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Original Article

Downregulation of serum miR-26a predicts poor clinical outcome of papillary thyroid carcinoma

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Abstract: Deregulation of miRNAs has been demonstrated to play an important role in the initiation and development of many types of cancers including papillary thyroid carcinoma (PTC). Currently the role of miR-26a and its potential clinical significance for PTC remains unknown. Eighty-four PTC patients and forty healthy controls were enrolled in this study. Quantitative real-time PCR (qRT-PCR) was performed to determine the expression level of serum miR-26a in all the participants. The association between serum miR-26a expression level and clinical outcome of PTC was investigated. Serum miR-26a expression level was reduced in PTC patients compared with the healthy controls (P<0.01) and significantly increased after receiving treatments (P<0.01). Serum miR-26a expression level was associated with lymph node metastasis (P=0.0173) and TNM stage (P=0.0022). In addition, PTC with lower serum miR-26a expression had poorer overall (P=0.029) and disease free survival rates (P=0.0004). Moreover, serum miR-26a expression was an independent risk factor for PTC (P<0.05). Collectively, down-regulation of serum miR-26a is associated with poor prognosis in PTC and it might be a promising biomarker for this malignancy.

Keywords: Papillary thyroid carcinoma, prognosis, serum miR-26a

Introduction

Thyroid cancers, the most common type of endocrine malignancy, have rapidly increased global prevalence [1]. Papillary thyroid carcinoma (PTC) accounts for 80-90% of thyroid carcinomas. Despite PTC is relatively indolent and highly curable in most cases, more than 10% of patients suffered from disease recurrence and PTC-related deaths [2]. Identifying the biomarkers that can predict the prognosis of PTC might not only allow a personalized and differentiated treatment, but also help monitor the therapy responses in real time.

MicroRNAs (miRNAs) are small (19-25 nucleotides) noncoding, single-stranded RNAs that regulate gene expression at the post-transcriptional level [3]. miRNAs play important roles in many biological processes including cell growth, differentiation, survival and apoptosis [4]. The aberrant expression of miRNAs has been implicated in many diseases such as cancer, cardiovascular diseases and autoimmune disorders [5]. Circulating miRNAs are very stable in serum and plasma samples, which enables them to become promising biomarkers for early detection as well as predicting the clinical outcome of diseases. The expression levels of miR-9 and miR-21 were significantly downregulated in PTC patients with recurrence compared to those without recurrence. In addition, miR-9 and miR-21 expression levels were associated with clinical parameters of PTC and independent prognostic factors for this malignancy [6]. Sun et al showed that the expression levels of miR-146a and miR-146b were significantly upregulated in PTC tissues in comparison with the nodular goiter tissues and perineoplastic thyroid tissues. They also found that increased miR-146a and miR-146b expression were associated with worse clinicopathologic features [7]. Liu et al showed that

Table 1. Association between serum miR-26a level and the clinical features of PTC

Parameters	No. of patients	Serum miR-26a expression		*p*
		Low	High	
Gender				
Male	32	14	18	0.6994
Female	52	25	27	
Age				
<50	38	17	21	0.7775
≥50	46	22	24	
Bilaterality				
Unilateral	51	26	25	0.2984
Bilateral	33	13	20	
Multifocality				
Solitary	44	21	23	0.8023
Multiple	40	18	22	
Tumor size (cm)				
<1	45	17	28	0.0877
≥1	39	22	17	
Lymph node metastasis				
No	62	24	38	0.0173
Yes	22	15	7	
Extrathyroidal extension				
No	57	24	33	0.2483
Yes	27	15	12	
TNM stage				
I-II	59	21	38	0.0022
III-IV	25	18	7	

ectopic expression of miR-204-5p could suppress PTC cell proliferation and induce cell cycle arrest and apoptosis, indicating miR-204-5p functions as a tumor suppressor during the progression of PTC [8].

miR-26a has been demonstrated to play important roles in the initiation and development of various types of cancers including PTC [9-11]. However, whether the expression level of serum miR-26a is downregulated in PTC patients and its potential clinical significance is poorly known. Therefore the purpose of this study was to reveal the clinical value of serum miR-26a for predicting the prognosis of PTC.

Materials and methods

Patients

The study was approved by the Ethic Committee of the Ninth People's Hospital of Shenzhen city and the written informed consent was obtained from all the participants. A total of 84 patients diagnosed with PTC who underwent surgery were enrolled in the study. The clinicopathological parameters of PTC such as age, gender, tumor size, multifocality, extrathyroidal extension, and lymph node metastasis were summarized in **Table 1**. Pathological staging was determined based on the TNM classification system of the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC).

Sample collection and storage

Serum samples were obtained from healthy controls and patients with PTC before receiving any types of treatments. Up to 5 mL of fasting venous blood were withdrawn from all participants. All samples were processed within 1 h after collection and separated by centrifugation at 3000 rpm for 15 min. The separated serum was divided into aliquots and cryopreserved at -80°C until use.

