

# Correlation between CT features of adrenocortical and adrenal medullary tumors and expression of miR-96 in serum

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**Abstract.** Correlation between CT features of adrenocortical and adrenal medullary tumors and the expression of miR-96 in serum were investigated. A total of 230 patients with adrenocortical tumors and 194 patients with adrenal medullary tumors were selected in Dongying People's Hospital from August 2013 to August 2017. The two groups of patients underwent CT examination, and the signs and symptoms were recorded. The expression of miR-96 in the serum of the two groups was detected by RT-PCR, and the correlation between the expression of serum miR-96 and CT features was analyzed. In patients with adrenocortical tumor, serum miR-96 expression levels were significantly higher in patients with tumor diameter  $\geq 5$  cm than those with tumor diameter  $< 5$  cm ( $p < 0.001$ ). In patients with adrenal medullary tumor, serum miR-96 expression levels were significantly higher in patients with tumor diameter  $\geq 3$  cm than those with tumor diameter  $< 3$  cm ( $p < 0.001$ ). In patients with adrenocortical or adrenal medullary tumor, serum miR-96 expression levels were significantly higher in patients with peripheral infiltration than those without peripheral infiltration ( $p < 0.001$ ), and serum miR-96 expression levels were also significantly higher in patients with distant metastasis than those without distant metastasis ( $p < 0.001$ ). Serum levels of miR-96 in patients with benign adrenocortical and adrenal medullary tumors were significantly lower than those with malignant tumors in the same group ( $p < 0.001$ ). miR-96 may have oncogenic functions in patients with adrenocortical or adrenal medullary tumors. Increased expression level of miR-96 may promote proliferation, invasion and metastasis of tumors, and serum levels of

miR-96 provide references for the diagnosis of adrenocortical and adrenal medullary tumors.

## Introduction

Adrenal gland secretes multiple hormones, and tumorigenesis in adrenal gland leads to a series of clinical syndromes of hormone secretion disorder, which in turn affects carbohydrate metabolism in the human body and even causes severe harm to the growth and development of human body (1,2). As a common type of tumor, incidence of adrenal tumor increases with age. It has been reported that incidence of adrenal tumor in people  $< 35$  years of age is 0.2%, while the incidence in people  $> 65$  years is as high as 7% (3,4). Therefore, accurate diagnosis of adrenal tumors is critical for the treatment of this disease.

CT as one of the preferred methods in imaging examination of adrenal tumors with the characteristics of high sensitivity and accuracy, and is sensitive to microcalcification (5). However, studies have shown that CT cannot be used to distinguish adrenocortical and adrenal medullary tumors (6,7). Therefore, we aim to include another indicator to improve the accuracy of CT diagnosis. Studies have shown that miRNAs have certain diagnostic and therapeutic values for a variety of tumors (8,9), suggesting that miRNAs may serve as new diagnostic and therapeutic targets for adrenal tumors. miR-96 has been proven to be differentially expressed in a variety of cancers. Zhang *et al* showed that miR-96 is highly expressed in breast cancer patients, and may promote the proliferation of breast cancer cells (10). Feng *et al* (11) demonstrated that downregulation of miR-96 expression significantly inhibited the migration and invasion of pancreatic cancer cells. However, the expression of miR-96 in the serum of patients with adrenal tumors has not been previously reported.

In this study, the expression of miR-96 in the serum of 424 patients with adrenal tumors was detected to explore its clinical significance. Our study provided references to assist CT diagnosis of adrenal tumors.

## Patients and methods

**Clinical data.** A total of 424 patients pathologically diagnosed with adrenal tumor were selected in Dongying People's Hospital (Dongying, China) from August 2013 to

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August 2017 and their age ranged from 20 to 70 years. Patients were divided into adrenocortical tumor group (n=230) and adrenal medullary tumor group (n=194) according to different tumor sites. Adrenocortical tumor group included 121 males and 109 females, and adrenal medullary tumor group included 101 males and 93 females. Inclusion criteria: patients with adrenal tumors confirmed by pathological examinations; serum samples were obtained prior to radiotherapy, chemotherapy and related immunotherapy; unilateral adrenal tumors. Exclusion criteria: patients with both adrenocortical tumor and adrenal medullary tumor; patients with active digestive diseases; patients with adrenocortical hyperthyroidism; patients with abnormal liver and kidney function; patients with a history of pre-eclampsia, diabetes, pregnancy-induced hypertension; patients with mental disorders or brain abnormalities. This study was approved by the Ethics Committee of Dongying People's Hospital, and all patients signed informed consent.

