MicroRNA Expression Profiles in the Management of Papillary Thyroid Cancer

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ABSTRACT

Papillary thyroid cancer (PTC) is the major contributor to the dramatically increasing incidence of thyroid cancer. Low-risk PTC shows the most rapid rate of increase because of changing trends in neck imaging and the use of fine needle aspiration to investigate thyroid nodules. The need for a paradigm shift in the management of these patients, to provide personalized treatment and surveillance plans, has led to the focus on molecular biomarker research. MicroRNAs (miRNAs) compose a class of molecules with promising applications for every stage

of PTC management, including diagnosis, prognosis, treatment, and surveillance. Although most of the miRNA studies are currently preclinical, given the rapid progress of scientific discovery, clinical trials will not be far away. Thyroid clinicians will be expected to have good insights into the current status of PTC-related molecular translational research. This article focuses on the potential roles of miRNA in PTC management in the context of contemporary recommended clinical practice. *The Oncologist* 2014;19:1141–1147

Implications for Practice: The rapid increase in the diagnosis of papillary thyroid cancer has led to a demand for strategies to improve risk stratification, in order to accurately tailor management. MicroRNA biomarkers are a promising tool to aide current clinicopathological features in preoperative and postoperative risk stratification. Furthermore, circulating miRNA biomarkers also have the potential to augment thyroglobulin in long-term, noninvasive surveillance after initial treatment. Other areas of miRNA research in the management of papillary thyroid cancer include improving diagnostic accuracy of cytology samples, and targeted therapy in advanced disease.

INTRODUCTION

The incidence of thyroid cancer has increased approximately threefold over the last three decades in many Western countries [1–4]. This increase is almost entirely attributable to an increase in the incidence of papillary thyroid cancer (PTC), which is the most common type of thyroid cancer. The American Thyroid Association (ATA) first published treatment guidelines for patients with thyroid nodule and differentiated thyroid cancer (DTC; including PTC) in 1996 and has since updated the guidelines in 2006 and 2009. The updated guidelines reflect the changing evidence from quality studies, and there have been significant advances in the diagnosis and therapy of DTC during this time.

The recommendations in the ATA guidelines were based predominantly on clinical studies [5]. There is limited application of molecular genetics in the current clinical management recommendations. In recent years, researchers have made dramatic advances in understanding the role of miRNAs in normal and malignant biological processes. A wealth of literature

supports the notion that miRNAs are involved in the modulation of a myriad of cellular processes, including cell proliferation, apoptosis, invasion, and metastasis. With improved understanding, investigators are working toward harnessing the potential of miRNA in the clinical management of malignant diseases.

This review gives a brief background on miRNA and summarizes the potential of the translational application of miRNA research in the context of current recommended management of PTC.

MICRORNA BACKGROUND

MicroRNA Discovery

MicroRNAs (miRNAs) belong to a class of noncoding small RNAs that are approximately 22 nucleotides in length. The founding member of this class of RNAs, lin-4, was discovered as early as 1993 in the worm *Caenorhabditis elegans* (*C. elegans*). It was noted that lin-4 did not code for a protein but instead had

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antisense complementarity with multiple sites in the lin-14 mRNA [6, 7]. Further studies subsequently demonstrated that the pairing of lin-4 RNA with the lin-14 mRNA resulted in translational repression of the lin-14 message. This, in turn, effected the transition from first to second larval stage [6, 7].

It was not until 2000 that the next member of this class of RNA was discovered. The let-7 RNA was also a gene in *C. elegans*, encoding another approximately 22-nucleotide regulatory RNA. The let-7 RNA had a role in promoting the transition from late-larval to adult stage, in a similar manner that lin-4 promoted progression from first to second larval stage [8, 9].

