Identification of Thyroid-Associated Serum microRNA Profiles and Their Potential Use in Thyroid Cancer Follow-Up

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Context: Trends toward more conservative management of papillary thyroid cancer (PTC) diminish the primacy of serum thyroglobulin (Tg) assays as a posttreatment surveillance tool.

Objective: To identify thyroid tumor-associated microRNAs (miRNAs) in the serum with potential for development as unique biomarkers of PTC recurrence.

Methods: We measured expression of 754 miRNAs in serum samples collected from 11 patients with PTC before and 30 days after thyroidectomy. Major candidates were then re-evaluated by absolute quantitative polymerase chain reaction analysis in an independent cohort of patients with PTC (n = 44) or benign nodules and 20 healthy controls (HCs). The 2 miRNAs most significantly associated with thyroid tumors were then assessed in matched serum samples (before and 30 days and 1 to 2 years after surgery) from the 20 PTC patients with complete follow-up datasets and results correlated with American Thyroid Association (ATA) responses to therapy.

Results: Eight miRNAs (miR-221-3p, miR-222-3p, miR-146a-5p, miR-24-3p, miR-146b-5p, miR-191-5p, miR-103a-3p, and miR-28-3p) displayed levels in prethyroidectomy serum samples from patients with PTC that significantly exceeded those measured after thyroidectomy and those found in samples from HCs. The 2 most promising candidates—miR-146a-5p and miR-221-3p—were further analyzed in the 20 PTC patients mentioned earlier. Serum levels of both miRNAs after 1 to 2 years of follow-up were consistent with ATA responses to therapy in all patients, including 2 with structural evidence of disease whose Tg assays remained negative (<1 ng/mL).

Conclusion: miR-146a-5p and miR-221-3p hold remarkable promise as serum biomarkers for post-treatment monitoring of PTC patients, especially when Tg assay results are uninformative.

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Freeform/Key Words: papillary thyroid carcinoma, microRNA, circulating, biomarker, follow-up

Serum thyroglobulin (Tg) is the main biomarker for detecting persistent/recurrent thyroid cancer [1–3], although its specificity is limited in the presence of anti-Tg autoantibodies and

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Abbreviations: ATA, American Thyroid Association; BN, benign nodule; cDNA, complementary DNA; Ct, cycle threshold; HC, healthy control; miRNA, microRNA; NF, normalization factor; PTC, papillary thyroid cancer; ROC, receiver-operating characteristic; RRA, radioiodine remnant ablation; Tg, thyroglobulin
residual normal thyroid tissue. The latter is being encountered with increasing frequency owing to the declining use of total thyroidectomy followed by radiiodine remnant ablation [4–13]. Consequently, the search is on for new, more specific tumor biomarkers to ensure optimal postoperative follow-up of today’s thyroid cancer patients.

One of the most promising solutions involves measuring the levels of tumor-derived microRNAs (miRNAs) in the bloodstream. The tissue-specific expression patterns of these RNA species and their high stability in biological fluids are well established [14]. Dysregulated miRNA expression has been documented and characterized at the tissue level in many malignancies, including papillary thyroid cancer (PTC), the most common form of differentiated thyroid cancer [15–19]. Less is known about the circulating miRNAs derived from normal and neoplastic thyroid tissues. Attempts have been made to identify miRNAs associated with thyroid tissue in general [20–22], thyroid tumor tissue [20–25], and specific clinicopathological variables of thyroid tumors [20, 22, 23, 25]. However, the few studies that have explored these issues have generated discordant results. The discrepancies can probably be attributed in large part to the variety of approaches used to quantify circulating miRNAs (e.g., RNA sequencing versus real-time polymerase chain reaction versus microarrays, as well as miRNA profiling versus selected miRNA panels) and other methodological differences (e.g., characteristics and size of patient and control groups, sampling time, or use of plasma or serum).

The aim of the current study was to identify circulating miRNAs that might be developed into thyroid cancer biomarkers for use during postoperative follow-up. To this end, we set out to identify (1) circulating thyroid-derived miRNAs by analyzing the expression of 754 miRNAs in preoperative and postoperative serum samples from patients with PTC; (2) thyroid tumor–derived miRNAs by analyzing serum levels of a selected panel of miRNAs in healthy controls (HCs), patients with benign thyroid nodules, and patients with PTC; and (3) the potential value of selected miRNAs as clinical biomarkers of PTC by analyzing their postoperative serum levels in a subset of patients with PTC classified according to the response-to-therapy criteria recommended by the American Thyroid Association (ATA) [1].

