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Down-regulation of miR-329-3p is associated with worse prognosis in patients with cervical cancer

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Abstract. – OBJECTIVE: miR-329-3p has been reported to serve as a tumor suppressor in the progression of cervical cancer (CC). The aim of this study was to investigate the clinical significance of miR-329-3p in human CC.

PATIENTS AND METHODS: Quantitative RT-PCR (qRT-PCR) was used to detect miR-329-3p expression in CC tissue samples and matched normal cervical tissues. The x^2 test was used to analyze the association between miR-329-3p expression and clinical features of CC patients. Moreover, we evaluated the prognostic value of miR-329-3p by Kaplan-Meier survival curve and Cox regression model.

RESULTS: We found that the mean expression level of miR-329-3p in CC tissues was significantly lower than the mean level in the adjacent normal tissues samples ($p < 0.01$). MiR-329-3p level was significantly associated with lymph node metastasis ($p = 0.013$), FIGO stage ($p = 0.024$) and distant metastasis ($p = 0.001$). Furthermore, a significant difference was found, that CC patients with low miR-329-3p expression level had distinctly shorter overall survival than patients with high miR-329-3p expression level ($p = 0.001$). Finally, multivariate analyses indicated that miR-329-3p represented an independent predictor for overall survival of CC ($p = 0.04$).

CONCLUSIONS: These results indicated, for the first time, that down-regulation of miR-329-3p was associated with poor prognosis in CC patients. MiR-329-3p can be used as an independent factor to predict survival of patients with CC.

Key Words:
MiR-329-3p, Cervical cancer, Prognosis.

Introduction

Cervical cancer (CC) is the fourth leading cause of cancer deaths among women worldwide, with increasing incidence globally each year[1,2]. CC accounts for 14% of female cancer death in China[3]. Surgery combined with chemotherapy and radiotherapy is the main treatment of CC and the patients who were early diagnosed have a satisfying outcome[4-6]. Despite the significant achievements that have been made in the treatment of CC, 5-year survival rate of CC patients diagnosed at advanced stages is still not satisfied[7]. Tumor metastases are responsible for approximately 90% of all cancer-related deaths and is a major obstacle to successful treatment[8,9]. Therefore, it is urgent to distinguish biomarkers and novel therapeutic targets for therapeutic strategy improvement.

MicroRNAs (miRNAs) are single-stranded, small noncoding RNAs of 18-25 nucleotides in length with limited protein-coding potential[10]. Growing evidence suggests that lncRNA was linked to very stage of cell life, including cell proliferation, differentiation, migration and apoptosis[11]. MiRNAs carry out their functions in a number of processes and can regulate gene expression various mechanisms[12,13]. Recently, studies reveal that miRNAs serve as regulatory molecules, acting as oncogenes or tumor suppressor genes in development and progression of various tumors, including CC. For instance, Wang et al[14] reported that over-expression of miR-429 suppressed invasion and migration of human CC cells through modulation of ZEB1

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and CRKL. Cong et al[15] found that miR-634 inhibited cell proliferation, migration and invasiveness in CC cells by targeting mTOR signal pathway. Recently, some studies reported that miRNAs was associated with prognosis of CC patients, such as miR-155[16] and miR-664[17]. These results indicated that miRNAs may represent a novel prognostic marker in CC.

MiR-329-3p is located on 14q32.31. Recent evidence showed that miR-329-3p can not only suppress tumor, but also take part in the proliferation and metastasis of various tumor cells, such as osteosarcoma[18], hepatocellular carcinoma[19] and non-small cell lung cancer[20]. More importantly, Li et al[21] firstly reported that miR-329-3p serve as a tumors suppressor by targeting MAPK1.

Patients and Methods

Patients

All patients were recruited between 2008 and 2011 in Shandong Maternal and Child Health Care. Tumor cervical tissues and cervical normal samples were immediately frozen in liquid nitrogen and kept at -80°C until total RNA was extracted. The CC diagnosis was histopathologically confirmed. There were no patients receiving radiotherapy or chemotherapy before operation. The following clinicopathological data were obtained from CC patients, such as age, tumor size, histological type and FIGO stage. The clinical features of GC patients are summarized in Table II. Written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of Shandong Maternal and Child Health Care.

QRT-PCR Analysis

Total RNA was obtained from the frozen tissues using the TRIzol Reagent (GenePharma, Beijing, China). cDNA was synthesized from 1 lg of purified total RNA using a PrimeScript RT reagent kit (BD Biosciences, Franklin Lakes, NJ, USA). The expression levels of miR-329-3p were quantified using the TaqMan miRNA assay kits (Applied Biosystems, Foster City, CA, USA) using a Light Cycler system. The reaction conditions were as follows: 40 cycles of 95˚C for 10 min, 95˚C for 15 sec and 60˚C for 1 min. GAPDH gene was amplified as an internal control. Primer sequence for QRT-PCR was shown in Table I. Each sample was measured in triplicate, and the $2^{-\Delta\Delta Ct}$ method

Table I. The primers for Real-time PCR.