Owing to their roles in the developmental transitions, lin-4 and let-7 RNAs were named small temporal RNAs (stRNAs) [10]. Although 7 years passed between the discovery of the first and second stRNAs, the explosion of discovery of new stRNAs had begun. Within a year of the discovery of let-7, more than 100 additional genes of similar characteristics were reported in humans, flies, and worms. Many of these newly identified stRNAs did not appear to be involved in the temporal developmental transition process; instead, they appeared to be differentially expressed in different cell types, and many had as yet undetermined functions. It was then that the term microRNA was coined to refer to the stRNAs and all the other tiny RNAs with similar features [11]. The growing discovery of new miRNAs is nothing short of an epic phenomenon. Over the past decade, more than 2,000 miRNAs have been sequenced in humans, with more in other species.

MicroRNA Expression and Maturation

The first step in the expression of a miRNA is the transcription of the miRNA gene into the primary miRNA transcript (Pri-miRNA) by an RNA polymerase (Fig. 1). Following transcription, still within the nucleus, the Drosha-DGCR8 (DiGeorge critical region 8) microprocessor complex cleaves both strands of the stem on the Pri-miRNA [12]. The product of the cleaved Pri-miRNA is the shortened precursor miRNA (Pre-miRNA) [13].

The Pre-miRNA produced by the nuclear processing is then exported into the cytoplasm by exportin-5 [12]. In the cytoplasm, the Pre-miRNA is cleaved into a double-stranded miRNA duplex by Dicer, an RNase enzyme [14]. One of the strands of the duplex is the guide strand, whereas the complementary strand is the passenger strand. The guide strand is incorporated into the miRNA effector machine, known as the RNA-induced silencing complex (RISC), and the passenger strand is degraded.

The RISC is the machinery responsible for gene silencing and consists of a miRNA strand, a member of the Argonaute (AGO) protein family, and other structural and facilitating proteins [12]. The guide miRNA strand recognizes the target mRNA transcript, whereas the AGO protein (with other facilitating proteins) mediates the corresponding action [12, 15]. The nature of the mediated action depends on the degree of complementarity between the miRNA and mRNA. If there is significant yet incomplete pairing of the miRNA and mRNA sequences, mRNA translation is repressed. It is this incomplete nature of miRNA-mRNA matching that allows a single miRNA to target multiple mRNAs and multiple miRNAs to target the same mRNA sequence [16]. In the less commonly occurring event of complete base pairing, the mRNA transcript is degraded [15].

MicroRNA Deregulation

MicroRNAs contribute to oncogenesis either as tumor suppressors or oncogenes. Their deregulation is a result of genomic abnormalities similar to those for protein-coding genes: chromosomal rearrangements, genomic amplifications or deletions, and mutations [17]. In any given cancer, a combination of abnormalities in protein-coding and non-coding genes can be identified [18]. Among the possible mechanisms, the contemporary view is that aberrant gene expression is the main mechanism underlying the functional changes of miRNAs in cancer cells. This is characterized by abnormal levels of expression for mature and/or precursor miRNA sequences compared with the normal tissue of that organ. The abnormal expression level is otherwise known as deregulation [17]. In addition, defects in miRNA processing and maturation also contribute to their deregulation and subsequently cause diseased states [12].

MicroRNA Versus mRNA

Several features of miRNAs make them attractive diagnostic biomarkers. They are regulators upstream from mRNAs, and each miRNA is able to target multiple protein-coding genes within or across pathways [19]. Unlike mRNAs, miRNAs do not need to be translated to proteins to exert their effects; therefore, their measured expression may correlate more closely to the functional status of the gene. Consequently, new miRNA markers can be tested for biological effects by generic sequence-based methods [19]. Furthermore, miRNAs show superior stability and maintain their expression profiles in archival formalin-fixed paraffin-embedded samples, allowing utilization of a vast amount of resources already available in most centers [19–22].

MICRORNA IN THE MANAGEMENT OF PTC

With the increased diagnosis of predominantly indolent PTC, biomarkers that can efficiently select the minority of patients with aggressive tumors are urgently needed. This would allow personalized treatment planning to adequately treat the minority with aggressive tumors and avoid overtreatment in the majority with indolent disease. The following discussion focuses on PTC; the potential utility of miRNA in other forms of thyroid cancer is not discussed.

