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Original Research Article

Expressions and clinic significance of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218 in cervical cancer tissues

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Abstract

Purpose: To search for novel biomarkers for early diagnosis of cervical cancer, as well as novel therapeutic target for cervical cancer.
Methods: A total of 96 cervical tissue specimens were collected from patients in the Second Affiliated Hospital of Zhengzhou University, out of which 10 were normal control. The remaining specimens (86) were cervical cancer specimens and were divided into 4 groups (A - D) based on tumor-biomarker levels of CA125 and SCC. Quantitative real-time polymerase chain reaction technology (qRT-PCR) was used to detect the expressions of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218 in the cervical cancer tissues.
Results: The levels of CA125 (U/mL) and SCC (ug/L) expressed in normal control group and groups A - D were 11.75 and 0.73 (n = 10), 382 and 2.72 (n = 25), 912.9 and 3.93 (n = 21), 1675 and 5.87 (n = 29), and 2120 and 6.66 (n = 11), respectively. Furthermore, qRT-PCR results showed that the expressions of miRNA-944 and miRNA-218 in cervical cancer tissues were markedly up-regulated compared to normal control tissues (p < 0.01). In contrast, the expression level of miRNA-143, miRNA-34A, and miRNA-101 were significantly decreased (p < 0.01).
Conclusion: The biomarkers, miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218, can be considered novel for early diagnosis of cervical cancer.

Keywords: Cervical cancer, Biomarkers, miRNA-143, miRNA-34A, miRNA-944, miRNA-101, miRNA-218

INTRODUCTION

Cervical cancer is a serious threat to the health of women, and is one of the leading causes of cancer-related death in the world due to delayed diagnosis and high risk of metastasis [1]. Furthermore, 85 % of the cervical cancers occur in developing countries, such as China and India [2]. Currently, chemotherapy is the commonly used strategy to prevent the relapse and metastasis of cervical cancer besides surgery [3]. However, early diagnosis is the most important strategy for treating cervical cancer [4].

MicroRNAs (miRNAs) are a class of non-coding RNAs (21 - 24 nucleotides in length) that are critical for many important processes such as development, differentiation and even

carcinogenesis, and can regulate the chemo sensitivity of tumor cells [5,6]. Accumulating evidence has demonstrated that dysregulation of miRNAs which function as tumor promoter or suppressor depending on the nature of its targets, occurs in various human cancers [7].

Currently, miRNAs involved in carcinogenesis and progression of cervical cancer have been widely investigated [8]. In our present study, we investigated the expressions of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218 in cervical cancer tissues, and explored their regulative significance on cervical cancer tissues. Our research on miRNAs is aimed to provide reference for the early clinical diagnosis of cervical cancer and provide novel therapeutic target for cervical cancer.

EXPERIMENTAL

Subjects and sample collection

A total of 86 fresh cervical tissue specimens were obtained from patients treated in the Second Affiliated Hospital of Zhengzhou University for squamous cell carcinoma. Additionally, normal cervical tissue specimens (group A) were also obtained from patients without cervical cancer (n = 10). The 86 cervical cancer patients were divided into 4 groups (B - E) based on the tumor-biomarker levels of CA125 and SCC, and the numbers of subjects were 25, 21, 29 and 10, respectively. All the patients enrolled were required to read and sign an informed consent form voluntarily before enrollment. All the experimental designs were carried out in accordance with the declaration of Helsinki promulgated in 1964 as amended in 1996 [9] and approved by the Ethics Committee of the Second Affiliated Hospital of Zhengzhou University (Ethical approval No.: S2013-06-23).

RNA extraction and reverse transcription

Total RNA was extracted from each of the experimental groups using Trizol Reagent (Shanghai Sangon, Shanghai, China) according to the manufacturer's instructions. Reverse transcription (RT) was performed on RNA samples, followed by polymerase chain reaction (PCR) amplification.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Quantitative RT-PCR was performed to detect the relative transcript levels of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218, respectively. PCR was performed under the following conditions: 94 °C for 4 min followed by 40 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min. U6 snRNA was used as an endogenous control to normalize the expression of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218, respectively. qRT-PCR was performed using FastStart Universal SYBR Green Master kit (Roche Diagnostics) and analyzed with an Applied Biosystems 7900 Real-Time PCR System. The specific primer pairs (Invitrogen, Shanghai, China) are shown in Table 1.

