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Reduced expression of miR-503 is associated with poor prognosis in cervical cancer

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Abstract. – OBJECTIVE: We wished to evaluate the association between expression of microRNA (miR) -503 and prognosis in patients with cervical cancer.

PATIENTS AND METHODS: 96 paired specimens of cervical cancer and adjacent normal cervical epithelial tissues were obtained. qPCR was used to evaluate expression levels of miR-503. We analyzed the associations between miR-503 expression levels and clinicopathological parameters, as well as recurrence-free and overall survival with Kaplan-Meier survival curves and proportional hazards model.

RESULTS: miR-503 levels were significantly ($p < 0.001$) lower in cervical cancer tissue compared with adjacent normal cervical epithelial tissue. We further observed significant associations of expression of this miR and recurrence of cervical cancer, lymph node metastasization, and International Federation of Gynecology and Obstetrics (FIGO) stage. In addition, in multivariate analysis, miR-503 expression level was found to be an independent prognostic factor for both recurrence-free and overall survival.

CONCLUSIONS: Reduced expression of miR-503 is an independent prognostic factor in cervical cancer indicating poor prognosis.

Key Words:
Cervical cancer, microRNA-503, qPCR, Recurrence-free survival, overall survival.

Introduction

Cervical cancer is the second most common cancer affecting women worldwide. Each year, there are about 529,800 new cases and 275,100 deaths per year[1,2]. Despite new methods of cervical cancer treatment, the prognosis is still not satisfactory[3]. The main reason for this is diagnosis at advanced stages of the disease and metastatic disease. It is reported that over 30% patients die from metastatisization of cervical cancer[4]. This highlights the need for biomarkers for earlier diagnosis.

MicroRNA (miRNA) are abundant small, endogenous, non-coding RNA which regulate translation of many genes[5,6]. It is estimated that the number of mature human miRNA is in the vicinity of 2000, and some miRNA can regulate multiple genes[7,8]. miRNA have been found to be involved in many biological processes associated with differentiation, cell type-specific function, and homeostasis. Recently, miRNA have also been found to be involved in the process of epithelial mesenchymal transition[9]. Moreover, abundant evidence demonstrates miRNA may function as either tumor suppressors or promoters of cancers[10-12].

Aberrant expression of miRNA-503 has been reported in several cancer types, including oral cancer, unicellular carcinoma, parathyroid carcinoma and nonidentical carcinoma[13-17]. However, potential prognostic value of miR-503 in cervical cancer has not been evaluated yet. This study reports that reduced expression of miR-503 is associated with poorer prognosis in patients with cervical cancer.

Patients and Methods

Patients and Tissue Specimens

We collected 96 paired specimens of primary cervical cancer and adjacent normal cervical epithelial tissues from patients who underwent surgery at our Hospital. Histopathogical diagnosis was made according to the pathological clas-

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sification system of the International Federation of Gynecology and Obstetrics (FIGO)[3]. Informed consents were obtained from all patients. The study protocol was approved by the institutional Research and Ethics Committee. All specimens were flash frozen in liquid nitrogen and stored at –70ºC until further use.

RNA Extraction and qPCR

Total RNA was extracted with using TRIzol reagent (Invitrogen; Carlsbad, CA, USA). The M-MLV Reverse Transcriptase kit (Promega; Peking, China) was used to reverse transcribe 2.0 μg of total RNA to cDNA as per manufacturer's instructions. qPCR was utilized to evaluate the levels of mature miRNA-503. For quantification, the comparative threshold cycle (Ct) method was used. miR-503 levels were normalized to U6B.

Statistical Analysis

Statistical analyses were conducted using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± SD. The Student's *t*-test or chi-square test, ANOVA test were utilized to compare the data. The Kaplan-Meier method was used to calculate survival curves which were compared using the log-rank test. The multivariate Cox regression analysis was used to evaluate survival data. The *p* value of < 0.05 was considered significant.

