in vivo *36*: 1551-1569 (2022)
doi:10.21873/invivo.12866

Review

Circulating microRNAs and Clinicopathological Findings of Papillary Thyroid Cancer: A Systematic Review

GEORGIOS GEROPOULOS[1,2], KYRIAKOS PSARRAS[2], MARIA PAPAIOANNOU[3], DIMITRIOS GIANNIS[4], MARIA MEITANIDOU[2], KONSTANTINOS KAPRINIOTIS[1], NIKOLAOS SYMEONIDIS[2], EFSTATHIOS T. PAVLIDIS[2], THEODOROS E. PAVLIDIS[2], KONSTANTINOS SAPALIDIS[5], NADA MABROUK AHMED[1,6], TAREK EZZAT ABDEL-AZIZ[1] and MOHAMMAD M. R. EDDAMA[1,7]

[1]*Department of General and Endocrine Surgery, University College London Hospitals, London, U.K.;*
[2]*2nd Propedeutical Department of Surgery, Hippokration Hospital, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece;*
[3]*Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, University Campus, Thessaloniki, Greece;*
[4]*Department of Surgery, North Shore University Hospital, Manhasset, NY, U.S.A.;*
[5]*3rd General Surgery Department, "AHEPA" University Hospital, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece;*
[6]*Department of Pathology, University of Alexandria, Alexandria, Egypt;*
[7]*Research Department of Surgical Biotechnology, University College London, London, U.K.*

Abstract. *Background/Aim: Papillary thyroid cancer (PTC) is the most common endocrine malignancy with a rising incidence. There is a need for a non-invasive preoperative test to enable better patient counselling. The aim of this systematic review was to investigate the potential role of circulating microRNAs (miRNAs) in the diagnosis and prognosis of PTC. Materials and Methods: A systematic literature search was performed using MEDLINE, Cochrane, and Scopus databases (last search date was December 1, 2021). Studies investigating the expression of miRNAs in the serum or plasma of patients with PTC were deemed eligible for inclusion. Results: Among the 1,533 screened studies, 39 studies met the inclusion criteria. In total, 108 miRNAs candidates were identified in the serum, plasma, or exosomes of patients suffering from PTC. Furthermore, association of circulating miRNAs with thyroid cancer-specific clinicopathological features, such as tumor size (13 miRNAs), location (3 miRNAs), extrathyroidal extension (9 miRNAs), pre- vs. postoperative period (31 miRNAs), lymph node metastasis (17 miRNAs), TNM stage (9 miRNAs), BRAF V600E mutation (6 miRNAs), serum thyroglobulin levels (2 miRNAs), ^{131}I avid metastases (13 miRNAs), and tumor recurrence (2 miRNAs) was also depicted in this study. Conclusion: MiRNAs provide a potentially promising role in the diagnosis and prognosis of PTC. There is a correlation between miRNA expression profiles and specific clinicopathological features of PTC. However, to enable their use in clinical practice, further clinical studies are required to validate the predictive value and utility of miRNAs as biomarkers.*

Correspondence to: Georgios Geropoulos, MSc, MRCS, Department of General and Endocrine Surgery, University College London Hospitals, Ground floor, GI Services, 250 Euston Road, London, NW1 2PG, U.K. Tel: +44 7491459960, e-mail: georgios.geropoulos@nhs.net

Key Words: miRNA, microRNA, clinicopathological features, papillary thyroid cancer, thyroid neoplasms, review.

Thyroid cancer is the 20th most common cancer in the UK, and the incidence rates are projected to rise to 74% by the year 2035 (1). Papillary thyroid carcinoma (PTC) is the most common histological type, accounting for approximately 80% of the cases (2). Although, the vast majority of patients presenting with thyroid nodules that are benign, the ability to characterize the malignant nodules is quite important to ensure appropriate patient counselling when discussing curative therapy and the extent of the primary resection (3).

In addition to the cytological assessment of fine needle aspiration (FNA), molecular DNA and RNA testing of known mutations has been shown to improve the diagnostic accuracy of thyroid cancer. Kinase-activating mutations in the V-Raf murine sarcoma viral oncogene homolog B1 (*BRAF*) and telomerase reverse transcriptase (*TERT*) promoter mutations are the most common, found in nearly half of all PTCs, and in almost 80% of patients with recurrent metastatic PTC (4, 5). A single amino acid substitution, from valine to glutamic acid at codon 600 (V600E), accounts for almost 90% of all BRAF mutations (6). Of clinicopathological significance, the combination of BRAF V600E and TERT promoter mutations appear to have a robust synergistic impact on the aggressiveness of PTC (7). Beyond FNA, circulating *BRAF* mutation in real-time liquid biopsy is now taken into account in some centres for both thyroid cancer diagnosis and to guide effective treatment strategy (8). Although these mutations as diagnostic tools significantly enhance the specificity of FNA cytology, their sensitivity to rule out cancerous nodules remains poor.

Furthermore, the prognostic value of circulating and immunohistopathology markers (including galectin-3, CK19, HBME-1, p27, p21, cyclin D1, osteopontin, and E-cadherin) have been investigated in the diagnosis and staging of PTC with promising results (9-14). However, their application is limited and further research is needed to support their clinical usefulness. For example, thyroglobulin, is an effective thyroid cancer recurrence biomarker for patients who underwent a total thyroidectomy, but it is unreliable in patients who have had a hemithyroidectomy and/or have residual normal thyroid tissue. Thyroglobulin is highly sensitive and specific for identifying patients with persistent or recurrent disease who underwent a total thyroidectomy and received radioactive iodine ablation (RAA); however, it may be unreliable in patients with circulating anti-thyroglobulin antibodies (15, 16).

MicroRNAs (miRNAs) are small endogenous noncoding RNAs. Each of them targets multiple mRNAs with complementary sequences (17-20). By doing this, these small sequences of RNA negatively regulate gene expression and influence protein production by degrading, destabilizing or translationally inhibiting mRNA (20). Studies have shown that over-expression of some miRNAs can reduce the expression of tumor suppressing genes and promote oncogenesis or down-regulate oncogenes and act as tumor suppressors (19). Using many methods of isolation, such as Northern hybridization or reverse transcription – PCR (RT-PCR), miRNAs have been found in serum and plasma. Several studies have reported circulating miRNAs as cancer biomarkers (18, 21). Currently, there are potential candidates that have been found to be highly expressed in PTC. Highlighting the scarcity of non-invasive diagnostic tests in thyroid cancer, there is an urgent need to identify novel biomarkers that may offer better sensitivity and specificity to distinguish between benign and malignant thyroid nodules, enabling better preoperative patient counselling and guidance to treatment. Hence, the aim of this study was to review the current literature regarding the potential role of circulating miRNAs in the diagnosis, prognosis, and staging of PTC patients.

Materials and Methods

Study design. This study was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines for systematic reviews (22). Original clinical studies, published in English, investigating the expression of circulating miRNAs in patients with papillary thyroid cancer were deemed eligible for inclusion. Exclusion criteria were: i) articles published in languages other than English, ii) narrative or systematic reviews and meta-analyses, iii) animal and *in-vitro* studies, iv) case reports, errata, comments, perspectives, letters to the editor, editorials that did not provide any primary patient data, v) published abstracts with no available full text, vi) studies that report tissue instead of serum or plasma miRNA expression in thyroid cancer patients, and vii) studies that included non-PTC patients or tumors with unclear/undetermined histology. No publication date, sample size restrictions, or any other search filters were applied. Studies originating from similar institutions were included only if different miRNAs were investigated.

Search strategy. The search strategy included terms relevant to miRNAs and thyroid cancer and was conducted on three databases (MEDLINE, Cohrane, and Scopus) with an end-of-search date on 01/12/2021. The following search algorithm was used: (plasma OR biomarker OR serum OR sera OR blood OR peripheral) AND (miRNAs OR miR OR microRNA OR exosomal OR exosomes) AND (thyroid OR PTC). Two independent researchers (GG and MM) performed the literature search and a third researcher (DG) participated in the resolution of any disagreements during the selection process. In addition, the reference lists of included studies were searched for any missed study that fulfilled the inclusion criteria (snowball methodology) (23).

