

Expression of serum Hsa-miR-93 in uterine cancer and its clinical significance

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Received September 8, 2017; Accepted March 22, 2018

DOI: 10.3892/ol.2018.8553

Abstract. The aim of the study was to investigate the differential expression of micro-ribonucleic acid (miRNA)-93 in serum of patients with uterine cancer, and to explore the clinical significance. A total of 176 patients with uterine cancer who received surgery from May, 2009 to January, 2011 in the Department of Oncology of Hubei Cancer Hospital were selected. At the same time, 100 healthy individuals selected from the Physical Examination Center of Hubei Cancer Hospital comprised the control group. Mean age of patients was 55 ± 11 years, and of the healthy individuals in the control group was 53 ± 9 years. Blood was extracted from each participant to prepare serum samples. Change in the expression of serum miRNA-93 was detected by reverse transcription-polymerase chain reaction (RT-PCR), and the correlation between the expression of miRNA-93 and clinicopathological features of uterine cancer was analyzed. Expression level of miRNA-93 in serum of patients with uterine cancer was significantly lower than that in the healthy controls ($P<0.05$). Expression level of miRNA-93 was significantly correlated with pathological staging and lymph node metastasis ($P<0.05$). Receiver operating characteristic curve analysis showed that the area under curve was 0.781 and 95% confidence interval was 0.724-0.842. Survival rate of the high miRNA-93 expression group was significantly higher than that in the low miRNA-93 expression group ($P=0.036$). These results indicate that change in the expression of miRNA-93 is related to the occurrence of uterine cancer, and its decreased expression level suggests tumorigenesis.

Introduction

At present, uterine cancer is the most common tumor in the female reproductive system, and is also one of the most common female malignant tumors (1). Changes in living conditions and the increased incidence of obesity have led to an increase in morbidity of uterine cancer, and this disease also tends to affect younger patients (2). According to the report of the World Health Organization, uterine cancer currently ranks fourth among all cancers in females (3). Thus, studies on the treatment of uterine cancer have attracted increasing attention. At present, serum molecular markers are not available in the diagnosis of uterine cancer. Therefore, this study aimed to identify new indicators for the diagnosis of uterine cancer, thereby improving early diagnosis and treatment.

Micro-ribonucleic acid (miRNA) is a class of long non-coding RNA with a length of about 18-22 bp. miRNA can regulate gene expression at the transcriptional level to regulate cell proliferation, differentiation and apoptosis. Findings have shown that miRNAs are closely-related to the occurrence, invasion and metastasis of tumors (4). Additionally, that miRNA-93 is abnormally expressed in breast (5), gastric (6), lung (7) and other malignant tumors, but its relationship with uterine cancer has yet to be reported. This study aimed to examine the differential expression of miRNA-93 in serum of patients with uterine cancer, and to analyze the correlation between the expression of miRNA-93 and the clinical features of this disease.

Materials and methods

General materials. A total of 176 patients who received uterine cancer surgery from May, 2009 to January, 2011 in Hubei Cancer Hospital were selected. At the same time, 100 healthy individuals were selected from the Physical Examination Center of Hubei Cancer Hospital (Hubei, China) to serve as the control group. The mean age of the patients was 55 ± 11 years, and the median age was 55 years, and the mean age of the control group was 53 ± 9 years, and the median age was 53 years. The difference in age between the two groups was not statistically significant ($P=0.08$). Inclusion criteria were: i) Patients with uterine cancer confirmed by histopathological examination; ii) patients

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Key words: micro-ribonucleic acid-93, uterine cancer, molecular marker, polymerase chain reaction, receiver operating characteristic

received preoperative radiotherapy, chemotherapy and drug therapy; and iii) operations were conducted in accordance with the 8th edition of 2017 American Joint Committee on Cancer Clinical Staging.

Sample collection. Blood (5 ml) was extracted from each patient. All blood samples were processed within 2 h after collection to prepare serum samples through centrifugation for 5 min at 3,800 x g at 4°C. Serum samples were stored at an ultra-low temperature refrigerator (-80°C) before use. This study was approved by the Ethics Committee of Hubei Cancer Hospital, and all participants signed informed consent.