**CT evaluation method.** CT images of all patients were analyzed by two chief physicians and a CT specialist at the same time using single-blind method. Content of evaluation included the nature of tumors (benign or malignant), the largest diameter of tumors, tumor morphology, boundary, invasion and metastasis (12). Researchers were not informed of the patients' pathology diagnosis results.

**RNA extraction.** TRIzol reagent was used for total RNA extraction from serum according to the instructions provided by Invitrogen (Invitrogen: Thermo Fisher Scientific, Inc., Waltham, MA, USA). UV spectrophotometer was used to determine the concentration and purity of RNA samples, and 3% agarose gel electrophoresis was performed to analyze RNA integrity.

**cDNA synthesis.** Total RNA was reversely transcribed into cDNA using TaqMan<sup>®</sup> MicroRNA Reverse Transcription kit (Thermo Fisher Scientific, Inc., Shanghai, China) according to the manufacturer's instructions. Reaction conditions: 45 min at 37°C, and 5 min at 95°C. cDNA samples were stored at -20°C.

**RT-qPCR.** Reaction system was 25  $\mu$ l in total. Reaction conditions were: pre-denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 45 sec, and 72°C for 3 min, and 72°C for 5 min. PCR reaction was performed using ABI Prism 7900 PCR instrument with U6 (Shanghai Meixuan Biological Science and Technology, Ltd., Shanghai, China) as endogenous control. Three replicate wells were set for each sample. Cq values were processed using  $2^{-\Delta\Delta Cq}$  method (13). Clinicopathological characteristics are shown in Table I.

**Statistical analysis.** SPSS 19.0 statistical software (Easybio, Beijing, China) was used for statistical analysis. Measurement data were expressed as mean  $\pm$  standard deviation (SD) and comparisons between the two groups were performed by t-test. Count data were expressed as % and processed by  $\chi^2$  test.  $P < 0.05$  was considered to be statistically significant.

## Results

**General information.** A total of 424 patients were included, and the age ranged from 20 to 70 years. There were 230 patients

Table I. Correlation between miR-210 expression and the clinicopathological characteristics of the osteosarcoma patients.

Clinicopathological features	Cases (n=54)	miR-210 <sup>a</sup> expression		P-value
		High (n=39)	Low (n=15)	
Age (years)				0.5768
>60	26	20	6	
≤60	28	19	9	
Sex				0.4937
Male	28	18	8	
Female	26	21	7	
Tumor size (cm)				0.3864
≥5.0	30	25	8	
<5.0	24	14	7	
TNM stage				0.0066 <sup>b</sup>
I-II	22	11	11	
III	32	28	4	
Lymph node metastasis				0.0042 <sup>b</sup>
Yes	33	31	4	
No	21	8	11	
Distant metastasis				0.5204
Yes	28	18	9	
No	26	21	6	

<sup>a</sup>The mean expression level of miR-210 was used as the cutoff.

<sup>b</sup>Statistically significant. TNM, tumor-node-metastasis.

with adrenocortical tumors, including 145 patients with benign tumors and 85 patients with malignant tumors, and 194 patients with adrenal medullary tumors, including 98 patients with benign tumors and 96 patients with malignant tumors. No significant differences in miR-96 expression were found between the two groups ( $p > 0.05$ ). There was no significant difference between the two groups in basic data, such as body weight, age, and exercise habits ( $p > 0.05$ ) (Table II).

**Correlation between CT features and serum miR-96 expression in adrenocortical tumor patients.** Serum miR-96 expression levels were significantly higher in patients with a tumor diameter  $\geq 5$  cm than those with tumor diameter  $< 5$  cm ( $p < 0.001$ ). There was no significant difference in the expression level of miR-96 between patients with regular and irregular morphology of tumors ( $p > 0.05$ ). There was no significant difference in the expression of miR-96 between patients with clear and unclear tumor boundary ( $p > 0.05$ ). Serum levels of miR-96 in patients with infiltrating tumor were significantly higher than those in patients without peripheral infiltration ( $p < 0.001$ ). Serum levels of miR-96 in patients with tumor metastasis were significantly higher than those in patients without tumor metastasis ( $p < 0.001$ ) (Table III).