Data extraction. Two main researchers (GG and MM) independently extracted relevant data through a standardized data extraction template. The extraction of the following variables was performed: i) expression of serum or plasma miRNAs (upregulated, downregulated or non-statistically significant) in PTC compared to benign thyroid disease or healthy controls and ii) Tumor specific data including tumor size, tumor location, pre- and postoperative status, tumor extrathyroidal extension, lymph node metastasis, TNM stage,

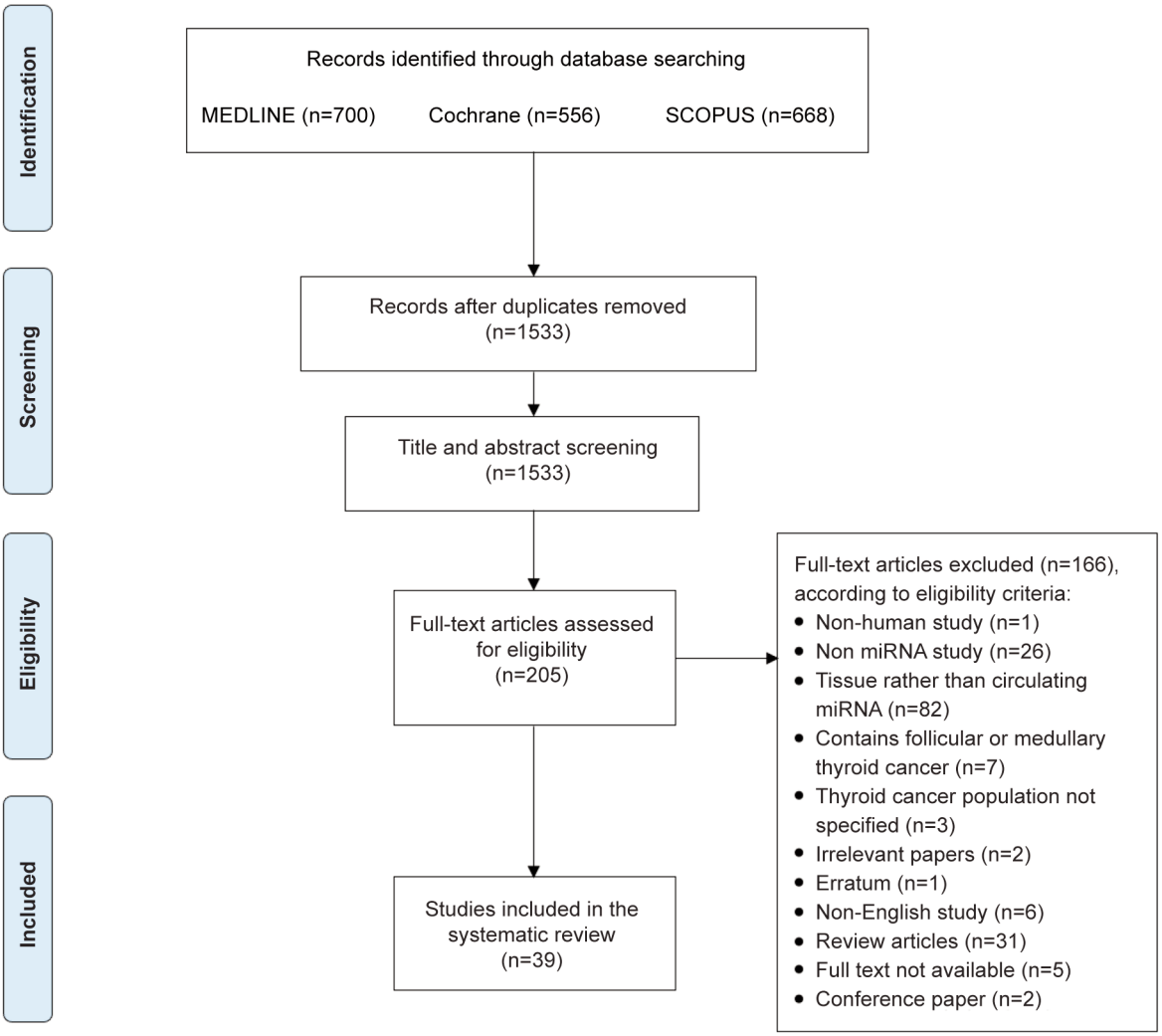

Figure 1. *PRISMA flowchart*.

BRAF V600E mutation, serum thyroglobulin levels, ^{131}I avid metastases, and tumor recurrence, and iii) sensitivity, specificity and area under the curve (AUC) as depicted by the receiver operating characteristic (ROC) curve of circulating miRNAs as a tool for PTC diagnosis. Any discrepancies during the data extraction process were solved by the rest of the authors.

Data presentation. Statistically significant differences in several miRNA expression levels in the plasma/serum of PTC patients compared to patients with benign thyroid disease or healthy controls are summarized in Supplementary Table I. Moreover, the clinicopathologic parameters of PTC patients are depicted in tabular form. The clinicopathologic features of PTC patients were also correlated with the serum/plasma miRNA expression.

Results

Study selection process. Our initial literature search yielded 1,533 unique articles, of which 205 articles were considered to be relevant and underwent full-text assessment. Following removal of the irrelevant, and non-eligible studies, thirty-nine studies were included in this systematic review (Figure 1 and Table I).

MicroRNA source. In the vast majority of the included studies the miRNAs were isolated from the serum or plasma of patients. However, some studies report that miRNAs were isolated from circulating exosomes (24-31). In one study, plasma and exosomes were used (26), whereas another study did not provide data regarding the blood component that was used to isolate miRNAs (32).

Table I. *Basic characteristics of included studies.*

Study	Year	Country	Study design	Sample size	Male/ Female	miRNA quantification method	Reference standard	miRNAs investigated
Hu *et al.* (33)	2017	The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China	Prospective	266 PTC 280 BN 300 HC	192/653	qRT-PCR	Small nuclear RNA U6	miR-940, miR-15a and miR-16
Jiang *et al.* (24)	2020	Sichuan University, China	Prospective	64 PTC	14/50	qRT-PCR	U6	miR-21-5p, miR-146b-5p, miR-204-5p, miR-221-3p, miR-222-3p, miR-451a, miR-7-5p, miR-30a-3p, miR-138-5p, miR-199a-3p
Rezaei *et al.* (51)	2019	Tabriz University of Medical Sciences, Imam Reza Hospital, Golgasht Street, Tabriz, Iran	Prospective	30 PTC 30 BN	28/32	qRT-PCR	Housekeeping miRNA	miR-222, miR-181a, miR-155-5p, miR-146a
Zhang *et al.* (34)	2018	Harbin Medical University, Harbin, Heilongjiang, China	Prospective	58 PTMC 47 PTC 35 BN 40 HC	–	qRT-PCR	–	miR-21
Zhang *et al.* (35)	2016	Jiao Tong University School of Medicine, Shanghai, China	Prospective	70 PTC 70 HC	28/42 (PTC)	qRT-PCR	RUN-44	miR-451
Boufraqech *et al.* (25)	2014	Jiao Tong University School of Medicine, Shanghai, China	Prospective	–	–	qRT-PCR	U6 snRNA	miR-145
Graham *et al.* (36)	2015	Queen Elizabeth II Health Sciences Centre and Dalhousie University, Halifax, NS, Canada	Prospective	87 PTC 118 BN 3 HC	68/137	qRT-PCR	UniSp2 and UniSp4	miR-150-5p, miR-342-3p, let-7b-50, miR-191-5p, miR-146a-5p, miR-93-5p, miR-10a-5p, miR-199b-3p
Maydanchi *et al.* (37)	2016	Azad University, Zanjan, Iran	Prospective	20 PTC 10 BN 10 HC	–	qRT-PCR	GAPDH	miR-146b
Molaei *et al.* (38)	2017	Azad University, Zanjan, Iran	Prospective	25 PTC	–	qRT-PCR	GAPDH	miR-146b and miR-221
Qiu *et al.* (60)	2015	Shanghai Jiao Tong University, Shanghai, China	Prospective	33 PTC	15/18	qRT-PCR	U6	miR-1249, miR-106a, miR-503, miR-34c-5p, miR-1281, miR-1959, miR-2861, miR-3196, miR-500, miR-572, miR-33b, miR-554, miR-18a
Shen *et al.* (61)	2016	Shanghai Jiao Tong University, Shanghai, China	Prospective	119 PTC	54/65	qRT-PCR	U6	miR- 106a, miR-34c-5p, miR-1281, miR-1915, miR-2861, miR-3196
Wang *et al.* (26)	2019	First Affiliated Hospital of Nanjing Medical University, Nanjing, China	Prospective	120 PTC 29 BN 131 HC	62/212	qRT-PCR	U6	miR-346, miR-10a-5p, miR-34a-5p
Dai *et al.* (27)	2020	The First Affiliated Hospital of Shenzhen University, Guangdong, China	Prospective	137 PTC 92 BN 51 HC	–	qRT-PCR	Cel-miR-39	miR-204-ep, miR-376a-3p, miR-4306, miR-4433a-5p, miR-485-3p