MicroRNA in PTC Diagnosis

Although the clinical diagnosis of PTC is not usually a problem, it is important to appreciate the development of miRNA research in PTC, as a foundation for other clinical applications.

He et al., in their seminal paper published in 2005, were the first to apply a miRNA profiling capability to PTC [23]. One of the significant findings was that in PTC, the key differentially expressed miRNAs appeared to be overexpressed, although global under-expression of miRNA was normally associated with cancers in general. This finding has since been confirmed in other reports [24, 25].

He et al. compared the miRNA expression of 30 PTC tumors and the normal tissue samples of their corresponding thyroid glands, using the Affymetrix HG-U133 plus two array chips [23]. The pairwise comparisons indicated that 23 miRNAs showed differential expression, with 17 of them overexpressed and 6 underexpressed. Of the overexpressed miRNAs, 6 demonstrated a fold change of >1.5-fold. The most overexpressed

Figure 1. Schematic diagram depicting miRNA production. The Pri-miRNA is transcribed from a miRNA gene within the nucleus. The Pri-miRNA is then cleaved to become the Pre-miRNA, which is exported into the cytoplasm. Dicer processes the Pre-miRNA into the mature miRNA duplex. One of the strands is the guide strand, which is incorporated into the miRNA effector machine, known as the RNA-induced silencing complex. Cleavage or translational repression of the target mRNA occurs depending on the complementarity of the miRNA to the mRNA. Figure copyright Beth Croce, Bioperspective. Reproduced with permission.

Abbreviations: AGO, Argonaute protein; miRNA, microRNA; Pre-miRNA, precursor microRNA; Pri-miRNA, primary microRNA; RISC, RNA-induced silencing complex.

miRNAs were miR-146b, miR-221, and miR-222, showing increases of 11- to 19-fold. These findings were further confirmed on semiquantitative reverse transcription polymerase chain reaction (RT-PCR) and Northern blotting. All underexpressed miRNAs had a fold-change of less than twofold [23]. Incidentally, in a 2006 publication, Calin and Croce pointed out that a less-than-twofold change in miRNA expression in some instances might also be biologically significant [17]. Using as few as 5 miRNAs, He et al. were able to define cancer status in 12 blind tumors samples with 100% accuracy [23].

He et al. also found that miR-221 expression was increased in nonmalignant adjacent thyroid tissue, albeit to a lesser degree [23]. This suggested that miR-221 overexpression was either an early genetic event in PTC carcinogenesis or that some carcinogenic miRNA changes were global phenomena within the thyroid gland [23].

Significant overexpression of miR-146b, miR-221, and miR-222, reported by He et al. [23], was confirmed in many subsequent studies by other groups [25–27]. Using microarray and Northern blotting, for example, Pallante et al. also showed

that miR-221 and miR-222 were significantly overexpressed in PTC tissue compared with normal thyroid tissue of the contralateral, unaffected lobe [26]. In their study, the third overexpressed miRNA reported was miR-181b [26]. Further confirmation of these findings was achieved by quantitative RT-PCR comparing the miRNA precursors in an external cohort of 39 PTC and 8 follicular adenoma tissue samples. Means of approximately 13-fold overexpression were seen in all 3 miRNA precursors in the PTC samples; they then extended this technique to fine needle aspiration (FNA) samples [26] (as discussed in MicroRNA in Thyroid FNA).

Between 2005 and 2013, eight publications reported on the differential miRNA expression profiles of human PTC samples compared with normal or benign thyroid tissue (Table 1); however, these publications were heterogeneous in terms of the included sample populations, tissue studied, and methodology. The heterogeneity arose from different study designs and questions, but the common ground was the use of microarray on at least 10 samples including PTC and control tissue types. Most studies included both classical and follicular variants of PTC, whereas some did not define the subtype of PTC samples included. Some studies also included other thyroid malignancies [28, 29]. The control or comparison groups varied from normal thyroid tissue [27, 30] to multinodular goiter (MNG) [25] or benign follicular lesions [28, 29]. Despite these inconsistencies, some similar trends of miRNA deregulation can be observed in PTC samples. The most consistently overexpressed miRNAs in PTC were miR-221, miR-222, and miR-146b. Other commonly reported overexpressed miRNAs were miR-155 and miR-181b. There was less consistency in the downregulated miRNA set.