1. Materials and Methods

A. Study Design and Patient Samples

We conducted a prospective observational study from 2012 to 2016 at the Sapienza University of Rome Hospital. Two cohorts were enrolled (Table 1). The screening cohort consisted of 11 patients who consecutively underwent total thyroidectomy for sporadic PTCs. The validation cohort included 44 patients with sporadic PTCs consecutively treated with total thyroidectomy and 39 age- and sex-matched controls. Nineteen of the 39 controls in the validation cohort were patients who underwent total thyroidectomy for nodules with indeterminate cytology (Bethesda Class IV) that were subsequently diagnosed as benign on the basis of surgical histology (benign nodule [BN] group). The other 20 were HCs seen in the hospital’s outpatient clinics for periodic health assessments or complaints unrelated to the thyroid. All the HCs had negative workups and no evidence of thyroid-disease based on the results of a screening examination performed by the study team (complete patient and family histories, neck ultrasound findings, and results of thyroid hormone and thyroid antibody assays).

The protocol called for collection of serum samples from enrolled participants according to the following schedule. For PTC patients of both cohorts and BN patients in the validation cohort, we collected a preoperative sample on the day of thyroidectomy, right before induction of anesthesia. A second sample was collected from PTC patients 30 days after surgery. For HCs in the validation cohort, a single sample was taken at study enrollment. Blood samples (8 to 10 mL) were collected in a BD Vacutainer® Tube (Becton, Dickinson and Company, Melbourne, Australia) and centrifuged at 3000 rpm for 10 minutes, and the serum obtained was stored at −80°C prior to analysis.

Clinical data on PTC patients were collected from hospital and outpatient clinic charts. Tumors were staged according to the American Joint Committee on Cancer criteria [26].
Risks of recurrence were classified as high, intermediate, or low using ATA-recommended criteria [27]. Posttreatment surveillance involved visits every 1 to 2 years, each including a serum Tg assay (DYNO test Tg-plus, Brahms Diagnostics GmbH, Berlin, Germany; functional sensitivity, 0.2 ng/mL), radioimmunometric assay of circulating Tg antibodies (Architect System Anti-Tg, Abbott Laboratories, Abbott Park, IL; functional sensitivity, 0.31 IU/mL), and high-resolution grayscale and color Doppler ultrasound of the thyroid bed and cervical lymph node compartments. Outcomes at the 1- to 2-year visit were classified according to the response-to-therapy criteria recommended by the ATA in its 2015 guidelines [1]. For the purposes of the study, 3 response categories were considered: (1) structural incomplete responses, (2) biochemical incomplete responses, and (3) excellent or indeterminate responses.

### Table 1. Characteristics of PTC Patients in the 2 Cohorts

| Clinicopathological Features | Screening Cohort (n = 11) | Validation Cohort (n = 44) |
|-----------------------------|--------------------------|---------------------------|
| Age in years at diagnosis (median, range) | 47 (23–67) | 46 (22–75) |
| Sex (n, rate) | | |
| Female | 7 (64%) | 34 (77%) |
| Male | 4 (36%) | 10 (23%) |
| Tumor size in millimeters (median, range) | 11 (5–33) | 10 (1–60) |
| Tumor foci (n, rate) | | |
| Unifocal | 9 (82%) | 31 (70%) |
| Multifocal | 2 (18%) | 10 (23%) |
| NA | 0 | 3 (7%) |
| Extrathyroidal extension (n, rate) | | |
| No | 5 (45%) | 23 (52%) |
| Yes, microscopic | 6 (55%) | 17 (39%) |
| Yes, macroscopic | 0 | 1 (2%) |
| NA | 0 | 3 (7%) |
| AJCC/UICC T class (n, rate) | | |
| T1a | 4 (36%) | 13 (30%) |
| T1b | 1 (9%) | 5 (11%) |
| T2 | 0 | 4 (9%) |
| T3 | 6 (55%) | 18 (41%) |
| T4a | 0 | 1 (2%) |
| NA | 0 | 3 (7%) |
| AJCC/UICC N class (n, rate) | | |
| Nx | 7 (64%) | 14 (32%) |
| N0 | 1 (9%) | 15 (34%) |
| N1 | 3 (27%) | 15 (34%) |
| N1a | 2 (18%) | 5 (11%) |
| N1b | 0 | 7 (16%) |
| NA | 1 (9%) | 3 (7%) |
| AJCC/UICC M class (n, rate) | | |
| Mx | 10 (91%) | 25 (57%) |
| M0 | 10% | 16 (36%) |
| M1 | 0 | 3 (7%) |
| 2009 ATA risk (n, rate) | | |
| Low | 4 (36%) | 19 (43%) |
| Intermediate | 7 (64%) | 21 (48%) |
| High | 0 | 4 (9%) |
| Radioiodine remnant ablation (n, rate) | | |
| Yes | 5 (45%) | 10 (23%) |
| No | 0 (0%) | 24 (54%) |
| NA | 6 (55%) | 10 (23%) |

