kappa B-induced kinase (NIK) by blocking its ubiquitination process. Neutralization of FAM167A in mouse tumor models reduces non-classical NF-kappa B activity and restores cellular sensitivity to TKI treatment.[46] GAS1 is an alpha receptor belonging to the glial cell-derived neurotrophic factor ligand family located on the cell membrane (GFR α).[47] GAS1 is expressed in various epithelial cells and fibroblasts, and it has been identified as a negative regulator of tumorigenesis and metastasis in colorectal cancer (CRC).[48] Therefore, investigating the molecular mechanisms underlying miR-182-5p-TNS3/TP53INP2 and miR-34a-5p-FAM167A/GAS1 interactions could provide insights into the occurrence and development of PTC. Conversely, highly expressed miR-222-3p and miR-135a-5p in PTC, along with BRAFV600E mutations, can enhance the metastatic potential of PTC by upregulating miR-222-3p expression.[49] Focal adhesion kinase is regulated at both post-transcriptional level by miR-7, miR-135a-3p, and miR-138 to modulate cellular communication and movement; while at post-translational level through Y397 autophosphorylation (pY397FAK).[50] To sum up, the five key miRNAs and their target molecules identified through bioinformatics prediction may play crucial roles in the tumorigenesis and progression, thus highlighting their potential application in PTC.

The significance of the five key miRNAs in PTC patients' blood samples was further confirmed through validation of their expression levels. The results demonstrated significant upregulation of three miRNAs, namely miR-222-3p, miR-182-5p, and miR-34a-5p, in the blood samples of PTC patients compared to normal blood samples. Notably, only miR-182-5p exhibited consistency with the predicted expression levels and survival curve results obtained from bioinformatics websites. While the role of five miRNA molecules in the diagnosis and treatment of PTC is analogous to that of the existing pathological tumor markers. Additionally, ROC analysis unveiled the diagnostic potential of two miRNAs:

miR-182-5p and miR-34a-5p. Thyroid status assessment is also an important part in transplant donors' evaluation. In this process, the rapid and accurate diagnosis of thyroid cancer is very important to ensure the success rate of transplantation and the health of recipients. Conventional evaluation methods such as ultrasound and puncture biopsy are already used methods for the diagnosis.[51–53] New tools are needed to speed up the process with safety. Therefore, specific miRNA such as miR-182-5p and miR-34a-5p are expected to become new tools that may improve the accuracy and efficiency of diagnosis of thyroid cancer, and thus can efficiently evaluate the thyroid status of donors.

The above results demonstrate that the regulation of specific target genes involved in the disease process by miRNAs is a complex and intricate regulatory mechanism, intricately intertwined within a signaling network. To gain comprehensive insights and validation, bioinformatics screening and prediction should be combined with extensive experimentation using human specimens, cells, and animal models. Therefore, further experimental investigations on these five types of miRNAs are imperative to elucidate their precise roles in PTC and their associations with prognosis.

Data Sharing Statement

The datasets GSE113629 for this study can be found in the GEO Accession viewer (nih.gov), The datasets GSE50901 for this study can be found in the GEO Accession viewer (nih.gov), The datasets GPL24741 for this study can be found in the GEO Accession viewer (nih.gov), The datasets GPL13607 for this study can be found in the GEO Accession viewer (nih.gov).

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Shandong Provincial Hospital Affiliated to Shandong First Medical University (SWYX: No.2021-526 on December 14, 2021) to be exempted from informed consent. The blood samples used in this study were left over from the participants' routine tests, as part of routine hospital procedure.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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