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Original Article

Tissue microRNA-182 expression level and its potential prognostic value for papillary thyroid carcinoma

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Abstract: Background: In previous study, qRT-PCR analysis revealed significantly higher miR-182 levels in papillary thyroid carcinoma (PTC) than matched normal tissues. However, the clinical significance and prognostic value of miR-182 have not been investigated in PTC until now. Methods: 151 pairs of PTC and adjacent normal thyroid tissues were obtained from Affiliated Hospital of Weifang Medical University from February 2008 to January 2015. The Chi square test was used to analyze the relationship between miR-182 expression and the clinicopathological characteristics. We used the Kaplan-Meier method and the log-rank test in univariate survival analysis, and we used the Cox proportional hazards regression model in our multivariate analysis. Results: The relative expression of miR-182 in PTC samples was significantly higher than that of matched normal tissues ($P<0.001$). The high expression level of tissue miR-182 was statistically correlated with extrathyroidal invasion ($P=0.009$), cervical lymphnode metastasis ($P=0.015$), and TNM staging ($P=0.001$). The Kaplan-Meier method revealed that higher miR-182 expression level was correlated with significantly reduced overall survival. Furthermore, multivariate survival analysis revealed that miR-182 expression level (HR=2.882, 95% CI: 1.289-10.928, $P=0.013$) was significantly correlated with the poor prognosis of PTC patients. Conclusions: Overexpression of miR-182 is associated with aggressive clinicopathologic characteristics of PTC, and miR-182 might be a novel prognostic molecular marker of PTC.

Keywords: MicroRNA-182, prognostic value, clinical significance, papillary thyroid carcinoma

Introduction

Papillary thyroid carcinoma (PTC) is a relatively indolent disease with low mortality. However, patients with cervical lymph node metastases, which frequently occur in PTC, might have a poor prognosis [1, 2]. Identification of novel molecular markers which can improve diagnosis and prognostic stratification and serve as possible therapeutic targets will be of great importance in the near future.

MicroRNAs (miRNAs) are small non-coding nucleotides which post-transcriptionally control the stability and translation of mRNAs. Today, we know more than 1500 different miRNAs, and each miRNA can regulate several genes [3]. The aberrant expression of miRNAs has been identified in many diseases including tumors, and its expression profiles are different among different types of tumor [4].

Dysregulated expression of microRNA-182 (miR-182) has been reported in a number of cancers, and it plays important roles in cancer tumorigenesis and progression and exert different effects in various types of cancer [5-14]. Previously, Zhu et al analyzed the expression of miR-182 in PTC specimens and adjacent normal thyroid tissues. qRT-PCR analysis revealed significantly higher miR-182 levels in PTC than matched normal tissues. Furthermore, miR-182 targeted CHL1 and controlled tumor growth and invasion in PTC. The results collectively support an oncogenic role of miR-182 in PTC cell proliferation and invasion through downregulation of CHL1 expression [15]. However, the clinical significance and prognostic value of

Table 1. The relationships between miR-182 expression and clinicopathologic features in 151 patients with PTC

		High miR-182 level		Low miR-182 level		
Variables	Number of patients	N	%	N	%	*P* value
Age (years)						
≥50	65	36	55.38	29	44.62	0.412
<50	86	41	47.67	45	52.33	
Gender						
Male	67	31	46.27	36	53.73	0.329
Female	84	46	54.76	38	45.24	
Tumor size (cm)						
≥1	91	51	56.04	40	43.96	0.138
<1	60	26	43.33	34	56.67	
Extrathyroidal invasion						
Yes	51	34	66.67	17	33.33	0.009
No	100	43	43.00	57	57.00	
Cervical lymphnode metastasis						
Yes	47	31	65.96	16	34.04	0.015
No	104	46	44.23	58	55.77	
TNM staging						
I and II	79	30	37.97	49	62.03	0.001
III and IV	72	47	65.28	25	34.72	

miR-182 have not been investigated in PTC until now.