Continuous variables are reported as median values and ranges; categorical variables are reported as absolute numbers and percentages.

Abbreviation: AJCC/UICC, American Joint Committee on Cancer/Union Internationale Contre le Cancer; M, metastasis; N, node; NA, information not available; T, tumor.
The study protocol was approved by the local ethics committee, and written informed consent was obtained from all patients whose serum samples were analyzed.

B. RNA Isolation From Serum Samples

Total RNA (containing small RNAs) was extracted from 200 μL of serum using the miRNeasy Serum/Plasma kit and cel-miR-39 as the spiked-in control (Qiagen, Hilden, Germany), in accordance with the manufacturer’s instructions. To perform miRNA profiling without the preamplification step, we used a multiple extraction approach, which involved isolation of total RNA from up to 5 200-μL aliquots of each serum sample. This solution allowed us to obtain over 1000 ng of total RNA from each sample. The quality and quantity of RNA samples were verified with a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA).

C. Circulating miRNA Analysis

C-1. Screening cohort

miRNA profiling was performed with TaqMan Array Human MicroRNA A+B Cards, version 3.0 (Thermo Fisher Scientific, Inc.), a set of 2 384-well microfluidic cards that allow quantitative expression analysis of 754 miRNAs. As noted earlier, reverse transcription and real-time polymerase chain reaction reactions were performed without a preamplification step, in accordance with the manufacturer’s instructions, which call for preamplification only if the total amount of RNA is <350 ng. Expression Suite software, version 1.0.3 (Thermo Fisher Scientific, Inc.), was used to calculate cycle threshold (Ct) values (cutoff, 35) and for data analysis. U6 was chosen as the endogenous control because it displayed the lowest between-sample variance, and relative miRNA expression levels were calculated using the comparative $2^{-\Delta\Delta Ct}$ method. Differences in preoperative and postoperative miRNA expression levels were considered significant if the fold change was $>2$ or $<0.5$ and $P \leq 0.05$.

C-2. Validation cohort

The miRNAs analyzed in the validation cohort were selected on the basis of the results of circulating miRNA profiling in the screening cohort and data in the literature (see “Results” for details). Expression levels were quantified in duplicate using specific TaqMan MicroRNA Assays (Thermo Fisher Scientific, Inc.) in accordance with the manufacturer’s instructions. Circulating miRNA abundance was quantified with the aid of standard curves constructed with miScript miRNA mimics (Qiagen), synthetic RNA oligonucleotides corresponding to mature miRNA sequences (miRBase Release, version 21). The mimics were reverse transcribed with specific reverse transcription primers (5x) over an empirically derived range of copy numbers, and 10-fold serial dilutions of the corresponding complementary DNA (cDNA) were used to generate standard curves for each miRNA TaqMan assay. The absolute number of miRNA copies was determined from each sample Ct using the following equation: $y = kx + m$, where $y$ is the Ct value, $k$ is the slope, $x$ is the logarithm of the number of miRNA copies, and $m$ is the $y$ intercept. A normalization factor (NF) was calculated for each sample using the following formula: $NF = 1/[2^{\text{median spike in Ct value}}] - (\text{spike in average Ct value of the given sample})$. A normalized copy number value was then obtained for each sample by multiplying the number of copies of the given miRNA by the NF. Normalized copies of each miRNA in the quantitative polymerase chain reaction were converted to normalized copies of miRNA per nanogram of cDNA loaded into the reaction mix (total: 0.4 ng per sample) and then to normalized copies of miRNA per milliliter of serum (considering the total amount of RNA isolated from 200 μL of serum).
D. Statistical Analysis

The Mann-Whitney test was used to assess differences in miRNA expression levels between preoperative and postoperative serum samples. The Kruskal Wallis test followed by Dunn’s multiple comparisons test was used when more than 2 groups were compared. P values lower than 0.05 were considered statistically significant. All these analyses were performed with GraphPad Prism software, version 5.0 (GraphPad Software Inc., San Diego, CA). Receiver-operating characteristic (ROC) curves and areas under the ROC curve were analyzed with the p-ROC package in R software, version 3.1.1 [28, 29], using the Youden Index and the DeLong method.