Table I. *Continued*

Table I. *Continued*

Study	Year	Country	Study design	Sample size	Male/ Female	miRNA quantification method	Reference standard	miRNAs investigated
Pan *et al*. (28)	2019	First Hospital of Quanzhou Affiliated to Fujian Medical University, China	Prospective	13 PTC 7 BN	2/18	RNA sequencing	–	miR-598-5p, miR-3161, miR-6516-5p, miR-4644, miR-1283, miR-1227-3p, miR-149-3p, miR-210-5p, miR-3662, miR-187-5p
Igci *et al*. (53)	2015	University of Gaziantep, Gaziantep, Turkey	Prospective	17 PTC 42 BN	9/50	qRT-PCR	U6	miR-30a-5p
Huang *et al*. (39)	2017	Nanjing Medical University, China	Prospective	87 PTC 50 BN 50 HC	18/69 (PTC)	qRT-PCR	–	miR-381
Lee *et al*. (54)	2015	University of Ulsan, College of Medicine, Seoul, Republic of Korea	Cross-Sectional	70 PTC 19 BN	15/54	qRT-PCR	Cel-miR-39	miR-146, miR-155, miR-221, miR-222
Sun *et al*. (32)	2015	Qingdao University, Qingdao, Shandong, China	Prospective	128 PTC 120 BN 120 HC	81/287	qRT-PCR	U6	miR-146a, miR-146b
Yoruker *et al*. (40)	2016	Istanbul University, Istanbul, Turkey	Prospective	31 PTC 31 BN 24 HC	–	qRT-PCR	miR-16	miR-146b, miR-21, miR-31, miR-151-5p, miR-221, miR-222, let-7e
Cantara *et al*. (41)	2014	University of Siena, Siena, Italy	Prospective	79 PTC 80 BN 41 HC	71/126	qRT-PCR	miR-159a, miR-451 and miR-16	mir-579, miR-190, miR-95, miR-29b, miR-362-3p, miR-501-3p, miR-518-5p, miR-548d-5p
Lee *et al*. (56)	2013	Royal North Shore Hospital and University of Sydney, Australia	Prospective	42 PTC 36 BN	16/62	qRT-PCR	miR-16	miR-221, miR-222, miR-146b, miR-155, miR-1299, miR-193b
Wang *et al*. (42)	2018	Hongqi Hospital Affiliated to Mudanjiang Medical University, Mudanjiang, Heilongjiang, China	Prospective	150 PTC 100 BN 40 HC	144/146	qRT-PCR	U6	miR-22
Yu *et al*. (43)	2016	Qilu Hospital of Shandong University, Jinan, Shandong, China	Prospective	50 PTC 50 BN 50 HC	31/119	qRT-PCR	miR-16	miR-124-3p, miR-9-3p, miR-196b-5p, miR4701, miR-196b-5p
Zhang *et al*. (34)	2016	The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China	Prospective	106 PTC 35 BN 40 HC	29/56 (PTC)	qRT-PCR	miR-16	miR-146b, miR-221, miR-222
Liu *et al*. (45)	2020	The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China	Prospective	100 PTC 50 BN 20 HC	84/86	qRT-PCR	U6	miR-323
Perdas *et al*. (57)	2020	Medical University of Lodz, Poland	Prospective	49 PTC 21 HC	14/56	ddPCR	–	let7a, let-7c, let-7d, let-7f, let-7i

Table I. *Continued*

Table I. *Continued*

Study	Year	Country	Study design	Sample size	Male/ Female	miRNA quantification method	Reference standard	miRNAs investigated
Wu *et al.* (29)	2019	Central South University, Changsha, China	Prospective	20 PTC 20 BN	–	qRT-PCR	miR-16-5p	miR-21-5p
Rosignolo *et al.* (16)	2016	University of Rome, Rome, Italy	Prospective	44 PTC 39 BN 20 HC	10/34 (PTC)	TaqMan MicroRNA Assays	–	miR-221-3p, miR-222-3p, miR-146a-5p, miR-24-3p, miR-191-5p, miR-103a-3p, miR-28-3p
Ye *et al.* (58)	2018	Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China	Prospective	60 PTC 60 HC	–	qRT-PCR	–	miR-423-5p
Wen *et al.* (30)	2020	The First Affiliated Hospital of Qiqihar Medical University, Qiqihar, China	Prospective	119 PTC 100 HC	57/62 (PTC)	qRT-PCR	miR-39	miR-29a
Kondrotiene *et al.* (46)	2020	Lithuanian University of Health Sciences, Kaunas, Lithuania	Prospective	49 PTC 23 BN 57 HC	22/107	qRT-PCR	miR-39	miR-146b, miR-221, miR-222, miR-21, miR-181b
Zou *et al.* (47)	2020	First Affiliated Hospital of Nanjing Medical University, Jiangsu, China	Prospective	100 PTC 30 BN 96 HC	74/152	qRT-PCR	U6	miR-25-3p, miR-296-5p, miR-92a-3p
Yin *et al.* (62)	2020	Affiliated Kunshan Hospital of Jiangsu University, Suzhou, China	Cross-Sectional	–	–	qRT-PCR	U6	miR-643
Ren *et al.* (59)	2016	Southern Medical University, Shenzhen, Guangdong, China	Prospective	84 PTC - HC	32/52	qRT-PCR	U6	miR-26a
Li *et al.* (48)	2015	First Affiliated Hospital, China Medical University, Shenyang, China	Prospective	56 PTC 95 BN 10 HC	–	qRT-PCR	U6	Let-7i, miR-21-5p, miR-29-3p, miR-106-5p, miR-140-3p, miR-1246, miR-25-3p, miR-451a
Liu *et al.* (55)	2018	The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China	Prospective	66 PTC 40 BN	–	qRT-PCR	U6	miR-431
Liang *et al.* (31)	2020	The Second Affiliated Hospital of Harbin Medical University, Harbin, China	Prospective	32 PTC 38 BN 38 HC	56/64	qRT-PCR	–	miR-16-2-3p, miR-223-3p, miR-34c-5p, miR-182-5p, miR-146b-5p
Yu *et al.* (50)	2012	Sun Yat-sen University, Guangzhou, China	Prospective	106 PTC 95 BN 44 HC	25/81 (PTC)	qRT-PCR	U6	Let-7e, miR-151-5p, miR-222
He *et al.* (49)	2019	Panzhihua Central Hospital, Panzhihua City, China	Prospective	60 PTC 60 BN 60 HC	48/132	qRT-PCR	U6	miR-199a-3p

PTC: Papillary thyroid cancer; BN: benign nodule; HC: healthy controls.