Methods

Patients and samples

Our study was approved by the Ethics Committee of Affiliated Hospital of Weifang Medical University. Written informed consent was obtained from all subjects. 151 pairs of PTC and adjacent normal thyroid tissues were obtained from Affiliated Hospital of Weifang Medical University from February 2008 to January 2015. All patients had no history of neck irradiation and underwent preoperative US and US-guided fine-needle aspiration biology (FNAB) whose diagnosis was PTC. Tissues were immediately stored at -80°C. Final histological classifications and findings were made by two pathologists according to the 2002 edition AJCC pathological classification of thyroid tumors. Clinicopathologic data were available, including tumor size, extrathyroid invasion, node metastasis, multifocality and disease stage. Details of the clinicopathologic features of the PTCs in this study are presented in **Table 1**.

RNA extraction and quantitative RT-PCR

Total RNA, including miRNA, was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was synthesized from 5 ng of total RNA by using the Taqman miRNA reverse transcription kit (Applied Biosystems, Foster City, CA), and the expression levels of miR-182 were quantified by using miRNA-specific TaqMan MiRNA Assay Kit (Applied Biosystems). Real-time RT-PCR was performed by using the Applied Biosystems 7500 Sequence Detection system. The expression of miRNA was defined based on the threshold cycle (Ct), and relative expression levels were calculated as $2^{-[(\text{Ct of miR-182}) - (\text{Ct of U6})]}$ after normalization with reference to expression of U6 small nuclear RNA.

Statistical analysis

The expression levels of miR-182 among different groups were compared by nonparametric Mann-Whitney U test or Kruskal-Wallis test, as appropriate. The Chi square test was used to analyze the relationship between miR-182 expression and the clinicopathological characteristics. We used the Kaplan-Meier method

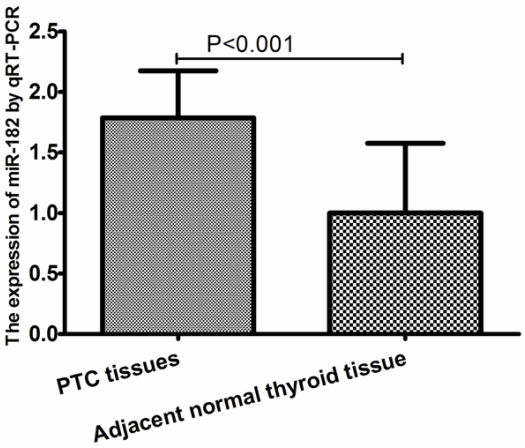

Figure 1. The tissue expression level of miR-182 in PTC tissues and adjacent noncancerous tissues.

and the log-rank test in univariate survival analysis, and we used the Cox proportional hazards regression model in our multivariate analysis. SPSS version 18.0 was used to perform our statistical analysis. Two-tailed *P* values <0.05 were considered statistically significant.

Results

Expression level of miR-182 in PTC tissues and adjacent noncancerous tissues

To investigate the role of miR-182 in PTC development, the expression levels of miR-182 in 151 paired human PTC tissues and adjacent noncancerous tissues were quantified by real-time PCR. The relative expression of miR-182 in PTC samples was significantly higher than that of matched normal tissues (*P*<0.001, **Figure 1**). Hence, we considered that the up-regulation of miR-182 may contribute to PTC tumorigenesis. To investigate the clinical relevance of miR-182 in PTC, the patients were divided into high expression group (n=77) and low expression group (n=74) used the median expression level as the cut-off value.

Correlation between miR-182 expression and clinicopathological features

We further evaluated the correlation between the tissue expression level of miR-182 and clinicopathological characteristics, and the data were presented in **Table 1**. The high expression level of tissue miR-182 was statistically correlated with extrathyroidal invasion (*P*=0.009), cervical lymphnode metastasis (*P*=0.015), and TNM staging (*P*=0.001), whereas no significant correlation between miR-182 expression and age, gender, and tumor size was observed (all *P*>0.05).