Quantitative real-time RT-PCR

Total RNA was isolated from serum by miR Vana PARIS kit (Life Technologies, Grand Island, NY, USA) according to the manufacturer's instructions. Reverse transcription was performed with the miRcute miRNA First-Strand cDNA Synthesis Kit (Tiangen Biotech, Beijing, China). Amplification of PCR products was quantified using Fast Start SYBR Green Master (Roche Applied Science, Indianapolis, IN, USA) on a Roche LC480 Light cycler (Roche). All PCR reactions were run in triplicate and the miR-26a expression value was expressed relative to that of U6 using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Two-tail paired students' t-test was used to compare the gene expression level of serum miR-26a between PTC patients and healthy volunteers. Chi-squared test was performed to evaluate the correlation between serum miR-26a and clinicopathological parameters of PTC patients. Survival curves were constructed with the Kaplan-Meier method and compared using log-rank test. Cox proportional hazards regression analysis was used for multivariate analyses of prognostic factors.

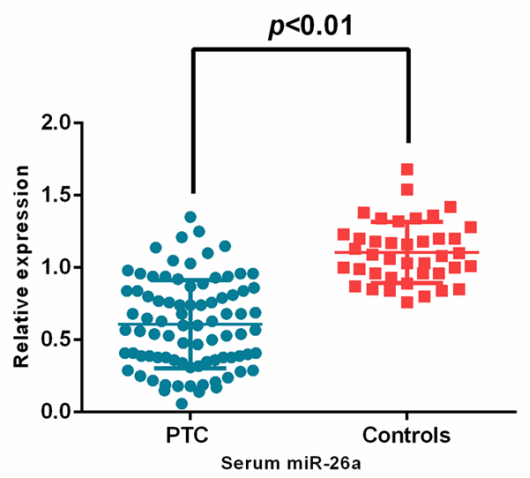

Figure 1. Expression level of serum miR-26a in PTC patients and controls.

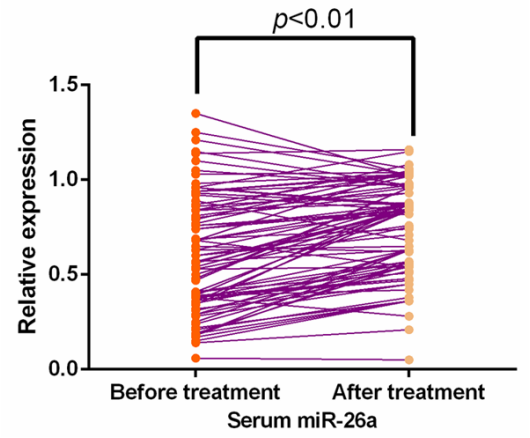

Figure 2. The expression level serum miR-26a in PTC patients after receiving treatments.

All Statistical analyses were performed using the SPSS 21.0 (SPSS Inc., Chicago, IL) and $P<0.05$ was considered to be statistically significant.

Results

Serum miR-26a expression in PTC patients

The qRT-PCR results showed that the expression level of serum miR-26a was significantly downregulated in patients with PTC in comparison with the healthy controls ($P<0.01$) (**Figure 1**). In addition, serum miR-26a level in PTC patients was remarkably increased after receiving treatments ($P<0.01$) (**Figure 2**).

Association between serum miR-26a and clinical features of PTC

Serum miR-26a expression level was associated with various clinicopathological parameters including lymph node metastasis ($P=0.0173$) and TNM stage ($P=0.0022$). However, it was not correlated with gender, age, bilaterality, multifocality, tumor size and extrathyroidal extension ($P>0.05$) (**Table 1**).

Low serum miR-26a expression was correlated with poorer survival rates

The mean value of serum miR-26a was used to divide all the PTC patients into two groups (high serum miR-26a expression group and low serum miR-26a expression group). The PTC patients in high serum miR-26a expression group had a better overall survival (OS) rate ($P=0.029$) and disease free survival (DFS) ($P=0.0004$) than people in the low serum miR-26a expression group (**Figures 3** and **4**).

Serum miR-26a was an independent prognosis factor for PTC

The multivariate analysis showed that TNM stage (OS: HR=4.358, 95% CI=1.747-7.532, $P=0.008$; DFS: HR=4.587, 95% CI=1.823-7.930, $P=0.005$) and serum miR-26a expression (OS: HR=2.528, 95% CI=1.128-4.068, $P=0.026$; DFS: HR=2.810, 95% CI=1.275-4.328, $P=0.021$) were independent risk factors for PTC (**Table 2**).