Table II. *Not significantly changed and significantly up- or down-regulated miRNAs in papillary thyroid cancer (PTC) vs. nodular goitre (NG) or benign nodules (BN) or healthy controls (HC).*

Blood levels	PTC *vs*. Healthy	PTC *vs*. (NG or BN)	(NG or BN) *vs*. Healthy
Up-regulation	Let-7a, Let-7b-5p, Let-7c, Let-7d, Let-7e, Let-7f, Let-7i miRNAs, miR-103-3p, miR-10a-5p, miR-124-3p, miR-140-3p, miR-145, miR-146b, miR-146b-5p, miR-151-5p, miR-181b, miR-190, miR-191-5p, miR-204-3p, miR-21-5p, miR-22, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-24-3p, miR-28-3p, miR-296-5p, miR-323, miR-346, miR-34a-5p, miR-376a-3p, miR-423-5p, miR-4306, miR-4433-5p, miR-451a, miR-485-3p, miR-9-3p, miR-93-5p	Let-7b-5p, Let-7e miRNA, Let-7i miRNA, miR-10a-5p, miR-124-3p, miR-1283, miR-140-3p, miR-145, miR-146a, miR-146b, miR-151-5p, miR-155, miR-16-2-3p, miR-181a, miR-190, miR-204-3p, miR-21, miR-22, miR-221, miR-222, miR-222-3p, miR-223-5p, miR-25-3p, miR-296-5p, miR-30a-3p, miR-3161, miR-323, miR-346, miR-34a-5p, miR-376a-3p, miR-4306, miR-4433-5p, miR-451a, miR-4644, miR-485-3p, miR-598-5p, miR-6516-5p, miR-9-3p, miR-92a-3p	Let-7b-5p. miR-103-3p, miR-146a-5p, miR-146b, miR-146b-5p, miR-181b, miR-191-5p, miR-196b-5p, miR-21, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-24-3p, miR-28-3p
Down-regulation	miR-146a-5p, miR-146b-5p, miR-150-5p, miR-16-2-3p, miR-182-5p, miR-199a-3p, miR-21, miR-223-3p, miR-223-5p, miR-25-3p, miR-26a, miR-29b, miR-342-3p, miR-34c-5p, miR-381, miR-579, miR-92a-3p, miR-95	miR-1227-3p, miR-146b-5p, miR-149-3p, miR-187-5p, miR-196b-5p, miR-199b-3p, miR-210-5p, miR-29b, miR-3662, miR-381,miR-431, miR-451, miR-5010-3p,miR-579, miR-95	miR-146b-5p, miR-150-5p, miR-16-2-3p, miR-182-5p, miR-21, miR-223-3p, miR-223-5p, miR-342-3p, miR-34c-5p
No statistically significant change	miR-100, miR-106b-5p, miR-1246 miR-146a, miR-146a-5p, miR-15a miR-16, miR-196b-5p, miR-29a-3p, miR-31, miR-362-3p, miR-39, miR-4701, miR-501-3p, miR-518-5p, miR-543, miR-548d-5p, miR-940	Let-7e miRNA, miR-100, miR-103-3p, miR-106b-5p, miR-1246, miR-146a, miR-146a-5p, miR-146b, miR-146b-5p, miR-151-5p, miR-155-5p, miR-15a, miR-16, miR-181b, miR-191-5p, miR-199a-3p, miR-21, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-24-3p, miR-28-3p, miR-29a-3p, miR-31, miR-362-3p, miR-39, miR-4701, miR-501-3p, miR-518-5p, miR-543, miR-548d-5p, miR-940	Let-7e miRNA, miR-100, miR-10a-5p, miR-124-3p, miR-145, miR-146a-5p, miR-146b, miR-151-5p, miR-15a, miR-16, miR-190, miR-199a-3p, miR-204-3p, miR-22, miR-221, miR-222, miR-25-3p, miR-296-5p, miR-29b, miR-31, miR-323, miR-346, miR-34a-5p, miR-362-3p, miR-376a-3p, miR-381, miR-39, miR-4306, miR-4433-5p, miR-4701, miR-485-3p, miR-501-3p, miR-518-5p, miR-543, miR-548d-5p, miR-579, miR-9-3p, miR-92a-3p, miR-940, miR-95

PTC vs. benign disease or healthy individuals. A total of 22 studies compared PTC against benign thyroid disease and healthy controls (16, 27, 31-50); six studies compared PTC to benign thyroid disease (28, 51-55), seven studies compared PTC to healthy controls (26, 29, 30, 56-59), and four studies (24, 60-62) did not include healthy or benign thyroid disease comparison groups. A total of 108 miRNAs were investigated in the serum, plasma, or circulating exosomes (Table II). Between PTC and healthy subjects, 17 miRNAs did not present a significant change, while 14 were down-regulated and 40 miRNAs were up-regulated. Between PTC and benign thyroid disease, 25 miRNAs did not change significantly, while 15 and 39 miRNAs were down and up-regulated, respectively. Finally, in the comparison of benign thyroid disease with healthy controls, 37 miRNAs did not change significantly, while 8 and 12 miRNAs were down and up-regulated, respectively (Table II).

Tumor size. In total, 17 studies investigated the association of circulating miRNAs with the thyroid tumor size (27, 30, 32-35, 37, 38, 44, 45, 51-54, 57, 59, 62). The included studies reported a non-significant association between 27 miRNAs, while 11 (miR-124-3p, miR-146b, miR-151-5p, miR-155, miR-181a, miR-204-3p, miR-221, miR-222, miR-30a-3p, miR-485-3p and miR-643) and 2 (miR-29b and miR-4306) miRNAs were found to be up- and down-regulated in patients with increased thyroid tumor size, respectively (Table III).

The cut-off tumor size varied among the included studies. Ten studies set the tumor diameter cut-ff at 2 cm (30, 32-34, 38, 44, 45, 51, 53, 62), four studies at 1 cm (27, 52, 59), while a two-level cut-off (1 cm and 2 cm) was reported by one study (37). The cut-off was not reported by two studies (35, 57). The linear correlation of miRNA expression and tumor size was investigated in one study (54). While miR-

Table III. *Not significantly changed and significantly up- or down-regulated miRNAs and clinicopathologic features of papillary thyroid cancer (PTC).*

Blood levels	Tumor size association	Bilateral *vs.* Unilateral location	Pre- *vs.* Postoperative in PTC	Extrathyroidal extension	Positive *vs.* negative LNM	Negative *vs.* positive DM	TNM stage association	BRAF V600E mutation	Serum TG levels	Non 131I avid metastasis	Recurrent PTC
Up-regulation	miR-124-3p, miR-146b, miR-151-5p, miR-155, miR-181a, miR-204-3p, miR-221, miR-222, miR-30a-3p, miR-485-3p and miR-643	miR-146, miR-21, miR-222	miR-103-3p, miR-146a, miR-146a-5p, miR-146b, miR-146b-5p, miR-151-5p, miR-155-5p, miR-181a, miR-181b, miR-191-5p, miR-21, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-24-3p, miR-25-3p, miR-28-3p, miR-30a-3p, miR-31, miR-323, miR-451a, miR-9-3p	miR-146a, miR-146b, miR-21, miR-21, miR-221, miR-222, miR-4433-5p, miR-485-3p	miR-146, miR-146a, miR-146b, miR-146b-5p, miR-151-5p, miR-204-5p, miR-21, miR-21-5p, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-26a, miR-423-5p, miR-4433-5p, miR-485-3p	miR-22, miR-323	miR-146a, miR-146b, miR-21, miR-221, miR-222, miR-26a, miR-4433-5p, miR-485-3p	miR-146a, miR-22, miR-222, miR-323, miR-4433-5p, miR-485-3p	miR-22, miR-323	miR-106a, miR-1249, miR-1281, miR-34c-5p and miR-503	miR-146b, miR-221, miR-222
Down-regulation	miR-29b, miR-4306	-	miR-26a, miR-29a, miR-381	miR-29a, miR-381	miR-381, miR-451	-	miR-29a, miR-381	-	-	miR-18a, miR-1915, miR-2861, miR-3196, miR-33b, miR-500, miR-554 and miR-572	
No statistically significant change	Let-7a miRNA, Let-7c miRNA, Let-7d miRNA, Let-7e miRNA, Let-7f miRNA, Let-7i miRNA, miR-146a, miR-146b, miR-151-5p,	miR-124-3p, miR-146a, miR-146b, miR-15a, miR-16, miR-181b, miR-190, miR-196b-5p, miR-21,									

