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

YONGYUN XU1*, ZHENFANG WANG1*, YANGANG BI1, ZUYUN DUAN2 and XUEWANG YUE1

1CT Room, Dongying People's Hospital; 2Department of Imaging, The Second People’s Hospital of Dongying, Dongying, Shandong 257091, P.R. China

Received January 29, 2018; Accepted May 25, 2018

DOI: 10.3892/ol.2018.8863

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 ≥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 ≥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
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 adrenocortical tumor group included 101 males and 93 females. Inclusion criteria: patients with adenocortical 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 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® 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 µl 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^ΔΔCT 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 ± standard deviation (SD) and comparisons between the two groups were performed by t-test. Count data were expressed as % and processed by χ² 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 with adenocortical 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 ≥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 I. Correlation between miR-210 expression and the clinicopathological characteristics of the osteosarcoma patients.

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

*The mean expression level of miR-210 was used as the cutoff.

Statistically significant. TNM, tumor-node-metastasis.
Correlations between CT features and serum miR-96 expression in patients with adrenal medullary tumor. Serum 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 nature of the tumor (benign or malignant). 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).

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

| Items              | Adrenocortical tumor (n=230) | Adrenal medullary tumor (n=194) | χ² | P-value |
|--------------------|-----------------------------|---------------------------------|----|---------|
| Sex                |                             |                                 |    |         |
| Male               | 121 (52.61)                 | 101 (50.06)                     | 0.177 | 0.962   |
| Female             | 109 (47.39)                 | 93 (47.94)                      |     |         |
| Age (years)        |                             |                                 |    |         |
| <48                | 112 (48.70)                 | 96 (49.48)                      | 0.876 | 0.411   |
| ≥48                | 118 (51.30)                 | 98 (50.52)                      |     |         |
| miR-96 expression  |                             |                                 |    |         |
| <5                 | 117 (50.87)                 | 93 (47.94)                      | 1.336 | 0.522   |
| ≥5                 | 113 (49.13)                 | 101 (52.06)                     |     |         |
| Weight (kg)        |                             |                                 |    |         |
| <55                | 166 (72.17)                 | 3.713±1.364                     | 1.070 | 0.285   |
| ≥55                | 64 (27.83)                  | 6.009±1.335                     |     |         |
| Exercise habits     |                             |                                 |    |         |
| Yes                | 115 (50.00)                 | 98 (50.52)                      | 0.933 | 0.393   |
| No                 | 115 (50.00)                 | 96 (49.48)                      |     |         |
| Smoking            |                             |                                 |    |         |
| Yes                | 60 (26.09)                  | 55 (28.35)                      | 0.976 | 0.381   |
| No                 | 170 (73.91)                 | 139 (71.65)                     |     |         |
| Drinking           |                             |                                 |    |         |
| Yes                | 53 (23.04)                  | 48 (24.74)                      | 1.147 | 0.311   |
| 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 expression | 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       |       |         |

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

| CT features                   | n (%)      | miR-96 expression | 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).

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

Correlation between serum levels of miR-96 and tumor nature (benign or malignant).
that in patients with malignant tumors in the same group (6.736±1.785, p<0.001). In adrenal medullary tumor group, the expression level of miR-96 in patients with benign tumor was (2.898±1.249), which was significantly lower than that in patients with malignant tumors in the same group (6.598±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 mRNA, 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.

Acknowledgements

Not applicable.

Funding

No funding was received.

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.
References

1. Slominski AT, Kim TK, Li W, Postlethwaite A, Tieu EW, Tang EK and Tuckey RC: Detection of novel CYP11A1-derived secosteroids in the human epidermis and serum and pig adrenal gland. Sci Rep 5: 14875, 2015.

2. Naan EC, Kirpensteijn J, Dupré GP, Galac S and Radlinsky MG: Innovative approach to laparoscopic adrenalectomy for treatment of unilateral adrenal gland tumors in dogs. Vet Surg 42: 710-715, 2013.

3. Chen W, Li F, Chen D, Zhu Y, He C, Du Y and Tan W: Retroperitoneal versus transperitoneal laparoscopic adrenalectomy in adrenal tumor: A meta-analysis. Surg Laparosc Endosc Percutan Tech 23: 121-127, 2013.

4. Libé R, Borget I, Ronchi CL, Zaggia B, Kerkhofs T, Bertherat J, Volante M, Quinkler M, Chabre O, et al; ENSAT network: Prognostic factors in stage III-IV adrenocortical carcinomas (ACC): An European Network for the Study of Adrenal Tumor (ENSAT) study. Ann Oncol 26: 2119-2125, 2015.

5. Ahmed M; Technology Assessment Committee of the Society of Interventional Radiology: Image-guided tumor ablation: standardization of terminology and reporting criteria - a 10-year update: Supplement to the consensus document. J Vasc Interv Radiol 25: 1706-1708, 2014.

6. Kao YH, Steinberg JD, Tay YS, Lim GKY, Yan J, Townsend DW, Budgeon CA, Boucek JA, Cheo TST, et al: Post-radioembolization ytrium-90 PET/CT - part 2: Dose-response and tumor predictive dosimetry for resin microspheres. EJNMMI Res 3: 57-57, 2013.

7. Uppot RN and Gervais DA: Imaging-guided adrenal tumor ablation. AJR Am J Roentgenol 200: 1226-1233, 2013.

8. Chandel R, Das A, Chawla YK and Kaur J: MiR-183/-96/-182 cluster is up-regulated in most breast cancers and increases cell proliferation and migration. Breast Cancer Res 16: 473, 2014.

9. Mall C, Rocke DM, Durbin-Johnson B and Weiss RH: Stability of miRNA in human urine supports its biomarker potential. Biomarkers Med 7: 623-631, 2013.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.