MicroRNA in Thyroid FNA

Although more histological subtypes of PTC were being profiled, work was also being done on thyroid FNA samples. Pallante et al. were the first to report three highly overexpressed miRNAs (miR-221, miR-222, and miR-181b) in thyroid tumors in the context of FNA cytology samples [26]. All three miRNAs showed higher expression levels in seven of eight PTC FNA samples compared with normal thyroid cells from FNA of non-neoplastic nodules. With this finding, new doors were opened for the utility of miRNA profiling in the diagnosis of thyroid malignancies. The potential role of miRNA profiling in thyroid pathology was transformed from postoperative confirmation or subtyping in tissue samples to preoperative diagnosis or subtyping in FNA samples. This development has major implications for the way PTC patients may be managed, if it enters clinical application.

Currently, approximately 3%–6% of FNA samples are considered indeterminate [32]. It is the management of this small subgroup that can be significantly improved with molecular diagnosis preoperatively. Keutgen et al. reported that by using a panel of 4 miRNAs (miR-222, miR-328, miR-197, miR-21), thyroid malignancies could be diagnosed in FNA samples of indeterminate lesions, with 100% sensitivity and 86% specificity and overall accuracy of 90% [33]. Other studies using a similar study design of indeterminate FNA samples, with single or a panel of miRNAs, failed to achieve such high accuracy [29, 34, 35]. Agretti et al. [35] and Shen et al. [34] were able to distinguish benign thyroid tissue from PTC using miRNA expression of FNA samples; however, the discriminatory power

of the technique was poorer in the subgroup with indeterminate cytology, limiting the clinical application [35]. Using 1 miRNA on 125 indeterminate FNA samples, Vriens et al. achieved accuracy of 75% and negative predictive value of 81% [29]. These rates are not yet suitable for clinical use. The consensus is that further work is required before miRNA expression in FNA samples can be of clinical utility [36].

MicroRNA in Staging and Prognostication

One of the challenges in managing PTC patients is identifying the small subgroup with aggressive disease and providing those patients with more extensive treatment and intensive long-term surveillance. As the number patients with indolent PTC increases, there is an even greater need for accurate and efficient identification of those with aggressive disease. Accurate stratification at initial diagnosis and treatment is essential to avoid overtreatment and unnecessary surveillance of the majority and to provide adequately intensive management for the minority with aggressive disease.

Various molecular markers have been investigated in the hope of more accurate risk stratification [37–40]. The most studied marker is *BRAF* (the B-isoform of the Raf gene). The *BRAF*^{V600E} mutation has been shown to be associated with aggressive clinical behaviors of PTC by some investigators [37]; however, the evidence is still inconclusive, with contradictory reports [38]. Other molecular markers being investigated include cell-cycle regulators p27, p21, and cyclin D1 and immunohistochemical markers such as CEACAM-1 (carcinoembryonic antigen-related cell adhesion molecule 1), OPN (osteopontin), and E-Cadherin (E-cadherin) [39, 40]. None of these markers have reached wide acceptance in clinical practice so far, including *BRAF*.

Following reports on the role that miRNAs play in PTC tumorigenesis, and thus their potential diagnostic utility, investigation also progressed to testing the prognostic ability of miRNA expression profiling. In one of the earliest studies on this subject, Gao et al. demonstrated miRNA differential expression in three subpopulations of a PTC cell line (IHH-4) with increased lymph node metastatic potency compared with the control subpopulations [41]. They found that 11 miRNAs from the array of 509 miRNAs were significantly differentially expressed among 3 pairs of subpopulations with high and low metastatic potential. Five of these miRNAs were upregulated in the metastatic subpopulations, and six were downregulated.