Instruments and reagents. The mirVana™ PARIS™ kit was purchased from Ambion, Inc.; Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Reverse transcription kit and Maxima SYBR-Green quantitative polymerase chain reaction (qPCR) kit were purchased from Thermo Fisher Scientific, Inc. Primers and internal references were produced by Guangzhou Shangeng Biotechnology Co., Ltd. (Guangzhou, China). The spectrophotometer (SMA5000) was purchased from Merinton Instrument, Ltd. (Beijing, China) and qPCR was purchased from Applied Biosystems; Thermo Fisher Scientific, Inc. Other conventional materials and instruments were provided by our hospital (Table I).

RNA extraction. Total RNA was extracted from serum according to instructions of the mirVana™ PARIS™ kit, and the purity and concentration of extracted miRNAs in serum were determined using the spectrophotometer. Only RNA samples with an A260/A280 ratio between 1.9 and 2.1 were used in reverse transcription to synthesize cDNA.

RT-PCR. Reverse transcription kit was used to synthesize cDNA. RTase M-MLV (RNase H-), dNTP Mixture, 5xM-MLV Buffer was used. Reaction volume was 20 µl. Reaction conditions were: 37°C for 60 min and 95°C for 5 min. cDNA samples were stored at -20°C before use.

qPCR. Maxima SYBR-Green qPCR kit was used to prepare reaction system according to the instructions. cDNA, (2 µl) 10 µl Maxima SYBR-Green qPCR Master Mix (2X), 0.5 µl upstream primer, 0.5 µl downstream primer and 7 µl RNase double-distilled water were mixed to make a final volume of 20 µl. PCR reaction conditions were: initial denaturation 95°C for 3 min, followed by 40 cycles of annealing 95°C for 15 sec and elongation of 60°C for 45 sec. Each reaction was repeated 3 times and the mean value was calculated. Cq values were processed using 2^{-ΔΔCq} method, and the relative expression of miRNA-93 was normalized to endogenous control U6.

Follow-up. All patients were followed up once every 2 months within 1 year after surgery. Then patients were followed up once every 3 months until the third year. Then patients were followed up once every six months until the fifth year. After that, patients were followed up once per year until December 31, 2016.

Statistical analysis. All data were analyzed by SPSS statistical software (SPSS, Inc., Chicago, IL, USA) and processed by the

Table I. Reverse transcription of miRNA-93 and primers of reverse transcription-polymerase chain reaction.

Items	Primer sequence
miRNA-93	
Reverse	5'-GTCGTATCCAGTGCAGGGTCCGAG GTATTGGCACTGGATACGACCTACC TGC-3'
Upstream	5'-CGTAGTTTCGCCAAAGTGCTGTTC-3'
Downstream	5'-ACGATGTAGGGTCCGAGGTATTC-3'
U6	
Reverse	5'-AACGCTTCACGAATTTGCGT-3'
Upstream	5'-CTCGCTTCGGCAGCAC-3'
Downstream	5'-AACGCTTCACGAATTTGCGT-3'

miRNA-93, micro-ribonucleic acid-93; RT-PCR, reverse transcription-polymerase chain reaction.

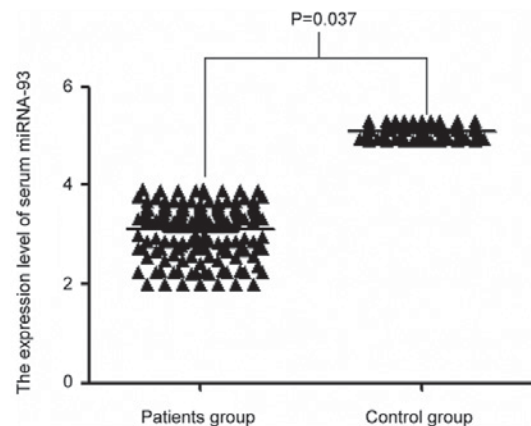


Figure 1. Comparison of the expression level of serum miRNA-93 between 176 patients and 100 healthy controls; miRNA-93, micro-ribonucleic acid-93.

non-parametric rank-sum test and independent-samples t-test. Data are expressed as mean ± standard deviation. Correlation between the expression level of miRNA-93 and clinical factors was analyzed using the Chi-square test. The survival analysis was conducted using the Kaplan-Meier survival analysis. P<0.05 indicated that the difference was statistically significant.

Results

Expression of miRNA-93 in patients with uterine cancer and healthy controls. As shown in Fig. 1, compared with the control group, the expression level of miRNA-93 in serum of patients with uterine cancer was significantly decreased (P=0.037).