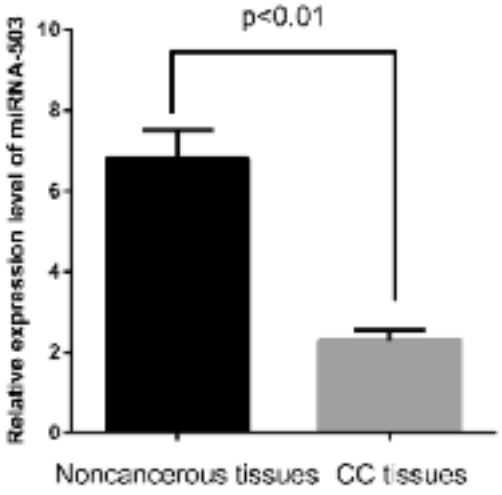

Figure 1. miR-503 expression levels in cervical cancer and adjacent non-tumour tissues.

Results

miR-503 is Significantly Downregulated in Cervical Cancer Tissue

Using qPCR, we compared expression of miR-503 in 96 cervical cancer specimens with matched noncancerous specimens. As shown in Figure 1, expression of miR-503 in cervical cancer tissue was significantly lower than that in normal cervical epithelial tissue ($p < 0.001$).

miR-503 Expression Correlates with Clinicopathological Characteristics in Cervical Cancer

We next tested the association between miR-503 and clinicopathological characteristics of study patients. The results of this analysis are presented in Table I. Specifically, low expression of miR-503 correlated with advanced FIGO stage, lymph node metastasis and cancer recurrence. However, there was no significant association between miR-503 and other tested parameters (Table I).

Correlation Between miR-503 Levels and Prognosis in Cervical Cancer

To analyse the prognostic value of miR-503 expression in study patients, we utilized the Kaplan-Meier test. The survival curves were evaluated by the log rank test. As shown in Figure 2A, the 5-year recurrence-free survival was significantly higher in patients with higher miR-503 expression ($p = 0.0052$ *vs.* patients with low miR-503 expression). Furthermore, patients with higher miR-503 expression had longer overall survival ($p = 0.0128$ *vs.* patients with low miR-503 expression; Figure 2B).

Next, the Cox proportional hazards regression model was used to conduct the multivariate analyses for both the 5-year recurrence-free survival and overall survival. The results are shown in Table II. The FIGO stage, lymph node metastasis and miR-503 expression were found to be independent determinants of the 5-year recurrence-free survival. In addition, miR-503 expression levels and lymph node metastases were independent prognostic factors for overall survival (Table II).

Discussion

Cervical cancer is the third most prevalent malignant gynecologic malignancy in women worldwide and one of the most frequent causes of can-

Table I. Association between miR-503 expression and clinicopathological parameters in study patients.

Parameters	Patients expressing low levels of miR-503 (n = 61)		Patients expressing high levels of miR-503 (n = 35)		*P*
	Absolute number	%	Absolute number	%	
Age (years)					
≤ 55	24	40.9	13	37.1	0.711
> 55	36	29.1	22	62.9	
Tumour diameter (cm)					
≤ 4.0	17	27.8	11	31.4	0.712
> 4.0	44	72.2	24	68.6	
HPV infection					
Negative	32	52.4	20	57.1	0.602
Positive	29	47.6	15	42.9	
Histological type					
AD	19	31.1	10	28.6	0.791
SCC	42	68.9	25	71.4	
Tumour differentiation					
Well-differentiated	10	16.4	8	22.9	0.362
Moderately differentiated	29	47.5	12	34.3	
Poorly differentiated	22	36.1	15	42.8	
FIGO stage					
I	20	32.8	22	62.9	0.004
II	41	67.2	13	37.1	
Lymph node metastasis					
Absent	22	36.1	21	60	0.023
Present	39	63.9	14	40	
Recurrence					
No	27	44.3	23	65.7	0.043
Yes	34	55.7	12	34.3	