Three miRNAs (miR-146b, miR-221, and miR-222) consistently found to be overexpressed in PTC tissue, compared with normal thyroid tissue, appeared to also confer high-risk features such as extrathyroidal extension, lymph node metastasis, distant metastasis, recurrence, and *BRAF*^{V600E} mutation. In 100 PTC samples, Chou et al. showed that tumors with the *BRAF*^{V600E} mutation had higher miR-146b expression than those without the mutation [42]. In a follow-up study, Chou et al. reported that patients whose PTC expressed high levels of miR-146b had poorer overall survival. They further demonstrated that the BCPAP human papillary thyroid cancer cell line showed increased ability with regard to cell migration and invasion when transfected with miR-146b mimics [43]. Yip et al. demonstrated that miR-146b and miR-222 were overexpressed in aggressive PTC, defined as PTC associated with distant metastasis or recurrence. They also demonstrated downregulation of miR-34b and miR-130b in the PTC samples associated with aggressive

Table 1. Overview of studies using miRNA microarray to compare PTC and control

Study	Samples	Microarray probe number	Validation samples ^a	Overexpressed miRNAs in PTC ^b	Underexpressed miRNAs in PTC
He et al. (2005) [23]	Fresh frozen, 15 PTC vs. 15 adjacent normal thyroid	460	Same cohort	(17) 146b, 221, 222 , 21, 155, 181a	(6)
Pallante et al. (2006) [26]	Fresh frozen, 30 PTC vs. 10 contralateral lobe normal thyroid	368	Independent 39 PTC vs. 8 FA	(15) 221, 222 , 213, 220, 181b	(7)
Tetzlaff et al. (2007) [25]	FFPE, 10 classic PTC vs. 10 MNG	754	Independent 10 classic PTC vs. 10 MNG	(13) 21, 221, 222 , 223, 31	(26) 345
Nikiforova et al. (2008) [27]	Fresh frozen, 9 PTC vs. 5 normal thyroid	158 ^c	No validation ^c	(47) ^d 187, 221, 222 , 181b, 146b, 155, 122a, 31, 205, 224	(57) ^d
Yip et al. (2011) [30]	Fresh frozen, 12 PTC vs. 4 normal thyroid	319	Independent 32 PTC ^e	(5) 146b, 221, 222, 155, 31	(11) 153, 448, 325, 382, 495, 34b, 1, 144, 367, 130b, 138
Kitano et al. (2011) [28]	Fresh frozen, 26 malignant vs. 21 benign ^f	1,263	Same cohort	(14) 146b-5p, 222, 221, 32* , 744, 30b*	(20) 451, 7, 144, 374a, 126, 143, 30e, 7g, 335, 101 , 26b, 7f, 30c, 193a-3p, 30b, 145, 30a, 195, 22
Vriens et al. (2012) [29]	Fresh frozen, 56 malignant vs. 48 benign ^g	850	Same cohort ^a	(NR) 628-3p, 550*, 564, 635, 584	(NR) 149, 100, 138, 125b, 768-3p
Huang et al. (2013) [31]	Fresh frozen, 12 PTC vs. 3 adjacent normal thyroid	955	No validation ⁱ	(12) 15a, 31*, 1274a, 15a*, 551b, 34a*, 221*, 34b*, 34a, 222, 221, 146b-5p	(6) 873, 876-3p, 7-2*, 7, 30a, 144*

Only studies with >10 microarray samples are included. Boldface text indicates differential expression confirmed on RT-PCR or Northern blot (miRNAs that did not show significant differential expression on validation studies are not indicated). Underlined text indicates top three. Numbers in parentheses indicate total number of miRNAs with differential expression that were statistically significant.

^aIf the validation group was not specified, it was assumed that the same cohort was used.

^bListed miRNAs were thought to be potentially important as interpreted by the authors and thus were not selected with a consistent set of criteria.

^cRT-PCR panel was used (strictly not a microarray); therefore, no validation was performed.