Table II. Comparison of general data between two groups [n (%)].

Items	Adrenocortical tumor (n=230)	Adrenal medullary tumor (n=194)	$\chi^2$	P-value
Sex			0.177	0.962
Male	121 (52.61)	101 (50.06)		
Female	109 (47.39)	93 (47.94)		
Age (years)			0.876	0.411
<48	112 (48.70)	96 (49.48)		
≥48	118 (51.30)	98 (50.52)		
miR-96 expression	4.907±1.519	4.748±1.531	1.070	0.285
Weight (kg)			1.336	0.522
<55	117 (50.87)	93 (47.94)		
≥55	113 (49.13)	101 (52.06)		
Exercise habits			0.933	0.393
Yes	115 (50.00)	98 (50.52)		
No	115 (50.00)	96 (49.48)		
Smoking			0.976	0.381
Yes	60 (26.09)	55 (28.35)		
No	170 (73.91)	139 (71.65)		
Drinking			1.147	0.311
Yes	53 (23.04)	48 (24.74)		
No	177 (76.96)	146 (75.36)		

Table III. Correlation between CT features and serum miR-96 expression in adrenocortical tumor patients.

CT features	n (%)	miR-96	t	P-value
Maximum diameter (cm)			11.510	<0.001
<5	166 (72.17)	3.713±1.364		
≥5	64 (27.83)	6.009±1.335		
Morphology			0.711	0.478
Regular	147 (63.91)	4.121±1.162		
Irregular	83 (36.09)	4.009±1.119		
Boundary			0.662	0.509
Clear	156 (67.83)	3.968±1.312		
Unclear	74 (32.17)	4.164±1.249		
Peripheral infiltration			13.170	<0.001
Yes	48 (20.87)	6.639±1.845		
No	182 (79.13)	3.658±1.252		
Metastasis			14.850	<0.001
Yes	67 (29.13)	6.922±2.164		
No	163 (70.87)	3.645±1.161		

Correlations between CT features and serum miR-96 expression in patients with adrenal medullary tumor. Serum

Table IV. Correlation between CT features and serum miR-96 expression in patients with adrenal medullary tumor.

CT features	n (%)	miR-96	t	P-value
Maximum diameter (cm)			11.000	<0.001
≥3	143 (73.71)	6.102±1.433		
<3	51 (26.29)	3.667±1.112		
Morphology			0.378	0.706
Regular	172 (88.66)	4.227±1.362		
Irregular	22 (11.34)	4.113±1.076		
Boundary			0.375	0.708
Clear	161 (82.99)	4.126±1.469		
Unclear	33 (17.01)	4.231±1.441		
Peripheral infiltration			8.283	<0.001
Yes	164 (84.54)	6.313±1.761		
No	30 (15.46)	3.512±1.331		
Metastasis			7.190	<0.001
Yes	177 (91.24)	6.516±1.656		
No	17 (8.76)	3.539±1.321		

Table V. Correlation between serum levels of miR-96 and nature of the tumor (benign or malignant).

	n	miR-96 expression		t	P-value
		Benign tumor	Malignant tumor		
Adrenocortical tumor	230	3.077±1.252	6.736±1.785	23.91	<0.001
Adrenal medullary tumor	194	2.898±1.249	6.598±1.813	23.41	<0.001
t		0.868	0.764		
P-value		0.385	0.445		

miR-96 expression levels were significantly higher in patients with tumor diameter ≥3 cm than those with tumor diameter <3 cm (p<0.001). There was no significant difference in the expression level of miR-96 between patients with regular and irregular tumor morphology (p>0.05). There was no difference in the expression level of miR-96 between patients with clear and unclear tumor boundary (p>0.05). Serum levels of miR-96 in patients with tumor infiltration were significantly higher than those in patients without peripheral infiltration (p<0.001). Serum levels of miR-96 in patients with tumor metastasis were significantly higher than those in patients without tumor metastasis (p<0.001) (Table IV).