Table III. *Continued*

Table III. *Continued*

Blood levels	Tumor size association	Bilateral *vs*. Unilateral location	Pre- *vs*. Postoperative in PTC	Extrathyroidal extension	Positive *vs*. negative LNM	Negative *vs*. positive DM	TNM stage association	BRAF V600E mutation	Serum TG levels	Non 131I avid metastasis	Recurrent PTC
No statistically significant change	miR-155-5p, miR-15a, miR-16, miR-190, miR-196b-5p, miR-21, miR-221, miR-222, miR-26a, miR-29b, miR-31, miR-362-3p, miR-376a-3p, miR-381, miR-4433-5p, miR-451, miR-501-3p, miR-518-5p, miR-548d-5p, miR-579, miR-9-3p, miR-940, miR-95	miR-221, miR-222, miR-26a, miR-29b, miR-362-3p, miR-451, miR-501-3p, miR-518-5p, miR-548d-5p, miR-579, miR-9-3p, miR-940, miR-95	Let-7e miRNA, miR-124-3p, miR-146b, miR-15a, miR-16, miR-21, miR-222, miR-940	miR-124-3p, miR-124-3p, miR-146, miR-146a, miR-146b, miR-155, miR-155-5p, miR-15a, miR-16, miR-181a, miR-181b, miR-196b-5p, miR-204-3p, miR-21, miR-221, miR-222, miR-26a, miR-376a-3p, miR-4306, miR-9-3p, miR-940	Let-7a miRNA, Let-7c miRNA, Let-7d miRNA, Let-7e miRNA, Let-7f miRNA, Let-7i miRNA, miR-124-3p, miR-138-5p, miR-146a, miR-146b, miR-155, miR-155-5p, miR-15a, miR-16, miR-181a, miR-181b, miR-190, miR-196b-5p, miR-199a-3p, miR-204-3p, miR-21, miR-221, miR-222, miR-29a, miR-29b, miR-30a-3p, miR-362-3p, miR-376a-3p, miR-4306, miR-451a, miR-501-3p, miR-518-5p, miR-548d-5p, miR-579, miR-7-5p, miR-9-3p, miR-940, miR-95	miR-15a, miR-16, miR-190, miR-29b, miR-362-3p, miR-501-3p, miR-518-5p, miR-548d-5p, miR-579, miR-940, miR-95	Let-7a miRNA, Let-7c miRNA, Let-7d miRNA, Let-7e miRNA, Let-7f miRNA, Let-7i miRNA, miR-10a-5p, miR-124-3p, miR-146, miR-146a, miR-146b, miR-151-5p, miR-15a, miR-16, miR-196b-5p, miR-204-3p, miR-21, miR-221, miR-31, miR-346, miR-34a-5p, miR-376a-3p, miR-4306, miR-9-3p, miR-940	Let-7e miRNA, miR-124-3p, miR-151-5p, miR-155-5p, miR-181a, miR-196b-5p, miR-204-3p, miR-222, miR-376a-3p, miR-4306, miR-9-3p	Let-7e miRNA, miR-151-5p, miR-222, miR-451		

ETE: Extrathyroid extension; LNM: lymph node metastasis; DM: distant metastasis.

222 and miR146b demonstrated a strong correlation between their expression and tumor size, miR-155 showed the strongest correlation (54).

Bilateral vs. unilateral and multifocality of the tumor. Six studies investigated the association of circulating miRNAs with bilateral or unilateral PTC (32, 35, 37, 45, 46, 59) and 20 miRNAs did not present significant changes between patients with bilateral and unilateral thyroid lesions. Further, three miRNAs (miR-146, miR-21, and miR-222) were found to be up-regulated in bilateral thyroid lesions, whereas no miRNAs were identified to be down-regulated in bilateral thyroid lesions (Table III).

PTC multifocality and miRNA expression were assessed by six studies (32, 35, 37, 45, 46, 59). PTC multifocality correlated with significantly increased expression of miR15a, miR16, miR-222, miR-146, and miR-21 compared to unifocal PTC cases (45, 46) (Table III). The expression of miR-940, miR-221, miR-146a, miR-146b, miR-124-3p, miR-9-3p, miR-196b-5p, and let-7e did not present significant changes in multifocal *vs.* unifocal PTC cases (32, 35, 37, 46, 63). Furthermore, the expression of miR-222 and miR-146b in patients with papillary thyroid microcarcinoma was not associated with multifocality (46). In addition, miR-26a did not present significant changes in multifocal *vs.* non multifocal thyroid cancer patients (59).

Lastly, patient with tumors located in thyroid isthmus, did not present significant changes in serum expression of miR-451 compared to right, left, and bilateral thyroid tumor location (52).

Pre- vs. postoperative changes. The change in circulating miRNAs occurring after thyroid surgery was investigated by 17 studies (16, 30, 33, 34, 37-40, 44-46, 49-51, 53, 56, 59). Out of 37 miRNAs, the preoperative levels of 28 (miR-103-3p, miR-146a, miR-146a-5p, miR-146b, miR-146b-5p, miR-151-5p, miR-155-5p, miR-181a, miR-181b, miR-191-5p, miR-21, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-24-3p, miR-25-3p, miR-28-3p, miR-30a-3p, miR-31, miR-323, miR-451a and miR-9-3p) and 3 (miR-26a, miR-29b and miR-381) miRNAs were up and down-regulated, respectively, compared to postoperative levels in PTC patients (Table III).

The interval between the pre- and postoperative serum/plasma sampling of miRNA levels was 30 (16, 33, 37, 46), 14 (39, 45), 14-42 (51, 56) and 5-15 days (44), respectively. Two studies reported more than one postoperative serum/plasma miRNA measurements; at 1, 3, 6, and 12 months postoperatively (38) or at 1 and 3 months (30). The interval between pre- and post-operative sampling was not reported by the rest of the studies.

In a longitudinal study by Rosignolo *et al.,* the authors collected serum at 1-2 years post thyroidectomy and despite an initial decrease in miRNA levels, tumor recurrence following surgery resulted in an increased expression of miR-146a-5p and miR-221-3p compared to pre-surgery levels. In contrast, the expression of miR-146a-5p and miR-221-3p remained stable and at low levels in recurrence-free patients (16).

The postoperative expression of miR-124-3p, miR-9-3p, miR-222, and miR-151-5p postoperatively was not significantly different in PTC patients compared to healthy controls (37, 44). Moreover, Hurthle cell cancer presented reduced miR-30a-5p levels postoperatively similar to non-Hurthle cell cancer (53).

Finally, hemithyroidectomy compared to total thyroidectomy did not affect significantly the expression of miR-221, miR-222, and miR-146b at 1, 3, 6, and 12 months postoperatively (38). Similarly, Kondrotient *et al.* supports the significantly reduced expression of miR-221 following hemi-thyroidectomy in PTC patients (46).

Lastly, four studies investigated the effect of surgery on the expression of circulating miRNAs in control groups (benign nodules) and reported that three miRNAs (miR-146a, miR-146b-5p and miR-222-3p) were significantly down-regulated in the postoperative period (38, 45, 51, 56).

Extrathyroidal extension. Twelve studies investigated the association between miRNA levels and extrathyroid extension of PTC (27, 30, 32, 33, 37, 38, 40, 45, 46, 51, 54, 59). Eight miRNAs did not change significantly in tumors with extrathyroidal extension compared to those without extension. However, seven (miR-146b, miR-21, miR-221, miR-222, miR-4433-5p, miR-485-3p) and two (miR-29b and miR-381) miRNAs were found to be up and down-regulated, respectively, in the serum or plasma of patients with extrathyroidal tumor extension compared to patients with limited disease (Table III).

Lymph node metastasis. A total of 18 studies examined the association between circulating miRNAs and lymph node metastasis (LNM) in PTC patients (24, 27, 30, 32, 33, 35, 37, 38, 40, 44-46, 51, 52, 54, 57-59). A total of 39 miRNAs were not associated with LNM. However, 15 (miR-146, miR-146b, miR-146b-5p, miR-151-5p, miR-204-5p, miR-21, miR-21-5p, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-26a, miR-423-5p, miR-4433-5p and miR-485-3p) and 2 (miR-381 and miR-451) miRNAs were found to be significantly up and down-regulated, respectively, in the serum/plasma of patient with LNM positive PTC (Table III).

The compartment of involved lymph nodes was reported by one study (54) in which the expression of miR-146b, miR-221, and miR-222 did not differ significantly between the central and lateral LNM compartment groups (54).

Distant metastasis and recurrence. The presence of distant metastasis in PTC patients and its association with aberrant

expression of miRNAs was assessed by four studies (35, 36, 39, 45). Eleven miRNAs were not associated with PTC metastasis. However, two miRNAs (miR-22 and miR-323) were significantly up-regulated in PTC metastasis (Table III).

In patients with PTC and ^{131}I avid metastasis, two studies reported significant up-regulation of five (miR-106a, miR-1249, miR-1281, miR-34c-5p, and miR-503) and down-regulation of eight (miR-18a, miR-1915, miR-2861, miR-3196, miR-33b, miR-500, miR-554, and miR-572) miRNAs (60, 61).