3. Results

A. Screening Analysis

miRNA profiling was performed on preoperative and 30-day postoperative serum samples from the PTC patients of the screening cohort. Of the 754 miRNAs that were quantified, 11 displayed significantly lower levels in the 30-day postoperative samples than in the samples collected right before thyroidectomy (Supplemental Table 1). None of the 754 miRNAs exhibited significantly increased expression after surgery.

B. Validation Analysis

The 6 miRNAs in Supplemental Table 1 whose expression declined most significantly after thyroidectomy (defined arbitrarily as P < 0.02 versus mean preoperative level) were investigated further in the validation cohort. Four additional miRNAs that did not meet this criterion were also included in the validation analysis because their association with PTC is supported by substantial evidence in the literature. They included miR-146b-5p and miR-221-3p, whose circulating levels have been reported by others to decrease significantly after surgery for PTC and whose expression in PTC tissues has proved to be substantially up-regulated with respect to normal thyroid tissue in multiple studies [19, 21, 24, 30], and miR-95-3p and miR-190a-5p, which were identified as circulating thyroid tumor miRNAs in a large, methodologically robust study that included both screening and validation steps [23]. Levels of these 10 miRNAs (miR-146a-5p, miR-28-3p, miR-103a-3p, miR-222-3p, miR-191-5p, miR-24-3p, miR-146b-5p, miR-221-3p, miR-95-3p, and miR-190a-5p) were then analyzed in serum samples from the 44 patients with sporadic PTCs (preoperative and 30-day postoperative), 19 patients with benign nodular disease (preoperative only), and 20 HCs (study-entry sample).

Levels of miR-95-3p and miR-190a-5p were very low in all the serum samples we tested—in many cases, below the detection limits of our assay (Ct values higher than 35). This precluded comparative assessment of their expression in the various sample subgroups. Analysis of data on the remaining 8 miRNAs revealed that all were less abundant in the 30-day postoperative serum samples than in those collected before thyroidectomy (Fig. 1). The most significant decreases were observed for miR-146a-5p, miR-221-3p, and miR-222-3p (P = 0.0007, P < 0.0001, and P < 0.0001, respectively).

All 8 miRNAs—including 3 (miR-146b-5p, miR-221-3p, and miR-222-3p) that are reported overexpressed in PTC tissues [18, 19, 30]—were significantly more abundant in the preoperative sera from the PTC group than in sera from HCs (Fig. 1). None of the 8 miRNAs displayed expression levels in preoperative PTC serum samples that differed significantly from those in the BN group, although the mean preoperative serum level of miR-221-3p in the PTC group appreciably exceeded that found in samples from patients with BN (653,242,460 ± 490,942,066 copies/mL versus 316,148,953 ± 153,115,869 [P = 0.076]).

The 3 miRNAs that decreased most markedly after surgery in the PTC group (i.e., miR-146a-5p, miR-221-3p, and miR-222-3p) were subjected to ROC curve analysis. All 3 displayed...
good accuracy in discriminating between PTC patients and HCs [Fig. 2(A)], but their performances declined substantially in the differentiation of PTCs and BNs [Fig. 2(B)]. In both comparisons, miR-146a-5p and miR-221-3p proved to be more accurate discriminators than miR-222-3p (Fig. 2).