Correlations of miR-182 expression with patient survival

The Kaplan-Meier method revealed that higher miR-182 expression level was correlated with significantly reduced overall survival (*P*=0.013, **Figure 2**). Multivariate survival analysis revealed that extrathyroidal invasion (HR=3.278, 95% CI: 1.532-8.991, *P*=0.009), cervical lymphnode metastasis (HR=3.018, 95% CI: 2.666-11.286, *P*=0.005), TNM staging (HR=3.283, 95% CI: 2.192-16.283, *P*=0.001), and miR-182 expression level (HR=2.882, 95% CI: 1.289-10.928, *P*=0.013) were significantly correlated with the poor prognosis of PTC patients (**Table 2**). The results showed that PTC cases with higher miR-182 levels had poor outcomes compared to those with lower miR-182 levels.

Discussion

Although most PTC could be managed successfully with a combination of radioiodine and levothyroxine treatment after complete thyroidectomy, tumors with more aggressive phenotype are associated with morbidity and mortality [16]. Identification of novel molecular markers which can improve diagnosis and prognostic stratification and serve as possible therapeutic targets will be of great importance in the near future.

miRNAs are involved in cancer development in many ways and have served as biomarkers for the diagnosis and prognosis in many cancers [4]. In recent reports, several miRNAs have been shown to be associated with aggressive clinicopathogenetic features of PTC, such as extrathyroidal invasion, advanced stages, and poor prognosis [17, 18]. Dysregulated expression of miR-182 has been reported in a number of cancers [6, 7, 9, 12, 14, 19]. MiR-182 is up-regulated in ovarian cancer, melanoma, and hepatocellular carcinoma, and it enhances their growth and metastasis [8, 10, 13]. By contrast, miR-182 is down-regulated in human gastric adenocarcinoma and lung cancer [5, 11], and it suppresses the growth of these cancer cells. These results suggested that miR-182 may play important roles in cancer tumori-

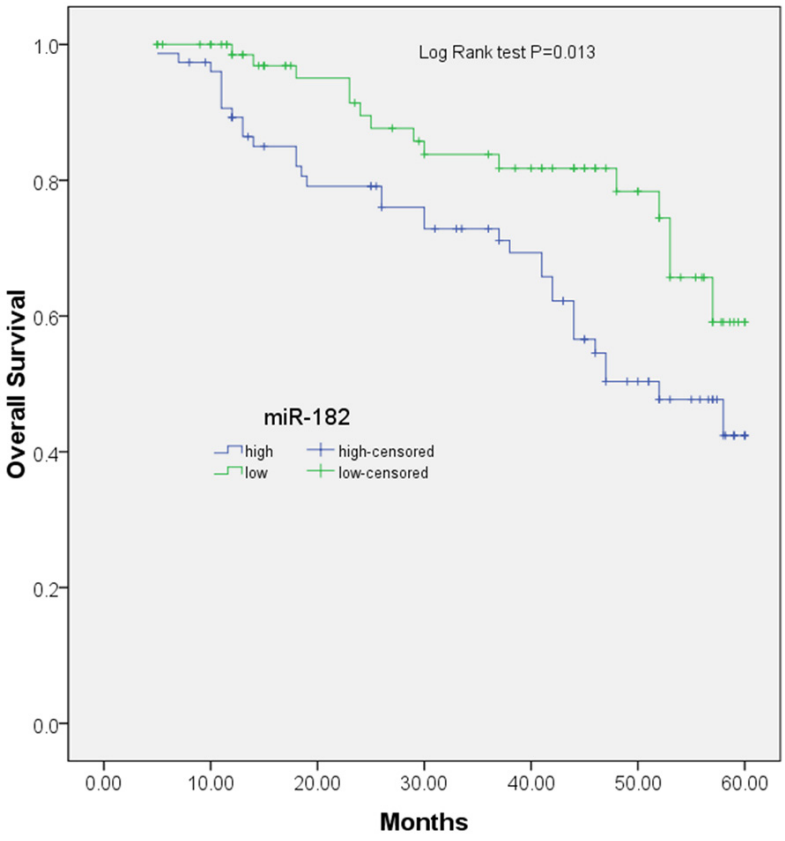

Figure 2. Kaplan-Meier curves for overall survival according to the tissue expression levels of miR-182.

