MiRNA in Papillary Thyroid Cancer

Subjects: Oncology

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Incidences of thyroid cancer have been increasing worldwide. Between 1992 and 2017, the incidence of TC in the USA increased from 5.7 to 13.3 cases per 100,000 people. Worldwide, nearly 300,000 cases of TC are diagnosed annually, causing nearly 40,000 deaths. Many studies have suggested the importance of miRNA abnormalities during PTC development.

miRNA papillary thyroid cancer

1. The Role of miRNAs in Fine-Needle Aspiration Biopsies

FNAB is the most frequently used diagnostic method, characterized by simplicity, high specificity, a low complication rate, and low cost [1]. However, it also has disadvantages, such as non-diagnostic or abnormal results and undefined significance in describing lesions [2]. In this case, the routine analysis of specific miRNAs would increase the sensitivity and specificity of FNAB when used for PTC diagnoses [3].

Castagna et al. demonstrated that a PTC diagnostic miRNA panel consisting of miR-146b, miR-221, and miR-222 would increase the diagnostic utility of FNAB [3]. The study was conducted on 174 samples obtained during FNABs from 168 patients. Another study showed that miR-181b, in combination with miR-146b, might be useful in differentiating between benign thyroid lesions and PTC lesions [4]. In a study performed on 20 malignant lesion samples and 20 samples containing benign lesions, Chen et al. showed that miR-146b could be a useful PTC-screening biomarker [5]. Santos et al. created a panel consisting of 11 miRNAs, including let-7a, miR-103, miR-125a-5p, let-7b, miR145, RNU48, miR-146b, miR152, miR-155, miR200b, and miR-181, and proved its diagnostic utility for differentiating between undefined changes obtained by FNAB examination [6]. The authors named this test mir-THYpe (miRNA-based thyroid molecular classifier for precision endocrinology). In order to validate this diagnostic procedure, 58 samples from benign tissues and 39 samples from malignant tissues were used. The proposed panel was characterized by 94.6% sensitivity, 81% specificity, a 95.9% positive predictive value, and a 76.1% negative predictive value. These results suggest that the mir-THYpe test is useful for differentiating between lesions of an undefined nature, which may reduce the number of unnecessary surgeries.

In a similar study, Mazeh et al. [7] identified a panel of miRNAs with potential diagnostic utility for differentiating between undefined lesions in FNABs. The research material consisted of 274 samples collected from 102 patients, and the miRNA expression levels were examined using Next Generation Sequencing (NGS). The Panel consisted of 19 miRNAs: miR-146b, miRNA-146, miR-222, miR-221, miR-134, miR-34a, miR-101, miR-143, miR-144, miR-615, miR-375, miR-181b, miR-194, miR-130a, miR-199a-3p, miR-30a, miR-424, miR-148a, and miR-24. Its

diagnostic usefulness was proved by its 91% sensitivity and 100% specificity, and the positive and negative predictive values were estimated at 94% and 100%, respectively. The limitations of the study included the analysis of ex vivo tissues, the selective use of malignant PTC tissues, and the coexistence of other thyroid diseases among the studied patients, which may have interfered with the obtained results.

In a subsequent study, Labourier et al. combined DNA, mRNA, and miRNA analyses into a specific PTC diagnostic panel [8]. The research was performed on 638 samples obtained during FNABs. Samples were evaluated to detect the presence of 17 genes and 10 miRNAs: miR-29b-1-5p, miR-31-5p, miR-138-1-3p, miR-139-6p, miR-146b-5p, miR-155, miR-204-5p, miR-222-3p, miR-375, and miR-551b-3p. The authors demonstrated that the effectiveness of molecular analysis was increased when genetic and miRNA tests were combined. The diagnostic usefulness of this panel was proved by its sensitivity and specificity, which were 89% and 85%, respectively.

The cited studies indicate that miRNA evaluations have a promising role in PTC diagnoses when combined with FNAB. It is important to underline that malignant tissues could also be differentiated from benign thyroid lesions using PTC miRNA diagnostic panels. Accordingly, a specific miRNA panel would increase both the sensitivity and specificity of FNAB, decreasing the number of undiagnostic results, and relatedly, the number of unnecessary surgeries. However, these studies are still considered preliminary. Further comparison with results obtained in groups with other thyroid malignancies and thyroid comorbidities, which may have an important impact on the isolated panel of miRNAs and subsequent diagnoses, should be performed.

2. PTC Screening Utility of Selected Plasma and Serum miRNAs

miRNAs can also be efficiently isolated from plasma and serum, and a specific miRNA can be investigated for potential PTC-screening utility. In a study performed by Wang et al., a panel consisting of three miRNAs isolated from plasma—miR-346, miR-34a-5p, and miR-10a-5p—was proposed as a useful tool for PTC screening [9]. The study was conducted on 30 samples obtained from PTC patients and 30 samples collected from healthy volunteers. The area under the ROC curve (AUC) of these three-miRNA panels was calculated at 0.816, which proved its great screening utility. Moreover, this study identified three miRNAs that were consistently upregulated in the exosomes obtained from PTC-patient plasma.

