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# Differential expression profiling of circulation microRNAs in PTC patients with non-$^{131}$I and $^{131}$I-avid lungs metastases: a pilot study☆

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ABSTRACT

*Introduction:* Loss of the ability to concentrate $^{131}$I is one of the important causes of radioiodine-refractory disease in papillary thyroid carcinoma (PTC). Recent advantages of serum microRNAs (miRNAs) open a new realm of possibilities for noninvasive diagnosis and prognosis of many cancers. The aim of the current study was to identify differential expression profiling of circulation miRNAs in PTC patients with non-$^{131}$I and $^{131}$I-avid lungs metastases.
*Methods:* The expressions of miRNAs were examined using miRNA microarray chip. The most significantly changed miRNAs from microarray were verified by using qRT-PCR. The potential miRNAs regulating target genes and their preliminary biological functions were forecasted by Bioinformatic analysis.
*Results:* Compared to $^{131}$I-avid lung metastases, 13 kinds of significantly differential serum miRNAs including 5 upregulated miRNAs (miR-1249, miR-106a, miR-503, miR-34c-5p, miR-1281) and 8 downregulated miRNAs (miR-1915, miR-2861, miR-3196, miR-500, miR-572, miR-33b, miR-554, miR-18a) in PTC patients with non-$^{131}$I-avid lung metastases were identified. Bioinformatic analysis demonstrated that miR-106a was the core miRNA regulating 193 genes in the network. The results of validation confirmed the up-regulation of miR-106a in non-$^{131}$I-avid lungs metastatic PTC patients.
*Conclusion:* Differentially expressed serum miRNA profiles between PTC patients with non-$^{131}$I and $^{131}$I-avid lungs metastases were analyzed. These findings in our present study could represent new clues for the diagnostic and therapeutic strategy in PTC patients with non-$^{131}$I-avid metastatic disease.

## 1. Introduction

Thyroid cancer is the most rapidly increasing cancer, and it is estimated that there are 600,360 individuals living with a previous thyroid cancer diagnosis in the United States, and an additional 62,980 cases will be diagnosed in 2014 [1]. Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer and account for 70–90 % of differentiated thyroid carcinomas (DTC). Its incidence has increased dramatically, by 189% over the past 30 years in industrialized countries [2]. Although most patients have a good prognosis, with 93% survival at 10-year follow-up, there is a wide range of aggressiveness, and some PTC progress to radioiodine-refractory [3,4].

Lung metastasis, the most common site of distant metastasis of DTC, is associated with a relatively poor prognosis [5], and it has been estimated that 30% of DTC patients with recurrent disease or metastases shows dedifferentiation of malignant cells [6]. Without $^{131}$I uptake, there is no obvious benefit from radioiodine therapy for those with non-$^{131}$I-avid tumors [5]. Patients with non-$^{131}$I-avid lung metastases subjected to unnecessary $^{131}$I treatment should be avoided the risk of high serum TSH stimulation after thyroxin withdrawal or injection of rhTSH. Thus, to guide $^{131}$I therapy, improve long-term survival, and reduce side effects, it is critical to screen for the $^{131}$I uptake ability of lung metastases before $^{131}$I therapy. Loss of ability to concentrate radioiodine in metastatic sites from DTC (one common kind of radioiodine refractory DTC) is most likely owning to less differentiated types of transformation (dedifferentiation) [7]. This creates a major obstacle in radioiodine treatment.

MicroRNAs (miRNAs) are an abundant class of short, noncoding RNAs ranging approximately from 17–25 nucleotides, which contain a seed sequence for binding to the 3'-untranslated region of specific target mRNAs to regulate biological events including individual development, apoptosis, proliferation, and differentiation [8]. The differential expression of miRNAs might have potential clinical value as biomarkers or treatment targets in the early diagnosis, treatment, and prognosis of

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☆ Advances in knowledge and implications for patient care: In the current study, the authors attempt to come up with a potential serum biomarker for the distinction between thyroid cancer patients whose lungs metastases are $^{131}$I-avid or not. In this research, 13 kinds of significantly differential serum miRNAs including 5 upregulated miRNAs (miR-1249, miR-106a, miR-503, miR-34c-5p, miR-1281) and 8 downregulated miRNAs (miR-1915, miR-2861, miR-3196, miR-500, miR-572, miR-33b, miR-554, miR-18a) in PTC patients with non-$^{131}$ I-avid lungs metastases were identified. These findings are very important because at present, the primary approach to identify the ability of concentration of $^{131}$I in distant metastasis depends on the results of posttherapy $^{131}$I whole-body scan. Patients with non-$^{131}$I-avid lungs metastases subjected to unnecessary $^{131}$I treatment should be avoided. These findings in our present study could represent new clues for the diagnostic and therapeutic strategy in PTC patients with non-$^{131}$I-avid metastatic disease.

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many carcinomas. Circulating miRNAs, known as stable cell-free miRNAs in serum or plasma, are passively and selectively released to blood by various cells, and may act as transmitter or messenger in cell communication. During disease, aberrantly expressed miRNAs in the diseased cells are released into the circulation, and the circulating miRNA profile is endued with the disease properties [9].

Recently, several authors [10–12] have reported that circulating miRNAs may have potential utility as diagnostic or prognostic markers for thyroid cancers. But to the best of our knowledge, circulating miRNA profiles between non-$^{131}$I-avid and $^{131}$I-avid lung metastases form papillary thyroid carcinoma have not been analyzed.

In the current study, we aimed to detect and identify differentially expressed serum miRNAs between PTC patients with non-$^{131}$I-avid lung metastases and those with $^{131}$I-avid lung metastases.