Lastly, thyroid tumor recurrence was assessed by two studies (38, 56). The circulating levels of miR-146b, miR-221, and miR-222 were significantly elevated in patients with recurrent PTC compared to the control group and non-recurrent PTC group (38).

TNM stage. TNM stage (Comparison of stage I/II *vs*. III/IV in all studies except two) and circulating miRNA association was examined by 13 studies (26, 27, 30, 32-34, 37, 38, 44-46, 57, 59). Advanced TNM stage (III/IV) was associated with significant up or down-regulation in the serum/plasma of seven (miR-146b, miR-21, miR-221, miR-222, miR-26a, miR-4433-5p, and miR-485-3p) and two (miR-29b and miR-381) miRNAs, respectively (Table III). Twenty-two miRNAs presented no significant association with PTC TNM stage. Lastly, one study compared discrete stages (I, II, III, and IV) without finding any significant change in the expression levels of miRNAs (45) (Table III).

BRAF V600E mutation and TG serum correlation. The association between the *BRAF V600E* mutation and circulating miRNAs was assessed by six studies (27, 36, 37, 39, 44, 51). Out of 18 miRNAs, six miRNAs (miR-146a, miR-22, miR-222, miR-323, miR-4433-5p, and miR-485-3p) were significantly up-regulated in patients with *BRAF V600E* mutation positive tumors (Table III).

The association between serum thyroglobulin (TG) levels and circulating miRNAs was examined by three studies (36, 39, 44). Two out of six investigated miRNAs (miR-22 and miR-323) were significantly up-regulated in patients with increased serum TG levels.

Diagnostic accuracy. Among the included studies, twenty studies investigated the diagnostic accuracy of circulating miRNAs (Table II) (16, 24, 26-28, 30, 31, 33, 36-42, 44, 46, 52, 54, 57). Circulating miRNAs could differentiate PTC from benign thyroid nodules with a sensitivity, specificity, and AUC of 40.9-95.6%, 52.6-100%, and 0.488-0.973, respectively. In contrast, the differentiation of PTC from healthy controls based on circulating miRNAs had a sensitivity, specificity, and AUC of 43-94.3%, 14-97.5%, and 0.640-0.980, respectively. Lastly, nine studies reported the diagnostic accuracy of circulating miRNAs as markers of PTC clinical characteristics, including lymph node metastasis (24, 52), papillary thyroid microcarcinoma (46), TNM stage (26), advanced clinical stage (28, 30), metastatic disease (39, 54), and recurrent disease (30) with variable results (Table IV).

Discussion

To the best of our knowledge, this is the first systematic review that focuses on whole blood circulating miRNAs and the clinicopathologic features of PTC. A recently published meta-analysis included studies with other subtypes of differentiated thyroid cancer as well as focused on exosomal miRNAs, excluding studies that investigate the whole serum or plasma (64). Several miRNAs were identified to differentiate healthy controls or patients with benign thyroid nodules from PTC patients. Moreover, our results show that specific PTC tumor characteristics could be associated with statistically significant up or down-regulation of circulating miRNAs: tumor size (13 miRNAs), tumor location (3 miRNAs), extrathyroid extension (9 miRNAs), lymph node metastasis (17 miRNAs), distant metastasis (2 miRNAs), TNM (9 miRNAs), *BRAF V600E* positivity (6 miRNAs), or serum TG levels (2 miRNAs). Furthermore, the thyroidectomy operation results in significant changes in 31 circulating miRNAs. Two miRNAs (miR-146a-5p and miR-221-3p) presented an association with PTC recurrence at one or two years following thyroidectomy. The aforementioned findings suggest that circulating miRNAs are a promising tool for thyroid cancer classification, diagnosis, and management.

The presence of a thyroid nodule in an otherwise anatomically and functionally normal thyroid alerts clinicians to order an array of further diagnostic tests. Thyroid assessment includes medical history, clinical examination, thyroid ultrasound, thyroid scintigraphy, and serum thyroid function tests. In the vast majority of the patients, these tests are used to determine if FNA is appropriate and whether a patient is a surgical candidate. Thyroid ultrasound findings that are suspicious for malignancy and thyroid nodule size are the main factors that determine the need for FNA examination (3, 65). However, it should be noted than up to 16% of the FNAs performed are non-diagnostic and of those, only 8% has been proven to be a PTC (66). Adjuvant immunocytochemical markers for PTC diagnosis including PAX 8, HBME1 positivity, CK19 positivity, galectin-3 positivity, and CD56 negativity have been investigated, but limitations in sensitivity and specificity preclude their widespread use (67). Lastly, given the aforementioned marker's high cost compared to qRT-PCR, miRNA detection could replace these modalities and potentially assist to PTC diagnosis and classification (68).

Currently, the histopathologic examination of thyroid is the gold standard for a definitive diagnosis (69). However, thyroidectomy is not a complication-free procedure, since all

Table IV. *Sensitivity, specificity, and area under the curve (AUC) of circulating miRNAs in papillary thyroid cancer (PTC) diagnosis.*

Population	Study	Serum miRNA	Serum/Blood change	Sensitivity	Specificity	AUC	Reference
PTC *vs.* benign thyroid nodules	Wang *et al.*, 2019	miR-10a-5p, miR-34a-5p and miR-346	Up-regulation	-	-	0.887	(26)
	Dai *et al.*, 2020	miR-485-3p	Up-regulation	85.4%	73.3%	0.858	(27)
		miR-4433a-5p	Up-regulation	83.3%	73.3%	0.812	
	Pan *et al.*, 2020	miR-5189-3p	Up-regulation	-	-	0.951	(28)
		miR-92b-3p, miR-24-2-5p, miR-548a-30, miR-1228-5p, miR-9-3p, miR-27a-3p, miR-4492, miR-4669, miR-1-3p and miR-196b-5p	-	-	-	Above 0.900	
	Lee *et al.*, 2015	miR-146b	Up-regulation	61.4%	57.9%	0.649	(54)
		miR-155	Up-regulation	74.3%	63.2%	0.695	
	Yu *et al.*, 2012	Let-7e	Up-regulation	63.2%	89.5%	0.782	(50)
		miR-151-5p	Up-regulation	59.4%	89.5%	0.780	
		miR-222	Up-regulation	81.1%	89.5%	0.906	
		Let-7e, miR-151-5p, miR-222	Up-regulation	87.8%	88.4%	0.917	
	Rosignolo *et al.*, 2017	miR-146a-5p	Up-regulation	79.5%	52.6%	0.653	(16)
		miR-221-3p	Up-regulation	40.9%	100%	0.730	
		miR-222-3p	Up-regulation	47.7%	84.2%	0.587	
	Yu *et al.*, 2016	miR-124-3p	Up-regulation	88%	76%	0.831	(37)
		miR-9-3p	Up-regulation	70%	64%	0.753	
		miR-196b-5p	Down-regulation	74%	66%	0.781	
	Zhang *et al.*, 2016	miR-146b	Up-regulation	94.3%	68.2%	0.873	(38)
		miR-221	Up-regulation	85.7%	52.9%	0.704	
		miR-222	Up-regulation	62.9%	88.2%	0.840	
		miR-146b, miR-221 and miR-222	Up-regulation	80%	97.5%	0.903	
	Liu *et al.*, 2020	miR-323	Up-regulation	-	-	0.912	(39)
	Liang *et al.*, 2020	miR-16-2-3p	Up-regulation	68.6%	66.7	0.690	(31)
		miR-223-5p	Up-regulation	57.1%	80%	0.680	
		miR-16-2-3p and miR-223-5p	Up-regulation	54.3%	90%	0.710	
		miR-16-2-3p, miR-223-5p and miR-34c-5p	Up-regulation, miR-34c-5p Down-regulation	60%	86.7%	0.720	
		miR-16-2-3p, miR-223-5p, miR-34c-5p, and miR-101-3p	Up-regulation, miR-101-3p Down-regulation	71.4%	73.3%	0.740	
		miR-223-5p, miR-34c-5p. and miR-101-3p and miR-146b-5p	Up-regulation, miR-146b-5p Down-regulation	74.3%	66.7%	0.730	
		miR-146b, miR-187, miR-138, miR-375, miR-222-3p and miR-151a-5p combined with ultrasound		-	-	0.973	