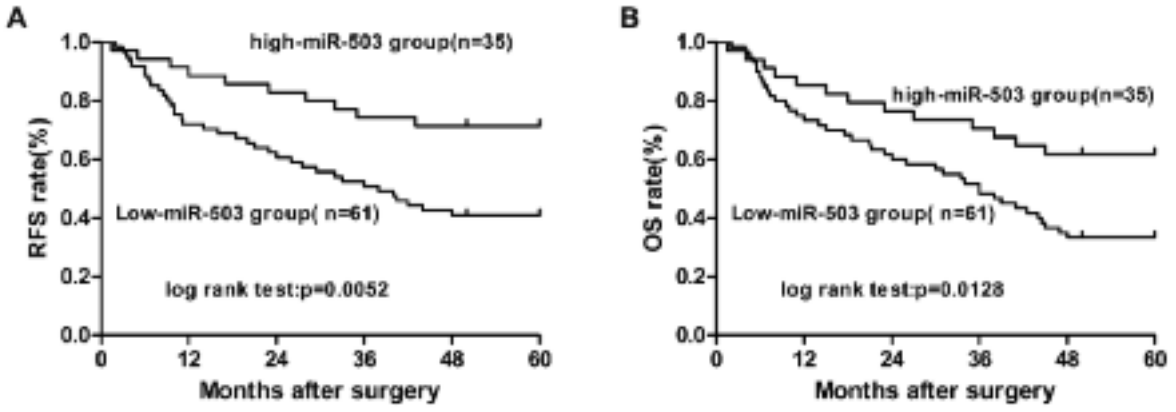

Figure 2. Kaplan-Meier curves for recurrence-free survival ***(A)*** and overall survival ***(B)*** in patients with cervical cancer based on miR-503 expression levels.

Table II. Multivariate Cox regression analysis of recurrence-free and overall survival in study patients.

Determinants	Recurrence-free survival HR (95% CI)	p	Overall survival HR (95% CI)	p
Age (>55 vs. ≤55 year)	2.615 (0.586-2.932)	0.189	2.126 (0.759-3.215)	0.286
Tumour diameter (>4.0 vs. ≤4.0 cm)	1.416 (0.825-1.967	0.253	1.477 (0.932-2.125	0.476
HPV infection (positive vs. negative)	2.326 (1.054-3.326)	0.626	3.842 (0.721-4.015)	0.312
Histology (SCC vs. AD)	1.421 (0.823-2.732)	0.271	2.326 (0.927-3.542)	0.207
Differentiation degree (moderately differentiated + poorly differentiated vs. well-differentiated)	1.842 (0.949-2.216)	0.461	1.526 (0.886-2.527)	0.206
FIGO stage (II vs. I)	2.362 (1.842-3.262)	0.026	1.954 (0.782-2.762)	0.327
Lymph node metastasis (yes vs. no)	1.842 (1.425-2.953)	0.032	2.446 (1.972-3.425)	0.016
miR-503 expression (low vs. high)	2.327 (1.922-3.436)	0.018	2.823 (1.476-2.631)	0.009

cer-related deaths in developing countries[18]. miR may function as prognostic factors in cancer. To date, more than 1900 of miR have been identified. It is estimated that expression of about 60% to 80% of the genes in humans are regulated by miR[19]. Thus, miR regulate many important biological functions, such as proliferation, metastasis, drug resistance of cancer[20,21]. It was further reported that cell cycle relating genes may be modulated by miR-142-5p and miR-9[22], and miR-155 and miR-148a are involved in regulation of the NF-κB pathway[23].

Aberrant expression of miR-503 was reported in several types of cancer (e.g., adrenocortical carcinoma, parathyroid carcinoma or retinoblastoma)[13,15,16]. However, potential association between miR-503 expression and cervical cancer has not been studied. In this study, we documented that expression of miR-503 in cervical cancer specimens was significantly lower than in normal tissue. Moreover, low expression of miR-503 correlated with high cancer recurrence rate. Therefore, miR-503 may be useful as a potential biomarker to predict the risk of recurrence.

Conclusions

The downregulation of miR-503 may serve as an indicator of unfavourable prognosis in patients with cervical cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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