These 2 miRs were therefore selected for further characterization. To this end, we analyzed their expression levels in additional serum samples collected 1 to 2 years after surgery from 20 of the 44 PTC patients (selected on the basis of availability alone). As shown in Fig. 3, at this point in time, 15 of the 20 patients had excellent or indeterminate responses to therapy as defined by 2015 ATA guidelines [1]. In these 15 cases, and in the single patient with a biochemical incomplete response, expression levels of miR-146a-5p and miR-221-3p decreased after surgery and remained low at the 1- to 2-year visit. Trends were very different in the 4 patients with structural incomplete responses at 1 to 2 years. In this subgroup, initial postoperative

Figure 2. ROC curve analyses performed to assess the diagnostic value of serum miR-146a-5p, miR-221-3p, and miR-222-3p levels for discriminating (A) between PTC patients and HCs and (B) between PTC patients and those with BNs. Tables on the right show areas under the curve (AUC) with 95% confidence intervals (95% CI) and cutoffs (indicated on curves as solid black circles and reported as copies per milliliter) with associated sensitivity and specificity.
declines in serum miR-146a-5p and miR-221-3p levels were followed by increases to levels at the 1- to 2-year visit that were similar to or higher than those found prior to surgery. Serum Tg levels measured in these 20 patients at the 30-day and 1- to 2-year visits were consistent with clinical findings and with circulating levels of the 2 miRNAs in all but 3 cases. The first exception (Fig. 3, case 1) was a patient with an indeterminate response consisting of negative cervical ultrasound findings and increasing serum Tg levels, which rose from 0.17 ng/mL (serum thyroid-stimulating hormone, 0.97 mIU/L) at 30 days to 0.95 ng/mL (serum thyroid-stimulating hormone, 0.2 mIU/L) at 1 year. Radioiodine remnant ablation (RRA) had been omitted in this case, and the patient’s Tg antibody assays were also positive but declining overtime (from 61.4 IU/mL to 14.3 IU/mL). More consistent with the negative imaging data was the steady postoperative decline in circulating levels of miR-146a-5p and miR-221-3p. The other 2 exceptions (Fig. 3, cases 2 and 3) were also patients whose thyroidectomies had not been followed by RRA. Both were classified as having structural incomplete responses owing to sonographic findings of lymph node lesions with suspicious features [31]. In case 3, the metastatic nature of the nodal lesions had also been confirmed cytologically. Serum Tg levels in both cases remained consistently below the 1-ng/mL cutoff and were even lower at the 1- to 2-year assessment (0.11 and 0.15 ng/mL) than they had been 30 days after surgery (0.7 and 0.36 ng/mL). Tg antibody titers were low and declining in case 2 and always negative in case 3. In both cases, the rising serum levels of miR-146a-5p and miR-221-3p were more consistent with the presence of structural disease.

Figure 3. Levels of miR-146a, miR-221-3p, and Tg in preoperative and postoperative (30 days and 1 to 2 years after thyroidectomy) serum samples of PTC patients classified according to response-to-therapy classes defined by the ATA. Case 1: Patient with an indeterminate response and increasing serum Tg levels. Cases 2 and 3: Patients with structural incomplete responses and decreasing serum Tg levels that were consistently less than 1 ng/mL.

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4. Discussion

For the growing population of patients with PTC, the 2015 ATA guidelines [1] recommend less extensive surgery, more selective use of RRA, and, in some cases, even active surveillance alone. One “side effect” of this change is a diagnostic gap in our posttreatment surveillance protocols. The reliability of serum Tg assay results declines substantially in patients with residual normal thyroid tissue. Consequently, clinicians are increasingly faced with a need for more accurate tumor biomarkers to monitor these patients for persistent/recurrent disease.