Relationship between the expression of miRNA-93 and clinical factors. Expression level of miRNA-93 was significantly correlated with pathological staging and lymph node metastasis (P<0.05). Lower expression level of serum miRNA-93 was detected in patients at higher pathological stage (P=0.010; P=0.026). Other clinicopathological factors such as age, tumor

Table II. Clinicopathological characteristics of patients with uterine cancer.

Characteristics	No.	Expression level of miRNA-93	P-value
Age (years)			0.573
<55	94	3.72±1.87	
≥55	82	3.64±1.55	
Pathological staging			0.010
Stage I	76	3.54±0.941	
Stage II	61	3.19±0.862	
Stage III	31	2.84±0.764	
Stage IV	9	2.53±0.841	
Tumor size			0.121
≤30 mm	124	2.87±1.21	
>30 mm	53	3.02±1.47	
TNM staging			0.061
I/II	131	2.94±1.47	
III/IV	45	2.76±1.51	
Lymph node metastasis			0.009
Positive	43	2.61±1.41	
Negative	133	3.24±1.71	
Distant metastasis			0.057
Positive	50	3.04±0.901	
Negative	216	3.41±0.857	
Smoking condition			0.173
Yes	20	3.25±1.24	
No	156	3.17±1.19	

TNM, tumor-node-metastasis staging.

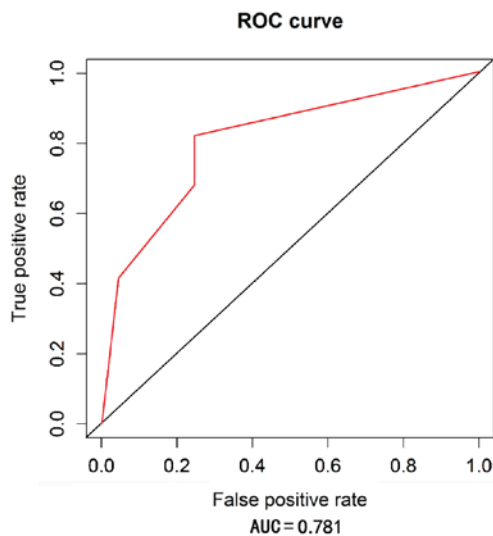


Figure 2. ROC curves analysis of the diagnostic value of serum miRNA-93 for uterine cancer (AUC=0.781). ROC, receiver operating characteristic curve; miRNA-93, micro-ribonucleic acid-93; AUC, area under curve.

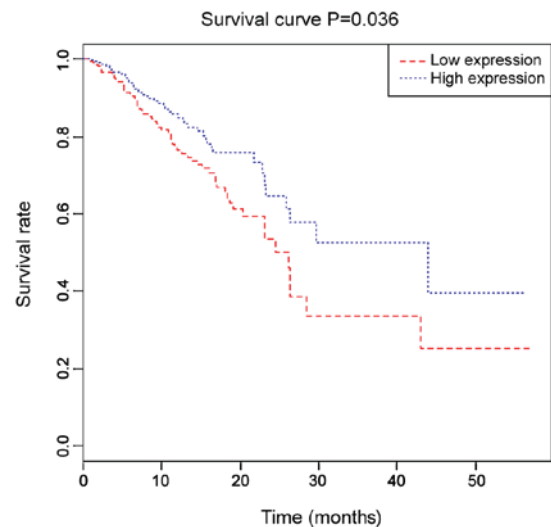


Figure 3. The Kaplan-Meier survival curve analysis. Survival rate of high expression is higher than that of the low expression group (P=0.036).

size, tumor-node-metastasis (TNM) staging, distant metastasis and smoking status were not significantly correlated with the expression level of miRNA-93 ($P>0.05$) (Table II).

Diagnostic value of miRNA-93 for uterine cancer. Receiver operating characteristic (ROC) curve analysis was performed to analyze the diagnostic value of miRNA-93 for uterine cancer. As shown in Fig. 2, area under curve (AUC) was 0.781,

and the 95% confidence interval was 0.724-0.842, indicating that miRNA-93 can be used to accurately predict uterine cancer.

Prognostic values of miRNA-93 for uterine cancer. Patients were divided into two groups based on the median expression level of miRNA-93. Survival rate of the high miRNA-93 expression group was significantly higher than that of the low miRNA-93 expression group ($P=0.036$). As shown in Fig. 3, the Kaplan-Meier survival curve showed that miRNA-93 expression was correlated with the prognosis of patients with uterine cancer.