Correlation between serum levels of miR-96 and tumor nature (benign or malignant). In adrenocortical tumor group, the expression level of miR-96 in patients with benign tumor was (3.077±1.252), which was significantly lower than

that in patients with malignant tumors in the same group ( $6.736 \pm 1.785$ ,  $p < 0.001$ ). In adrenal medullary tumor group, the expression level of miR-96 in patients with benign tumor was ( $2.898 \pm 1.249$ ), which was significantly lower than that in patients with malignant tumors in the same group ( $6.598 \pm 1.813$ ,  $p < 0.001$ ). However, there was no significant difference in the expression of miR-96 between benign adrenocortical tumor patients and benign adrenal medullary tumor, and between patients with malignant adrenocortical tumor patients and patients with malignant adrenal medullary tumor ( $p > 0.05$ ) (Table V).

## Discussion

Adrenal tumors can be divided into adrenocortical and adrenal medullary tumor according to tumor locations. Different types of adrenal tumors should be treated differently, so correct diagnosis of the tumor is particularly important. However, CT cannot be used to distinguish benign adrenal tumor from malignant tumors (14,15). In this study, the expression of miR-96 in 424 patients with adrenal tumors was detected with an expectation of providing references to assist CT diagnosis of adrenal tumors.

Results of this study showed that the serum miR-96 expression levels were not related to the morphology and boundary of tumors, but were related to tumor diameter, peripheral tissue infiltration and tumor metastasis. miR-96 expression level in serum may assist CT diagnosis of benign and malignant adrenal tumors. The role of miR-96 in tumorigenesis of different types of tumors is quite different. Xu *et al* (16) found that miR-96 was overexpressed in patients with highly-metastatic HCC, and miR-96 downregulation significantly inhibited the invasion and migration of HCCLM6 cells. In a study carried out by Wang *et al* (17), miR-96 was found to be upregulated in bladder cancer. miR-96 overexpression was observed in breast cancer patients (18), and the upregulation of miR-96 expression in animal model promoted the growth of breast tumors. Consistent results were found in this study. miR-96 may promote the growth, invasion and metastasis of adrenal tumors, and serum miR-96 expression was upregulated in patients with bigger tumor diameter. Chandel *et al* (19) showed that miR-96 was highly expressed in HCC cell lines. Increased expression level of miR-96 not only promoted the migration but also enhanced the invasion ability of HCC cells by inhibiting the expression of miRNA PTPN9. Those results suggest that increased expression level of miR-96 is very likely to promote proliferation, invasion and metastasis of adrenal tumors through the regulation of PTPN9 miRNA, but the mechanism remains to be further studied. In both adrenocortical and adrenal medullary tumor groups, the relative expression level of serum miR-96 was significantly higher in patients with malignant tumors than in those with benign tumors, indicating that miR-96 may promote the malignant proliferation of adrenal tumors and further validated that the detection of miR-96 expression may provide references to assist CT diagnosis of benign and malignant adrenal tumors. However, no significant difference in the serum level of miR-96 was found between adrenocortical and adrenal medullary tumor groups, and there was also no significant difference in the expression level of miR-96 between benign

adrenocortical and benign adrenal medullary tumor patients, and between malignant adrenocortical and malignant adrenal medullary tumor patients. Therefore, the expression level of miR-96 may not be related to the sites of adrenal tumors, but the correlations with other types of adrenal tumors based on different typing methods need to be further verified.

This study also has some shortcomings. In this study, the expression of miR-96 in adrenal tumors was not correlated with the morphology and boundary of tumor, but tumor morphology and boundary are closely related to the nature of tumors (benign or malignant) (20). The possible explanations may include subjective factors and instability of miR-96 (21). We will include larger number of samples in our future study. In addition, this study is a prospective analysis, and the results need to be further verified by more experiments and clinical data. We did not explore the diagnostic value of CT and miR-96, therefore, it is necessary to further verify whether the detection of miR-96 level can assist CT diagnosis of adrenal tumors.

In summary, miR-96 may play an oncogenic role in adrenal tumors. The increased expression of miR-96 may promote malignant proliferation, infiltration and metastasis of adrenal tumors, which may provide references for the diagnosis of adrenal tumors.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

YX analyzed and interpreted patients' data. ZW contributed in designing the methods. YB performed the experiment and participated in the design of the study. ZD participated in the analysis and discussion of the data. XY contributed in the conception of this study and was also responsible for reviewing. YX and ZW contributed in writing the manuscript. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

This study was approved by the Ethics Committee of Dongying People's Hospital (Dongying, China). Signed informed consents were obtained from the patients or guardians.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.



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