Table 2. Multivariate analyses of prognostic variables of overall survival in PTC patients

Variables	HR	95% CI	*P* value
Age (years)	1.839	0.736-3.927	0.162
Gender	0.827	0.267-1.923	0.728
Tumor size (cm)	2.565	0.887-3.282	0.081
Extrathyroidal invasion	3.278	1.532-8.991	0.009
Cervical lymphnode metastasis	3.018	2.666-11.286	0.005
TNM staging	3.283	2.192-16.283	0.001
miR-182 expression level	2.882	1.289-10.928	0.013

genesis and progression and exert different effects in various types of cancer. The prognostic value of miR-182 has also been investigated in several cancers. For example, Stenvold et al found that high tumor cell miR-182 expression is an independent positive prognostic factor for non-small cell lung cancer (NSCLC) [20]. In the study by Wang et al, the Kaplan-Meier method revealed that higher miR-182 expression level correlated with significantly reduced disease-free survival in HCC (P= 0.039). Multivariate survival analysis revealed that high expression of miR-182 (P=0.022) was significantly correlated with the poor prognosis of HCC patients [13]. MiR-182 was also upregulated in colorectal cancer tissues and correlated with adverse clinical characteristics and poor prognosis, indicating that miR-182 might be involved in colorectal cancer progression and could be used as a potential prognostic biomarker and therapeutic target in the management of colorectal cancer [21]. In the study by Jiang et al, the cumulative 5-year survival rate of glioma patients was 51.54% (95% confidence interval, 0.435 to 0.596) in the low miR-182-expression group, whereas it was only 7.23% (95% confidence interval, 0.027 to 0.118) in the high miR-182-expression group (P<0.001), and multivariate Cox regression analysis indicated that miR-182 expression was an independent prognostic indicator for the survival of glioma patients [22].

Previously, Zhu et al analyzed the expression of miR-182 in PTC specimens and adjacent normal thyroid tissues. qRT-PCR analysis revealed significantly higher miR-182 levels in PTC than matched normal tissues. Furthermore, miR-182 targeted CHL1 and controlled tumor growth and invasion in PTC. The results collectively support an oncogenic role of miR-182 in PTC cell proliferation and invasion through downregulation of CHL1 expression [15]. However, the clinical significance and prognostic value of miR-182 have not been investigated in PTC until now. In the present study, to investigate the role of miR-

182 in PTC development, the expression levels of miR-182 in 151 paired human PTC tissues and adjacent noncancerous tissues were quantified by real-time PCR. The relative expression of miR-182 in PTC samples was significantly higher than that of matched normal tissues. Hence, we considered that the up-regulation of miR-182 may contribute to PTC tumorigenesis. We further evaluated the correlation between the tissue expression level of miR-182 and clinicopathological characteristics. The high expression level of tissue miR-182 was statistically correlated with extrathyroidal invasion, cervical lymphnode metastasis, and TNM staging. These results provided further support for the idea that altered miR-182 expression may be an important regulator of aggressive biological behavior in PTC. The Kaplan-Meier method revealed that higher miR-182 expression level was correlated with significantly reduced overall survival. Furthermore, multivariate survival analysis revealed that extrathyroidal invasion, cervical lymphnode metastasis, TNM staging, and miR-182 expression level were significantly correlated with the poor prognosis of PTC patients. The results showed that PTC cases with higher miR-182 levels had poor outcomes compared to those with lower miR-182 levels. In conclusion, overexpression of miR-182 is associated with aggressive clinicopathologic characteristics including extrathyroidal invasion, cervical lymphnode metastasis, and advance TNM staging in PTC, and miR-182 might be a novel prognostic molecular marker of PTC.

Disclosure of conflict of interest

None.

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