^dNumber reflecting deregulated miRNAs from all tested tumor types, including PTC, FTC, MTC, ATC, PDTC, and FA.

^eValidation was performed for aggressive vs. nonaggressive PTC.

^fMalignant samples included 6 PTC, 6 follicular-variant PTC, 6 FTC, and 8 HCC. Benign samples included 6 follicular adenoma, 8 MNG, and 7 Hurthle cell adenoma.

^gMalignant samples included 12 follicular variant PTC, 8 PTC, 12 FTC, 20 HCC, 4 ATC; benign samples included 7 normal thyroid, 14 MNG, 15 follicular adenoma, 12 Hurthle cell adenoma.

^hEqual third.

ⁱNo validation comparing PTC and normal.

Abbreviations: ATC, anaplastic thyroid cancer; FA, follicular adenoma; FFPE, formalin-fixed paraffin-embedded; FTC, follicular thyroid cancer; HCC, Hurthle cell cancer; miRNA, microRNA; MNG, multinodular goiter; MTC, medullary thyroid cancer; NR, not reported; PDTC, poorly differentiated thyroid cancer; PTC, papillary thyroid cancer; RT-PCR, reverse transcription polymerase chain reaction.

biology [30]. Lee et al. reported that miR-146b and miR-222 were overexpressed in PTC with recurrence and were more strongly associated with PTC recurrence than BRAF expression. They postulated that the quantifiable nature of miRNA expression is more suited to the role of prognostication than the dichotomous nature of BRAF expression [44]. Zhou et al. found that overexpression of miR-221 was associated with extra-thyroidal extension, lymph node metastasis, advanced disease stages III and IV, and the BRAF mutation [45].

Three miRNAs (miR-146b, miR-221, and miR-222) consistently found to be overexpressed in PTC tissue, compared with normal thyroid tissue, appeared to also confer high-risk features such as extrathyroidal extension, lymph node metastasis, distant metastasis, recurrence, and BRAF^{V600E} mutation.

The findings in these studies are exciting, although further refinement through larger, externally validated studies is necessary to uncover the true potential of prognosticating with miRNA expression profiling [46]. Although few studies have reported miRNA profiling as a prognostic tool in PTC risk stratification, miRNA classifiers with promising prognostic potentials have been reported consistently across a variety of other cancers. In a 2012 systematic review of 43 studies, Nair et al. reported that several miRNA classifiers provided more accurate prognostic information, for diverse cancers, than the traditional clinicopathologically based tools [46]. They also reported consistencies in the deregulation of specific miRNAs across classifiers for different cancers, leading them to postulate that some miRNA-coordinated regulatory pathways are common to many cancers. They cautioned that errors and biases can occur at every step of the appraisal of miRNAs for prognostic purposes and concluded that the clinical application of miRNA expression measurement as a biomarker for prognosis has been limited so far.

MicroRNA in Treatment

The majority of nonaggressive PTC is adequately treated by surgery, radioactive iodine ablation, and thyroid-stimulating hormone-suppressive therapy; however, a subgroup of patients present with aggressive disease that can be locally invasive, widely metastatic, or repeatedly recurrent. Disappointingly, external-beam radiotherapy and chemotherapy have shown limited success. Recent trials of tyrosine kinase inhibitors have shown promising results and indicate the need for alternative treatment options for advanced PTC [47, 48].

The therapeutic potential of miRNAs as agents of targeted therapies is currently being explored. The ability of a single miRNA to target multiple genes is of great therapeutic advantage. Simultaneous targeting of multiple components of the same pathway, leading to synergistic effects, would confer therapeutic advantage. However, achieving target organ specificity while limiting off-target effects is a major challenge in the translation from bench to bedside [49]. Although the prospect of manipulating miRNAs to complement current therapeutic strategies in cancer is appealing, the practicality is complex.