The results of our study suggest that serum miRNA assays are a promising tool for meeting this need. Our screening analysis of expression levels of 754 miRNAs in pre- and postthyroidectomy serum samples from PTC patients pinpointed 11 miRNAs (Supplemental Table 1) whose serum levels dropped significantly after thyroidectomy—a pattern suggestive of miRNAs derived largely from thyroid tissue. In the validation cohort, this list was refined to include 8 miRNAs: In addition to displaying down-regulated expression after thyroidectomy, these miRNAs appeared more likely to be closely associated with thyroid tumorigenesis, because their preoperative serum levels in PTC as well as in BN were significantly higher than those found in HCs (Fig. 1). Moreover, 3 (miR-146b-5p, miR-221-3p, and miR-222-3p) out of 8 miRNAs were previously reported overexpressed in PTC tissues [18, 19, 30]. In our series, expression of none of the 8 miRNAs was significantly different between BN and PTC patients, although the mean preoperative serum level of miR-221-3p in the PTC group was appreciably higher than that found in samples from patients with BN (653,242,460 ± 490,942,066 copies/mL versus 316,148,953 ± 153,115,869 copies/mL). ROC curve analyses revealed that miR-221-3p and miR-146a-5p were the most accurate in discriminating between PTC patients and HCs [Fig. 2(A)], but their performances declined substantially in the differentiation of PTCs and BNs [Fig. 2(B)]. To definitely ascertain the diagnostic role of miRNA, larger cohorts of patients will be needed. The 2 most promising miRNAs—miR-146a-5p and miR-221-3p—were subjected to additional analyses to further evaluate their potential roles in predicting tumor persistence or relapse. We measured their serum levels 1 to 2 years after surgery in a subset of 20 patients classified according to the response to treatment [1]. In the patients in this subset with excellent/indeterminate responses or biochemical incomplete responses, circulating levels of both miRNAs continued to decline between the 30-day and 1- to 2-year observation points. In contrast, in the patients with structural incomplete responses, serum miRNA levels at the 1- to 2-year visit were both higher than those at 30 days (Fig. 3). Serum levels of miR-146a-5p and miR-221-3p were also consistent with serum Tg assay results in all but 3 (15%) of the 20 patients analyzed (Fig. 3). Thus, both miRNA assays accurately predicted the disease status of all patients, and both were better for detecting lymph node metastases than serum Tg assays, which were consistently negative and even revealed decreasing levels between the 30-day and 1- to 2-year observation points. These findings are consistent with reports of late increases in the serum Tg level in certain settings characterized by documented recurrence/persistence [32]. Of note, alterations of miR-146a-5p and miR-221-3p circulating levels were previously reported in patients with cardiovascular diseases, acute cerebral infarction, systemic inflammation, and osteoarthritis [33, 34]. In our series, only 1 patient had a history of ischemic cardiovascular disease, and none had a history of stroke, systemic inflammation, or osteoarthritis; thus we can exclude a role of systemic disorders in affecting miRNA expression levels.

Our study attempts to document the kinetics of circulating miRNA levels in PTC patients during the first 1 to 2 years of follow-up and to analyze these results in light of the patients’ clinical status. The cohorts we studied were admittedly small, and much larger studies are needed to confirm our findings. An important strength of our study, however, is the quality of the protocol we used to assay circulating miRNA levels. To minimize intrinsic interindividual variability related to thyroid-independent factors (e.g., sex, age, individual genetic background, diet, lifestyle, and comorbidities), our initial screening analysis involved comparison of post-thyroidectomy miRNA levels with those found in preoperative samples from the same PTC patient population. Intersample variability can also be introduced by technical factors,
such as sample preparation, the addition of a preamplification step, and normalization strategies [35–38]. We selected the miRNeasy Serum/Plasma kit (Qiagen), which facilitates the detection of low-abundance miRNAs and allows the isolation of more concentrated RNA samples than other kits of this type [37, 39]. In addition, the multiple-extraction approach we adopted for miRNA profiling allowed us to obtain over 1,000 ng total RNA from each serum sample, thereby eliminating the need for cDNA preamplification. Because consensus is lacking on reliable endogenous controls for circulating miRNA analysis [37], we used absolute quantitative polymerase chain reaction for all the analyses carried out in the validation cohort.

Preamplification is an important potential source of technical bias [35, 36], and it might at least partially explain the discrepancy that emerged between our findings regarding miR-95-3p and miR-190a-5p and those of other researchers [23]. Using a protocol that involved cDNA preamplification, Cantara et al. [23] found serum miR-95-3p and miR-190a-5p levels in PTC patients to be significantly different (lower and higher, respectively) from those in HCs. In our study, however, the levels of both miRNAs in all the serum samples were so low (in many cases, below the detection limits of our assay) that they had to be excluded from subsequent analyses. More sensitive techniques are needed to allow reliable quantification of even low-abundance circulating miRNAs without the use of the preamplification step and endogenous controls.

In summary, postoperative changes in circulating levels of miR-146a-5p and miR-221-3p in PTC patients display good correlation with ATA-defined response-to-therapy classes, even in cases in which serum Tg assay results are unreliable or difficult to interpret. Studies are already underway in our laboratory to obtain additional validation for our findings in a larger PTC patient population that includes cases with locoregional spread at diagnosis as well as groups with and without evidence of recurrence/persistence during follow-up. Our current results suggest, however, that serum levels of miR-146a-5p and miR-221-3p might one day be used as complementary biomarkers for the early noninvasive detection of persistent/recurrent PTC, particularly in the expanding population of patients undergoing more conservative treatment of these tumors.

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