Another study performed by Liang et al. proposed two combined, plasma-isolated miRNA screening panels. The first consisted of two miRNAs: miR-16-2-3p and miR-223-5p; the second consisted of six miRNAs: miR-16-2-3p, miR34c-5p, miR223 -3p, miR223-5p, miR182-5p, and miR146b-5 [10]. The study included 24 patients during the testing phase and 91 patients during validation. This study revealed that a panel consisting of miR-223-5p, miR-34c-5p, miR101-3p, and miR-16-2-3 may be particularly useful in differentiating between malignant and benign lesions. The AUC was estimated at 0.735, with 71.43% sensitivity and 73.33% specificity.

Dai et al. analyzed the plasma of 119 PTC patients, 51 healthy subjects, and 82 patients with benign thyroid nodules. The study showed the potential PTC-screening utility of a panel consisting of miR-485-3p and miR-4433a-

5p [11]. Additionally, it has been shown that the level of miR-485-3p expression could be considered as a prognostic marker, differentiating low-risk cancer from high-risk cancer. Another study performed by Li et al. confirmed the diagnostic usefulness of these measurements, demonstrating 92.8% sensitivity and 88.9% specificity [12]. The study sample was comprised of 56 patients with PTC and 95 patients with benign thyroid nodules. The control group consisted of 10 healthy volunteers, which was a notable limitation of this research.

Many authors have emphasized the potential measurement of plasma miR-222 and miR-146b levels in the PTC screening [13][14][15]. Kondrotiene et al. analyzed the plasma levels of five miRNAs—miR-221, miR-222, miR-146b, miR-21, and miR-181b—of which miR-222 had the highest screening utility. The study included 49 patients with PTC, 23 patients with benign thyroid nodules, and 57 healthy individuals. The study showed the significant overexpression of miR-221, miR-222, miR-146b, miR-21, and miR-181b [16].

Furthermore, the study performed by Perdas et al. suggested that the screening panel, consisting of four miRNAs, such as let-7a, let-7c, let-7d, and let-7f, whose levels were elevated in plasma, have a higher PTC screening utility [17]. Accordingly, Ricarte-Filho et al. showed that the let-7 family affects growth and differentiation of PTCs. In particular, let-7f might attenuate a neoplastic process of RET/PTC papillary thyroid oncogenesis through impairment of MAPK signaling pathway activation [18]. **Table 3** shows plasma-delivered downregulated and overregulated miRNAs than may be considered for PTC screening (**Table 3**).

Table 3. Novel potential screening biomarkers determined by miRNA profiling.

Overexpressed miRNA	Underexpressed miRNA	Origin of Samples	Reference
miR-221, miR-222, miR-146b, miR-21 and miR-181b		Plasma	[16]
miR-346, miR-34a-5p, miR-10a-5p		Plasma and tissues	[18]
miR-16-2-3p, miR-223-5p	miR-34c-5p, miR-101-3p, miR-381-3p	Plasma	[10]
let-7a, let-7c, let-7d, let-7f		Plasma	[17]

Due to the rapid development of promising miRNA evaluation methods, the clinical effectiveness of PTC screening could be improved. These measurement methods are characterized by high sensitivity, specificity, and reproducibility. Due to of differences in the types of miRNAs reported by different authors and the relatively small number of samples and difficulties in validating the tests, there is still a need for further investigations of the PTC screening utility of miRNAs. Due to the increasing number of thyroid lesions found on ultrasound, the use of miRNA as a biomarker of PTC may help to accelerate diagnosis and treatment of PTC patients. However, additional plasma/serum measurement of miRNAs would be a practical, noninvasive method for screening and for follow-up observations after thyroidectomy.

3. The Importance of miRNAs in the Prognosis of the Course of Papillary Thyroid Cancer

Despite a good prognosis, the frequency of PTC recurrence is estimated at 20% [19]. Many studies indicate the potential importance of miRNAs in the prognostic assessment of PTC. In the study performed by Chen-Kai Chou et al., it was shown that the overexpression of miR-146b was associated with a significant deterioration of overall survival rates. Moreover, the overexpression of miR-146b was further correlated with an increased percentage of nodal metastases and tumor invasiveness [20]. Furthermore, the polymorphism of miR-146a-3p among patients with an increased mortality rate was observed [21]. In this study, the HR of death (after adjustments for age) was 6.21 (95% CI, 1.38-27.93; $p = 0.006$).

Moreover, miR-221 and miR-222 dysregulation was observed to be more common in patients with PTC who were also diagnosed with distant metastases [22]. The study performed by Lei et al. included 78 patients diagnosed with PTC as the study group, which was subsequently divided into two subgroups: the first group consisted of 54 patients diagnosed with relapses; the second group consisted of 24 patients with no cancer recurrence. The authors identified miR-221 as a potential biomarker for PTC relapse [23]. A study performed by Pamedityde et al. on 400 PTC tissue samples obtained from paraffin blocks showed that the overexpression of five miRNAs—miR-146b, miR-222, miR-21, miR-221, and miR-181b—occurred more frequently in recurrent PTC [24].

Certainly, in a meta-analysis of 18 studies concerned on the role of miRNA in PTC screening, Silaghi et al. showed that miR-146b, miR-221, and miR-222 could be considered as potential screening/prognostic biomarkers of recurrent TC, and are particularly useful when referred to PTC [25]. The authors of these studies underlined the beneficial prognostic role of miRNAs in PTC screening, diagnosis and prognosis.

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