Statistical analysis

All the data are presented as mean ± standard deviation (SD) and group comparison was performed by ANOVA (SPSS 16.0). $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathologic characteristics of subjects

Ten normal women and eighty six women with cervical cancer were included in the study.

Table 1: Primers used for quantitative real-time PCR

U6	F	5'GCTTCGGCAGCACATATACTAAAAT3'
	R	5'CGCTTCACGAATTTGCGTGTCAT3'
miRNA-143	F	GGGCGGGTGCAGTGCT
	R	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACCAGA
miRNA-34A	F	GGGCGGTGGCAGTGTCTT
	R	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACAACC
miRNA-944	F	GGGCGGAAATTATTGTA
	R	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCTCATCC
miRNA-101	F	GGGCGGCAGTTATCACAG
	R	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAGCATC
miRNA-218	F	GGGCGGTTGTGCTTGAT
	R	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACATGGT

Basic characteristics of five groups are given in Table 2. A represented normal control, and B-E represented the 4 groups of cervical cancer patients divided according to the biomarker expression levels of cancer antigen 125 (CA125) and squamous cell carcinoma antigen (SCC). Overall, no obvious difference was observed for the height, age, weight, and fasting blood-glucose (FBG) in the five groups ($p > 0.05$). However, the values of CA125 and SCC in the 5 groups were significantly different. As shown in Table 2, the CA125 and SCC values of the 4 groups of patients with cervical cancer were significantly higher than the normal values of CA125.

PCR results

To further validate the altered expressions of miRNA-143, miRNA-34A, miRNA-944, miRNA-

101 and miRNA-218 in cervical cancer, we tested the expressions of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218 in the cervical cancer tissues and normal tissues by using qRT-PCR assay. The amplification plots of qRT-PCR are shown in Figure 1. As shown in Table 3, miRNA-944 and miRNA-218 in the 4 groups of cervical cancer tissues were found to be expressed at significantly higher levels when compared to the normal control tissues ($p < 0.01$). Meanwhile, the expression levels of miRNA-143, miRNA-34A, and miRNA-101 in the 4 groups of patients with cervical cancer were relatively down-regulated ($p < 0.01$). The results showed that the expressions of miRNA-944 and miRNA-218 changed dramatically with the serious extent of cervical cancer.

Table 2: Baseline demographics and disease characteristics

Group	Height (cm)	Age	Weight	FBG (mmol/L)	CA125 (U/mL)	SCC (ug/L)
A	159.91±4.65	43.62±10.33	53.41±4.17	5.04±1.25	11.75±2.98	0.73±0.28
B	162.76±4.54	41.16±9.12	55.04±6.19	4.94±0.64	382±97.29	2.72±0.88
C	162.76±3.97	41.16±8.44	55.04±6.13	4.9412±0.93	912.9±99.76	3.93±0.93
D	162.03±4.73	42.03±8.26	58.72±5.07	4.69±0.89	1675±246.78	5.87±1.14
E	160.82±5.40	43.09±9.28	53.91±4.83	4.61±0.89	2120±234.45	6.66±1.28

Key: FBG: Fasting blood-glucose; A: normal control; B-E represented the 4 groups of cervical cancer patients, and cervical cancer patients were divided according to the biomarkers expression levels of CA125 and SCC
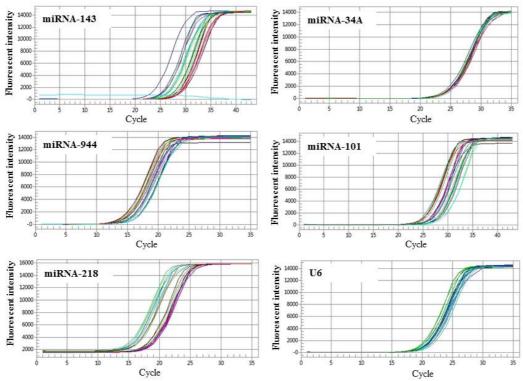

Figure 1: The amplification plots of real-time quantitative PCR.