## 2. Materials and method

### 2.1. Patients and serum samples

Venous blood samples were obtained from PTC patients just before $^{131}$I therapy at the Nuclear Medicine Department of Shanghai Jiao Tong University affiliated Sixth People's Hospital, a major $^{131}$I treatment center in China. The study was approved by the institutional ethics review board of our hospital, and informed consent was obtained from each patient.

The patients included in the study were divided into two age- and sex-matched groups: a non-$^{131}$I-avid lungs metastases group (group A) and a $^{131}$I-avid lungs metastases group (group B). Inclusion criteria: (1) pathology confirmed PTC in the primary tumor, (2) prior total/near total thyroidectomy without a history of $^{131}$I therapy, (3) without any other distant metastases except to lungs, (4) a negative history of other tumors, (5) lymph node metastasis was not diagnosed both in post-therapeutic $^{131}$I scintigraphy and neck ultrasonography. The diagnosis of lung metastases was made on the basis of the presence of diffuse bilateral pulmonary nodules confirmed by a computed tomography scan with significantly increased serum thyroglobulin (Tg) levels. Exclusion criteria: (1) other metastatic sites like lymph node, bone and other soft tissue were diagnosed, (2) a positive history of other tumors, (3) other subtypes of DTC.

The post-therapeutic $^{131}$I whole-body scan (RxWBS) was performed 3–5 days after the administration of $^{131}$I at a dosage of 150–200 mCi. Patients showing no $^{131}$I uptake (defined as a level of $^{131}$I uptake in the lung that was similar to that in the upper limbs) were placed in group A. Conversely, patients with diffuse $^{131}$I uptake were placed in group B. 5 mL of venous blood from each participant was centrifuged at 4,000 rpm for 10 min at 4 °C within 2 hr of collection, followed by a second centrifugation at 12,000 rpm for 15 min at 4 °C to eliminate any residual cell debris. The supernatant serum was then stored at −80 °C until use. Serum samples from 3 paired patients (clinical characteristics matched) selected in group A and group B (subgroup A-1 and B-1) were subjected to MicroRNA microarrays to identify miRNAs that were significantly differentially expressed.

### 2.2. MicroRNA microarrays chip analysis

Total RNA was extracted and purified using the miRNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. miRNA expression profiling microarray was completed by using GeneChip miRNA 2.0 (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions.

### 2.3. Bioinformatic analysis

Potential target genes prediction was performed by searching against the miRBase 17.0 to identify the known mature miRNAs. Gene ontology (GO) analysis was applied to analyze the main function of the differential expression genes according to the gene ontology which is the key functional classification of NCBI. Similarly, pathway analysis was used to find out the significant pathway of the differential genes according to Kyoto Encyclopedia of Genes and Genomes (KEGG), Biocarta and Reatome. The relationship of the MicroRNA and genes were counted by their differential expression values, and according to the interactions of MicroRNA and genes in Sanger MicroRNA database to build the MicroRNA-Gene-Network.

### 2.4. Validation

To validate the differentially expressed, the candidates were tested with qRT-PCR in an independent cohort from the left patients in group A and group B (subgroup A-2 and B-2).

Total RNA was extracted and purified using the miRNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, and stored at −80 °C. RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, K1622) was used for reverse transcription. qRT-PCR was performed in the ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) using the SYBR Green RT-PCR kit (Qiagen, Germany). All values were normalized using an internal reference (U6, for miRNAs).

### 2.5. Statistics analysis

Two class Dif-Two Factor RVM was used to screen of miRNA differences between A-1 and B-1 ($P < 0.05$, fold-change $\geq 1.1$ or $\leq 0.9$ were considered a statistically significant difference). Fisher's exact test and $\chi^2$ test were used to GO analysis and pathway analysis, and the false discovery rate (FDR) was calculated to correct the P-value (a $P < 0.001$ was considered a statistically significant difference). Comparisons of continuous variables between two groups were performed using the Student's t test while categorical variables were performed using the chi-square test. (a $P < 0.05$ was considered a statistically significant difference.)

## 3. Results

Thirty three PTC patients with non-$^{131}$I-avid lungs metastases (group A) and 33 PTC patients with $^{131}$I-avid lungs metastases (group B) were identified between January 2007 and July 2010. Demographic and clinical features of the two groups are summarized in Table 1. The two groups had no significant differences in parameters such as age or gender.

**Table 1**
Clinical and demographic features of group A and group B in this study.

| Clinical characteristics | Groups | | P value |
|---|---|---|---|
| | A (N = 33) | B (N = 33) | |
| Age/years | | | 0.42 |
| Median (range) | 42 (30–55) | 41 (29–53) | |
| Gender | | | 0.62 |
| Male (%) | 13 (39.4) | 15 (45.5) | |
| Female (%) | 20 (60.6) | 18 (54.5) | |
| Tg level (ng/ml) | | | |
| Median (range) | 1613.54 (265.8–3070.3) | 1751.50 (351.6–3151.4) | 0.79 |
| TgAb level (IU/ml) | | | 0.67 |
| Median (range) | 48.34 (10–150.8) | 47.53 (10–120.9) | |
| Histological diagnosis | PTC | PTC | – |
| Prior $^{131}$I treatment | NO | NO | – |
| Chest CT scan | Diffuse small nodules | Diffuse small nodules | – |
| $^{131}$I up take in lungs | Close to upper limbs | Diffuse lung uptake | – |
| Extent of disease | No other metastasis | No other metastasis | – |
| Other cancer history | No | No | – |