Discussion

Uterine cancer is currently one of the most common malignant tumors in the female reproductive system and poses a serious threat to women's health and life (8-11). Early diagnosis is still the key for the treatment of this disease. Radiotherapy combined with chemotherapy is the first choice of treatment of uterine cancer after surgery (12). However, the early diagnosis of uterine cancer is performed through colposcopy and visual observation, which mainly depends on clinician's experience. Consequently, the rate of misdiagnosis is high. This study aimed to identify novel molecular markers for the diagnosis of uterine cancer to improve the diagnosis and treatment of this disease. miRNA expression is closely-related to the occurrence and development of tumors (13). Findings have shown that miRNAs can be stably expressed in urine, serum and other body fluids (14-16). Expression level of miRNAs in cancer tissues is basically the same as that in plasma, suggesting that circulating miRNAs can reflect miRNA expression level in tumor tissues (17). Serum is the most convenient and relatively non-invasive biological sample. Test with serum samples can be performed *in vitro* and avoid the side effects caused by surgeries.

miRNA-93 is located on human chromosome 7q.22.1 and is a miRNA produced by the transcription of miRNA-106b-25. miRNA-93 can participate in many inflammatory and immune reactions through the interactions with downstream target proteins MMP-2, integrin- β 8 and E2F1 (18). To the best of our knowledge, the expression of miRNA-93 in uterine cancer has yet to be reported. Therefore, we detected the differential expression of miRNA-93 in patients with uterine cancer to identify a new biomarker for this disease.

At present, correlation between miRNA-93 expression and clinicopathological characteristics of uterine cancer has not been reported. To demonstrate the potential relationship between miRNA-93 and uterine cancer, RT-qPCR was conducted to detect the expression of miRNA-93 in serum of each participant. miRNA-93 was significantly downregulated in serum of patients with uterine cancer compared with the control group. Expression level of miRNA-93 in pathological stage III/IV was significantly lower than that in stage I/II. Expression level of miRNA-93 in patients with lymph node metastasis was also downregulated compared with the healthy controls. The above results suggested that the low expression of miRNA-93 is closely related to the occurrence and development of uterine cancer. It has been reported that miRNA-93 can mediate the downregulation of transforming growth factor β

receptor 2, thus participating in nasopharyngeal carcinoma aggressiveness (18). Singh *et al* (19) reported that miRNA-93 reduced apoptosis of mammary epithelial cells and increased colony formation, mammary ball formation and cell migration. Silencing of miRNA-93 in these cells inhibited the development of cancer. Li *et al* (20) showed that miRNA-93 could promote angiogenesis by increasing endothelial cell proliferation and migration, and inhibition of miRNA-93 expression inhibited the secretion of vascular endothelial growth factor. The downregulation of c-Myc expression by TSA (acetylase inhibitor) can directly regulate the expression of miRNA-93 host gene MCM7 to induce cell cycle arrest and apoptosis (21). This finding can be explained by the changed miRNA-93 gene locus, which can affect the function of miRNA-93 in the tumor.

In this experiment, analysis of the Kaplan-Meier survival prognosis and ROC curve analysis were conducted. The Kaplan-Meier survival analysis showed that the survival rate of the miRNA-93 high expression group was higher than that in the low expression group ($P=0.036$). AUC (0.781) of ROC curve indicated that miRNA-93 could be used as a clinical indicator for uterine cancer.

This study is still limited by the small sample size, and in addition, this study only detected the expression level of miRNA-93 in serum. The mechanism of the function of miRNA-93 in uterine cancer was not investigated. Thus, further studies are needed.

In summary, expression level of miRNA-93 is significantly higher in the serum of patients with uterine cancer than in the healthy controls, and the expression level of miRNA-93 is significantly correlated with clinical stage and other pathological characteristics of patients with uterine cancer. miRNA-93 can be used as a potential molecular marker for the diagnosis of uterine cancer. However, the clinical applications of miRNA-93 needs to be further studied and confirmed.

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

SF wrote the manuscript, treated patients and collected blood sample. MG helped with RNA extraction. SX and QC performed PCR and qPCR. HZ recorded and analyzed follow-up. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Hubei Cancer Hospital (Hubei, China), and all participants signed informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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