Discussion

In this study, our result showed that the expression level of serum miR-26a was significantly downregulated in PTC patients and it was very sensitive to monitor therapeutic responses. In addition, reduced serum miR-26a expression was associated worse clinicopathological parameters and shorter overall/disease free survival. Moreover, serum miR-26a was demonstrated to be an independent prognosis factor for PTC. miR-26a might function as a tumor suppressor in PTC and loss of miR-26a promotes the development of this malignancy. Consistent with our findings, Lv et al reported that the expression level of miR-26a was significantly decreased in the tissue samples derived from PTC patients compared with the controls. In addition, ectopic

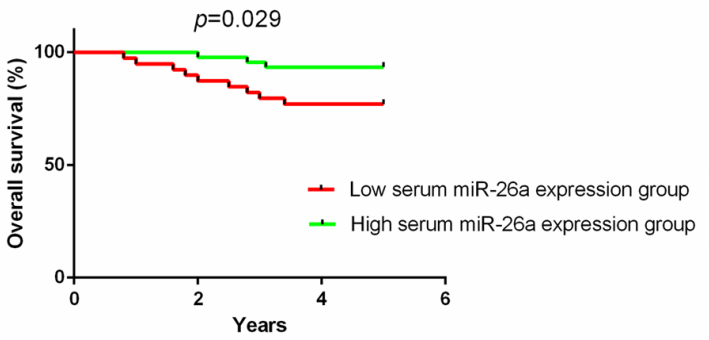

Figure 3. Association between serum miR-26a expression level and overall survival.

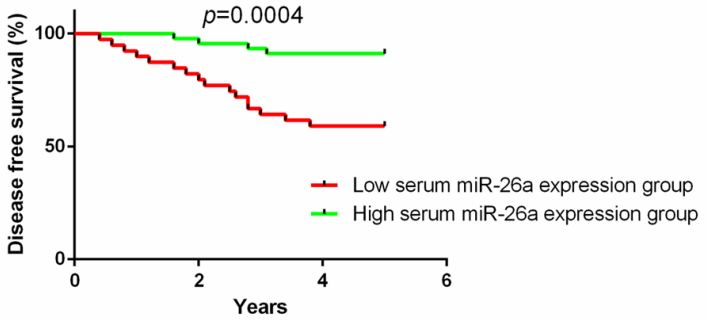

Figure 4. The association between serum miR-26a expression level and disease free survival.

expression of miR-26a could induce G_2 phase-arrest in PTC cell lines and opposite results were observed following miR-26a inhibition. Moreover, CKS2 was identified as a downstream target of miR-26a [9]. Similarly, Visone et al showed that a significant decrease in tissue miR-26a expression was found in anaplastic thyroid carcinoma which was an aggressive form of cancer of the thyroid gland. Overexpression of miR-26a could significantly suppress cell proliferation, suggesting miR-26a played a tumor suppressive role in PTC [12]. However, the molecular mechanisms responsible for the reduced miR-26a expression in PTC are poorly known. In addition, due to the relative small sample size in this study, large scale cohort studies are needed to perform to further corroborate the clinical significance of tissue/serum miR-26a in predicting the prognosis of PTC.

Downregulation of miR-26a has also been reported in many types of cancers. Liu et al showed that the expression level of tissue miR-26a was downregulated in triple-negative breast cancer (TNBC), and its expression levels were correlated with lymph node metastasis and overall survival. In addition, upregulation of miR-26a inhibited proliferation and metastasis of breast cancer cell lines both *in vitro* and *in vivo* by downregulating MTDH [13]. miR-26a expression was reduced in prostate cancer and cell lines. Overexpression of miR-26a inhibited cell proliferation, metastasis, and epithelial mesenchymal transition and induced G_1 phase arrest in prostate cancer. Moreover, Wnt5a was demonstrated to be a target of miR-26a [14].

Some miRNAs may function as oncogenes or tumor suppressors in different types of cancer or even in the same type of cancer [15-17]. The role of miR-26a in carcinogenesis appears to be very complicated, in the sense that it has also been suggested to play an oncogenic role in cancers. Huse et al showed that miR-26a DNA is frequently amplified in human glioma. In addition, overexpression of miR-26a could enhance *de novo* tumor formation *in vivo* by targeting PTEN, which was a validated tumor suppressor [18]. Chen et al reported that the expression level of miR-26a was increased in colorectal cancer (CRC) cells, especially in CRC cells with high metastasis capacity. Moreover, miR-26a played an important role in regulating glucose metabolism by direct suppressing PDHX in CRC cells, which inhibited the conversion of pyruvate to acetyl CoA in tricarboxylic acid cycle [19]. Therefore, the controversial properties of miR-26a in various cancers indicate that the concrete functions of miR-26a are cell type dependent and closely correlated with the tumor environment. It is also possible that miR-26a has distinct functions in different stages of cancer pathogenesis and progression, while the underlying mechanisms need further validation.

Taken together, our results provide the convincing evidence that miR-26a might involve in the

Table 2. Multivariate analysis of 5-year overall and disease-free survival in patients with PTC

Parameters	Overall survival			Disease-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
TNM stage (III-IV vs. I-II)	4.358	1.747-7.532	0.008	4.587	1.823-7.930	0.005
Serum miR-26a (Low vs. High)	2.528	1.128-4.068	0.026	2.810	1.275-4.328	0.021

progression and recurrence of PTC. More importantly, the serum level of miR-26a may be a noninvasive and novel prognostic biomarker for the patients with PTC.

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Disclosure of conflict of interest

None.

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