Table IV. *Continued*

Table IV. *Continued*

Population	Study	Serum miRNA	Serum/Blood change	Sensitivity	Specificity	AUC	Reference
	Kondrotiene *et al*., 2020	miR-146b	Up-regulation	-	-	0.631	(46)
		miR-222	Up-regulation	-	-	0.711	
		miR-21	Up-regulation	-	-	0.595	
		miR-221	Up-regulation	-	-	0.612	
		miR-181b	Up-regulation	-	-	0.488	
	Li *et al*., 2015	miR-25-3p	Up-regulation	92.8%	68.8%	0.835	(48)
		miR-451a	Up-regulation	88.9%	66.7%	0.857	
		miR-25-3p and miR-451a	Up-regulation	95.6%	64.1%	0.683	
PTC patient *vs*. healthy controls	Wang *et al*., 2019	miR-346	Up-regulation	-	-	0.834	(26)
		miR-10a-5p	Up-regulation	-	-	0.774	
		miR-34a-5p	Up-regulation	-	-	0.760	
	Dai *et al*., 2020	miR-485-3p	Up-regulation	80.2%	71.2%	0.866	(27)
		miR-4433a-5p	Up-regulation	81.2%	72.9%	0.863	
	Huang *et al*., 2017	miR-381	Down-regulation	81.6%	82%	0.851	(33)
	Yu *et al*., 2012	Let-7e	Up-regulation	78.3%	72.7%	0.786	(44)
		miR-151-5p	Up-regulation	79.2%	68.2%	0.776	
		miR-222	Up-regulation	94.3%	70.5%	0.882	
		Let-7e, miR-151-5p, miR-222	Up-regulation	86.8%	79.5%	0.897	
	Wang *et al*., 2019	miR-22	Up-regulation	-	-	0.942	(36)
	Yu *et al*., 2016	miR-124-3p	Up-regulation	88%	78.8%	0.859	(43)
		miR-9-3p	Up-regulation	80%	73.7%	0.823	
	Zhang *et al*., 2016	miR-146b	Up-regulation	69.4%	97.5%	0.896	(34)
		miR-221	Up-regulation	83.5%	87.5%	0.918	
		miR-222	Up-regulation	74.1%	90%	0.876	
		miR-146b, miR-221 and miR-222	Up-regulation	72.9%	94.3%	0.956	
	Perdas *et al*., 2020	Let-7a	Up-regulation	74%	38%	0.660	(57)
		Let-7c	Up-regulation	50.5%	14%	0.670	
		Let-7d	Up-regulation	71%	33%	0.700	
		Let-7f	Up-regulation	59%	19%	0.690	
		Let-7i	Up-regulation	43%	14%	0.640	
	Liu *et al*., 2020	miR-323	Up-regulation	-	-	0.942	(45)
	Wen *et al*., 2021	miR-29a	Down-regulation	79%	85.7%	0.884	(30)
	Liang *et al*., 2020	miR-16-2-3p	Down-regulation	93.8%	89.3%	0.940	(31)

patients who require a total thyroidectomy require postoperative lifelong thyroidal hormone replacement therapy, as well as complications associated with damage to the recurrent laryngeal nerve have a significant impact in patients quality of life (44, 70). Based on these findings, the circulating miRNAs added to the existing diagnostic modalities may potentiate the discrimination between malignant and benign thyroid disease and reduce the unnecessary thyroidectomies.

Although microRNAs constitute about 3% of the whole genome, their function is of utmost importance in the regulation of gene expression (71). Following a maturation process taking place in the nucleus, miRNAs are transferred to the cytoplasm where they form complexes with proteins and mediate their regulatory action (20, 72). Their ribonucleotide sequence is usually complementary with more than one messenger RNA (mRNA) and their interaction results in the inhibition of mRNA translation. As a result, miRNAs may act indirectly as tumor suppressors, or oncogenic factors, through their interference with the expression of oncogenes, or tumor suppressor genes, respectively (73) (Figure 2).

Recently, further evidence on the importance of miRNA dysregulation in thyroid tumorigenesis has been uncovered.

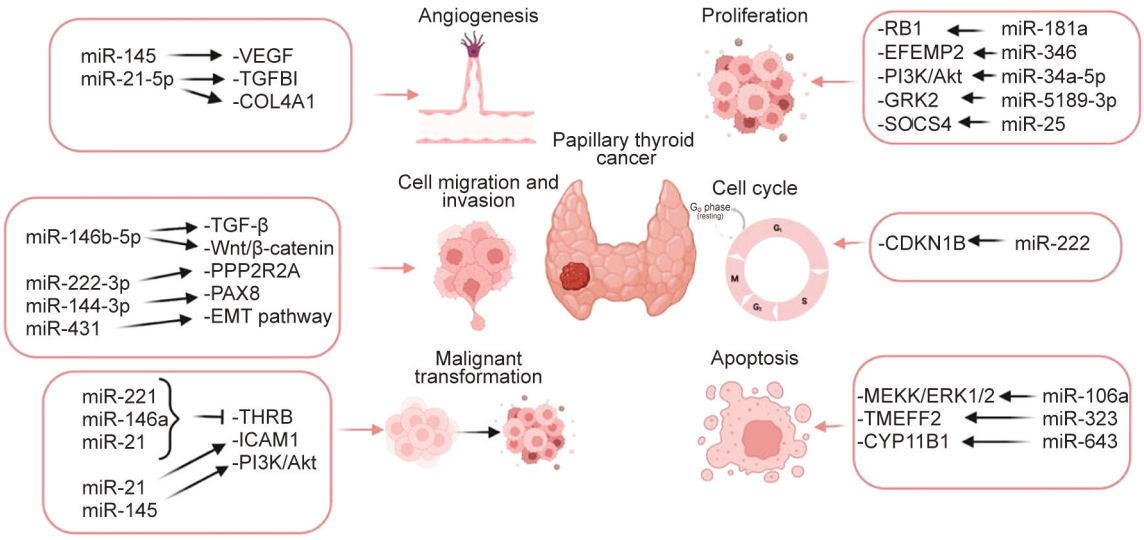

Figure 2. *The role of circulating microRNAs in the cellular pathways involved in papillary thyroid carcinoma. Protein phosphatase 2 regulatory subunit B alpha (PPP2R2A) (51), tumor growth factor β (TGF-β) (2), WNT/β-catenin (24), thyroid hormone receptor β (THRB) (51), Retinoblastoma 1 gene (51), intracellular cell adhesion molecular-1 (ICAM1) (34), PI3K/Akt (25), vascular endothelial growth factor (25), MEKK/ERK1/2 (61), EGF-containing fibulin-like extracellular matrix protein 2 (EFEMP2) (26), complex G protein-coupled receptor kinase 2 (GRK2) (28), PAX 8 (55), transmembrane protein with EGF-like and 2 follistatin domain (TMEFF2) (55), gene encoding type IV collagen α1 (COL4A1) (29), cyclin-dependent kinase inhibitor 1B (CDKN1B) (46), suppressor of cytokine signaling 4 (SOCS4) (47), cytochrome P450 Family 11 Subfamily B Member 1 (CYP11B1) (62), epithelial to mesenchymal transition (EMT) (31) (Created with BioRender.com).*

The *DICER1* gene product, DICER1, is a protein that normally functions as an endoribonuclease that plays a major role in regulating miRNA processing and maturation (74). It has been found that when *DICER1* gene is mutated, through inherited or acquired mutation, patients develop an increased predisposition to thyroid tumor development. Patients with germline inherited *DICER1* loss-of-function mutations could suffer from a wide variety of rare life-threatening childhood-onset malignancies, such as benign and malignant thyroid neoplasms including PTC, collectively called *DICER1* syndrome (75). Also, acquired somatic mutations or down-regulation of *DICER1* expression have been found in early-adulthood-onset well differentiated thyroid cancer (PTC and follicular thyroid cancer) and poorly differentiated thyroid carcinoma. The *DICER1* mutated thyroid tumors in both adult and pediatric patients have been shown by several groups to be mutually exclusive of any of the well-known driver mutations of thyroid cancer (including *BRAF* and *RAS* among others). This provides evidence that *DICER1* mutation can be a driver of thyroid tumor development (76, 77) and establishes another link between miRNA dysregulation and thyroid cancer. Furthermore, the role of DICER1 down-regulation and subsequently miRNA dysregulation has been shown *in vitro*. Ramírez-Moya *et al*., found that DICER1 protein expression was lower in thyroid cancer cell lines in comparison to normal thyroid follicular cell lines. In their study, silencing of *DICER1* in PTC cell lines led to a reduction in several miRNA levels including miR-221-3p, miR-30a-5p, miR-21-5p, miR-146b-5p, miR-100-5p, and miR-204-5p, which ultimately led to an increase in proliferation, invasion, migration, and epithelial-mesenchymal transition of PTC cells (78). In conclusion, *DICER1* acts as a tumor suppressor of thyroid cancer through increased miRNA expression (78).