Despite the challenges, in April 2013, for the first time, a miRNA mimic reached phase I clinical study. MRX34 aimed to restore the lost tumor suppressor function of endogenous miR-34 in patients with primary liver cancer or metastatic tumors to the liver. By restoring the tumor suppressor pathway via miR-34 replacement therapy, apoptosis was induced in tumor cells in vitro and in mouse models; however, off-target effects, dosing, and pharmacokinetics were all areas that required further study [50]. Currently, there is limited research on the miRNA-targeted treatment of PTC. In a recent early stage in vitro study, Lin et al. showed that restoration of miR-101 expression in the K1 PTC cell line significantly reduced proliferation [51].

MicroRNA in Follow-Up

In recent years, there has been intense interest in the feasibility of using miRNAs or miRNA panels as circulating biomarkers for the presence of malignant and nonmalignant diseases. Cellular miRNAs can be released into the circulation or surrounding microenvironment membrane free, protein bound, or packaged within microvesicles (0.1–1 μ m) or nanovesicles (<100 nm). Free miRNAs are rapidly degraded by RNases, whereas vesicular or protein-bound miRNAs are protected from degradation. These circulating miRNAs released by diseased cells, however they escape degradation, are currently being investigated as potential noninvasive biomarkers of disease for diagnosis or recurrence. Some of these circulating miRNA biomarkers also double as prognostic factors for survival, staging tools, extracellular communicators, and markers of pathological progression in various cancer types.

In PTC, circulating miRNAs are potential alternatives to serum thyroglobulin (Tg) measurements. Serum Tg measurement as a long-term noninvasive surveillance tool is not applicable to up to 25% of PTC patients because of the presence of Tg antibodies, the performance of less than total thyroidectomy, or the lack of postoperative radioactive iodine ablation. Preliminary studies have demonstrated that circulating levels of let-7e, miR-151-5p, miR-146b, miR-221, and

miR-222 are higher in PTC patients compared with healthy subjects [44, 52]. Yu et al. measured miRNA expression in the serum of 106 patients with PTC, 95 patients with benign thyroid nodules, and 44 healthy subjects. They found that serum levels of let-7e, miR-151-5p, and miR-222 were significantly overexpressed in PTC patients compared with patients with benign nodules and healthy subjects. No significant difference was found in the serum levels of these miRNAs between patients with benign nodules and healthy subjects [52].

Preliminary studies have demonstrated that circulating levels of let-7e, miR-151-5p, miR-146b, miR-221, and miR-222 are higher in PTC patients compared with healthy subjects.

In a study comparing plasma miRNA expression before and after total thyroidectomy, Lee et al. reported that plasma levels of miR-146b, miR-221, and miR-222 were overexpressed in 42 PTC patients compared with healthy individuals [44]. They further reported that the plasma levels of all three miRNAs in PTC patients fell to levels comparable to those of healthy subjects. However, in addition to the healthy subjects, Lee et al. also included a third group of patients with multinodular goiter. They found that both preoperative and postoperative plasma miR-146b, miR-221, and miR-222 levels in MNG patients were comparable to those of PTC patients. Consequently, they concluded that plasma miRNA expression is not suitable for de novo diagnosis of PTC but may be a useful adjunct to Tg in long-term surveillance [44]. A noninvasive, miRNA alternative to thyroglobulin would simplify the long-term recurrence surveillance for the subgroup of patients who are unable to make use of serum Tg levels.

CONCLUSION

There has been significant improvement and standardization in the clinical management of PTC in recent years; however, molecular markers to further improve risk stratification are still anticipated for clinicians to be able to provide biomarker-driven, personalized treatment. Not only are microRNAs potential biomarkers for PTC recurrence and metastasis (miR-146b and miR-222), they also have been shown to be potential tools for long-term surveillance (let-7e, miR-151-5p, miR-146b, miR-221, and miR-222). Work is in progress to ascertain prognostic information by profiling miRNA expression in thyroid FNA samples and by using miRNAs as targeted therapy for treatment-resistant PTC tumors.

AUTHOR CONTRIBUTIONS

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Data analysis and interpretation: Justin S. Gundara, Anthony Glover
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DISCLOSURES

The authors indicated no financial relationships.

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