Table 3: Expressions of the miRNA-143, 34A, 944, 101 and 218 in patients with cervical cancer

	miRNA-143	miRNA-34A	miRNA-944
A	0.744863±0.156102	0.725107±0.194532	0.695712±0.190653
B	0.000413±0.000113 **	0.092564±0.012031 **	25.22961±5.987026 **
C	0.000384±0.000124 **	0.087595±0.019765 **	27.50011±5.976513 **
D	0.000343±0.000123 **	0.065139±0.015563 **	32.41054±11.74653 **
E	0.000341±0.000115 **	0.063897±0.021022 **	39.55085±12.65385 **

	miRNA-101	miRNA-218
A	1.265348±0.344745	0.732692±0.243097
B	0.373367±0.098217 **	21.18917±2.687207 **
C	0.398324±0.176539 **	24.84102±5.210923 **
D	0.448022±0.138603 **	25.87216±7.864375 **
E	0.546068±0.176068 **	35.21287±7.412874 **

A: normal control; B-E represented the 4 groups of cervical cancer patients, and cervical cancer patients were divided according to the biomarkers expression levels of CA125 and SCC; ** significant difference at $p < 0.01$, respectively *vs.* normal control

DISCUSSION

In the present study, we systematically investigated the expression of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218 in cervical cancer tissues, and found that in cervical cancer tissues, miRNA-944 and miRNA-218 were dramatically up-regulated, whereas miRNA-143, miRNA-34A and miRNA-101 were decreased.

A number of studies have identified miRNA-143 as a tumor suppressor gene lost expression significantly in several cancer types, including breast and cervical cancers [10-13], which is consistent with our results on the expression of miRNA-143 in cervical cancer tissues. miRNA-143 was expressed at significantly lower levels when compared to the normal control tissues. In addition, miRNA-34A belongs to the miRNA-34 family and acts as a tumor suppressor in several cancer types, and ectopic miRNA-34 expression induces apoptosis, cell-cycle arrest or senescence [14,15]. Recently, many studies have reported that miRNA-34A was down-regulated in many human cancers through hypermethylation of promoter DNA to silence this miRNA [14,16]. In the present study, miRNA-34A in patients with cervical cancer was also down-regulated.

The gene coding for miRNA-944 is located in the intron of TP63 gene, which encodes tumor protein 63 (p63). And the expression of miRNA-944 may correlate with TP63 expression [17]. miRNA-944, which targets an mRNA of SOCS (suppressor of cytokine signaling) family tumor suppressor genes, can promote tumor growth, proliferation, and squamous differentiation [18]. In addition, miRNA-944 was first identified in human cervical cells using a small RNA cloning approach and it was reported that it is significantly more abundant in cervical cancer tissues than their normal counterparts [18,19]. In the present study, the expression of miRNA-944 in cervical cancer tissues had significantly changed compared to other miRNAs and miRNA-944 was expressed at significantly higher levels compared to normal tissues. Thus, the miRNA-944 in cervical cancer tissues might be regarded as an important biomarker in the process of diagnosing cervical cancer.

miRNA-101 is one of the down-regulated miRNAs in a diverse of malignancies relative to their non-tumor counterparts, such as colorectal cancer, ovarian carcinoma, and non-small cell lung cancer, etc [20-22]. Furthermore, down-regulated expression of miRNA-101 might be involved in the progression of malignant cancers [23]. The results show that the expression of miRNA-101 in patients with cervical cancer is down-regulated when compared with the normal tissues. As a broadly conserved miRNA, miR-218 is considered a tumor suppressor in multiple carcinomas by targeting different genes, such as bladder cancer, liver cancer, and breast cancer, etc [24-26].

Many researchers have reported that the expression of miRNA-218 was down-regulated in various human tumor cells and tissues. However, miRNA-218 expression was up-regulated in cervical cancer tissues. The results demonstrate that miRNA-944 and miRNA-218 significantly increase in cervical cancer tissues. On the contrary, miRNA-143, miRNA-34A, and miRNA-101 in cervical cancer tissues are down-regulated.

CONCLUSION

The findings of the present work indicate that the expressions of miRNA-143, miRNA-34A, miRNA-944, miRNA-101 and miRNA-218 in cervical cancer tissues are different from those of normal tissues. Thus, these miRNAs are suitable as novel biomarkers for early diagnosis of cervical cancer.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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