Deregulation of gene expression by *de novo* or preexisting genetic mutations is a common characteristic of carcinogenesis. Chromosomal instability and genetic alterations commonly seen in cancer cells may affect miRNA expression (79). Contrarywise, the deregulation of miRNA expression may affect the expression of genes. This vicious cycle is part of the deranged gene expression seen in tumorigenesis (20). Several studies have pointed out the association of deregulated miRNA expression and cancer development as well as the association of miRNA signatures/profile with human tumors including thyroid cancer (54, 80, 81).

The underlying mechanisms behind the secretion of miRNAs in body fluids is still under investigation. Several theories have been proposed, including miRNA release following tumor cell death, secretion of tumor exosomes, microvesicles, or secretion induced by tumor-targeting immune response (82, 83). In the serum, miRNAs may be present either as free miRNAs combined with protective proteins or in envelopes consisting of microparticles, such as

exosomes or apoptotic bodies. These proteins facilitate the exosome-cell membrane interaction and RNA material exchange (49). High density lipoprotein (HDL) and nucleophosmin 1 (Npm1) belong to the serum protein microRNA stabilizers (57). However, the measured amount of circulating miRNAs has to be interpreted with caution, as additional sources of circulating miRNAs should also be considered. Circulating tumor cells as well as other cells in the bloodstream may contribute to the total circulating volume of miRNAs. These miRNAs could act as confounders and mistakenly attributed to the actual tumor activity (84, 85). Several methods have been proposed to avoid this restriction, including the division of the tumor-derived miRNAs and the bloodstream-derived ones, without any clinical application at present (85).

Furthermore, the expression and/or aberrant regulation of miRNAs in serum and thyroid neoplastic tissue (including PTC) and the evaluation of their role as diagnostic biomarkers have also been reported (48). Although the association between tissue and serum miRNA expression is yet to be determined, important differences have been previously described (54, 86, 87). Cantara *et al*. compared the expression of four miRNAs (miR-29b, miR-579, miR-95, and miR-190) in tissue and serum, and all except miR-29b presented significant changes in both the serum and tissue specimens of patients diagnosed with PTC (41). Further, Yu *et al*. reported that the serum expression of let-7e was not consistent with that in tissue, (50). Similar differences were described by Wang *et al*., who reported that the miR-10a-5p, in contrast to miR-346 and miR-34a-5p, was not significantly increased in the tissue samples of PTC patients (26). Lastly, interesting findings emerged upon the investigation of miR-146 expression in tissue and serum (54). Two isoforms of miR-146 have been described; namely miR-146a and miR-146b, which are located in different chromosomes. Although the structural difference of the protein products is only two nucleotides, it is hypothesized that the over-expression of miR-146b is associated with poor thyroid cancer outcomes compared with the expression of miR-146a (25, 50). Sun *et al*. reported that the expression of miR-146a and miR146b were significantly higher in PTC tissue compared to normal goiter and perineoplastic tissue specimens (32).

The ability of miRNAs to interact with multiple mRNA transcripts and the dysregulation of several miRNAs in thyroid cancer give rise to a molecular network that affects a wide group of gene products. This network could drive multiple cellular functions and pathways towards tumorigenesis (19, 20, 88). However, knowledge of these PTC miRNA "signatures" could be associated with specific oncogenic pathways facilitating the implementation of novel PTC therapeutic targets. In addition, future guidelines may include panels of circulating miRNAs that are associated with specific thyroid cancer subtypes and characteristics, such as extrathyroidal extension, lymph node infiltration, vascular invasion, and distant metastasis. The hypothesis that miRNAs are secreted by tumor cells enhances the potential use of miRNAs as thyroid cancer biomarkers (50). Although some of them may not be tumor-derived, such as let-7e, their presence in plasma could be used as a tumor fingerprint for the differential diagnosis of thyroid nodules (50).

An interesting diagnostic approach was proposed by Zhang *et al*., who combined the serum expression of four miRNAs (miR-222, miR-221, miR-146b, and miR-21) with ultrasound. This novel diagnostic combination achieved a cumulative sensitivity of 91.4% and specificity of 91.1% in the diagnosis of PTC. The study concluded that the combination of these four miRNAs and thyroid ultrasound, improved the PTC diagnosis when compared to thyroid ultrasound alone (34). Several other studies combined multiple circulating miRNAs for PTC diagnosis and reported that sensitivity and specificity markedly improved compared to single miRNA use (26, 28, 31, 38, 44, 46). However, in the study of Perdas *et al*., the combination of the let family of miRNAs did not provide an additional increase in sensitivity and specificity of PTC diagnosis (57). Table II summarizes the sensitivity, specificity and AUC of several miRNAs and their combination as reported in the literature. These results could support the hypothesis that a combination of the miRNAs reported in this study could enhance the sensitivity and specificity of PTC diagnosis.

Despite its strengths (systematic literature search, high number of included studies), our study has several limitations. The high heterogeneity of the included studies precludes the quantification and generalizability of our findings. Our analysis focused on the most common thyroid cancer subtype and did not investigate the role of miRNAs in other less common subtypes, including follicular, anaplastic, and medullary thyroid cancer. Caution should be taken when interpreting our results as 27 out of 39 included studies originated from China. This issue may increase the risk of generalizability bias and preclude the application of our findings to other ethnic groups. Furthermore, some included studies follow the World Health Organization (WHO) 2004 TNM staging criteria as well as the American Joint Committee on Cancer (AJCC) 7th edition for extrathyroid tumor extension characterization while newer versions of both AJCC and WHO staging criteria have been published thereafter. Lastly, the variation in the specimens that were used to extract miRNAs, such as serum, plasma, and thyroid tissues, further complicates the interpretation of our results and the development of an ideal biomarker diagnostic panel.

In conclusion, our study focused on the miRNA expression patterns that may be utilized in the diagnosis of thyroid nodules with a PTC potential. The expression of

miRNAs was shown to be associated with PTC clinical characteristics, including lymph node or distant metastasis, extrathyroidal extension, bilateral location, *BRAF* mutation, TNM stage, serum thyroglobulin levels, and/or ^{131}I avid thyroid cancer metastasis. Specifically, among the miRNAs investigated in this study, circulating miR-146, miR-221, and miR-222 were frequently encountered in the literature and presented the strongest association with PTC. Among the included studies and identified miRNAs, it is reported that in some populations miR-221-3p presented 100% specificity, miR-146b presented 94.3% sensitivity, while the combination of miR-146b, miR-187, miR-138, miR-375, miR-222-3p, miR-151a-3p and thyroid ultrasound had the highest AUC. The development of a miRNA assay for the detection of the most common aberrantly regulated miRNAs in the serum could be a promising diagnostic minimally invasive tool for the early diagnosis of PTC. This approach may increase the diagnostic sensitivity and specificity and improve preoperative patient counselling and guidance to treatment.

Supplementary Material

Available at: https://docs.google.com/document/d/1kjxRohBlKG0YpeUw_mLmT_h1U05r7srE/

Conflicts of Interest

The Authors have no conflicts of interest to declare that are relevant to the content of this article.

Authors' Contributions

All Authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by GG, MP, KP, NMA, KK, MM and DG. The first draft of the manuscript was written by GG, ME, and DG and all Authors commented on previous versions of the manuscript. All Authors read and approved the final manuscript. The final editing was performed by ETP, DG, TEP, KS, TEA, and ME.

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Received March 20, 2022
Revised April 24, 2022
Accepted April 26, 2022