Primer	Primer sequence(5′-3′)
MiR-329-3p F	GGGAACACACCTGGTTAAC
MiR-329-3p R	CAGTGCGTGTCGTGGAGT
GAPDH F	AATGGGCAGCCGTTAGGAAA
GAPDH R	TGAAGGGGTCATTGATGGCA

($\Delta CT = CT^{miR\text{-}329\text{-}3p} - CT^{GAPDH}$) was used to quantify the relative amount of miR-329-3p.

Statistical Analysis

SPSS software, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The differences between two groups were analyzed using the Student's *t* test. Correlations between clinicopathological parameters and miR-329-3p expression were determined using x^2 tests. Survival curves were constructed with the Kaplan-Meier method and compared by log-rank test. The significance of different variables was analyzed using the univariate and multivariate Cox regression analyses. A two-sided *p*-value < 0.05 was considered statistically significant.

Results

miR-329-3p is Down-Regulated in Human CC Specimens

To investigate the expression of miR-329-3p and its significance in CC, we first explored the expression of miR-329-3p by RT-PCR. As shown in Figure 1, we found that miR-153 was significantly

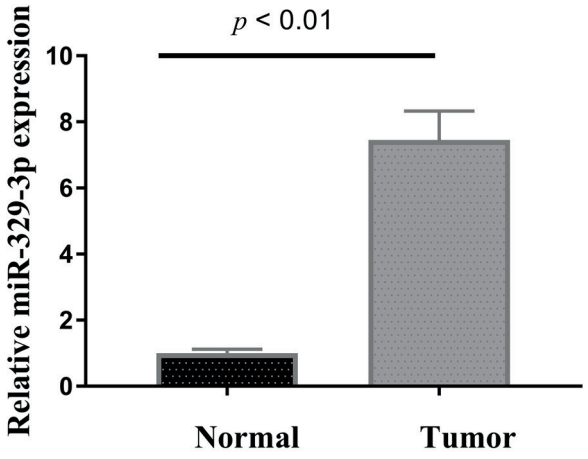

Figure 1. MiR-329-3p expression determined by Real-time PCR. MiR-329-3p expression was significantly higher in CC tissues compared with normal cervical tissues ($p < 0.01$).

Table II. The relationship of miR-329-3p expression with clinicopathological features of patients with CC.

Clinicopathological features	No. of cases	miR-329-3p-low (n, %)	miR-329-3p-high (n, %)	p
Age				0.303
< 45	91	43 (47.3)	48 (52.7)	
≥ 45	93	51 (54.8)	42 (45.2)	
Tumor size (cm)				0.468
< 4.0	122	60 (49.2)	62 (50.8)	
≥ 4.0	62	34 (54.8)	28 (45.2)	
Histological type				0.376
Squamous carcinoma	92	44(47.8)	48 (52.2)	
Adenocarcinoma	92	50(54.3)	42 (45.7)	
Tumor differentiation				0.211
Well + moderate	108	51 (47.2)	57 (52.8)	
Poor	76	43 (56.6)	33 (43.4)	
Lymph node metastasis				0.013
Yes	59	38 (64.4)	21 (35.6)	
No	125	56 (44.8)	69 (55.2)	
Distant metastasis				0.001
Yes	58	40 (69)	18 (31)	
No	126	54	72	
FIGO stage				0.024
I/II	131	60 (45.8)	71 (54.2)	
III/IV	53	34 (64.2)	19 (35.8)	

downregulated in the CC tissues compared with the normal cervical tissues ($p < 0.01$). These findings suggested that downregulation of miR-329-3p may play an important role in CC development.

Association of Expression Level of miR-329-3p with the Clinicopathological Parameters of CC

In order to assess the clinical role of miR-329-3p in CC, we further explored the association between miR-329-3p and the clinicopathological parameters of CC. According to the mean expression of miR-329-3p, CC patients was divided into two groups(high miR-329-3p expression group and low miR-329-3p expression group). As shown in Table II, MiR-329-3p level was significantly associated with lymph node metastasis ($p = 0.013$), FIGO stage ($p = 0.024$) and distant metastasis ($p = 0.001$). However, there were no significant associations between miR-329-3p expression and other clinical features including age, tumor size, histological type and tumor differentiation (all $p > 0.05$).

The Prognostic Value of miR-329-3p Expression in CC

To evaluate whether miR-329-3p expression levels can predict prognosis of patients with CC, we next performed Kaplan-Meier method. As shown in Figure 2, the results showed that patients with CC expressing a lower level of miR-329-3p had significantly shorter overall survival ($p = 0.0003$). Also, univariate and multivariate analysis were performed to determine whether miR-329-3p expression level was independent factors for prognostic prediction in CC patients. As shown in Table III, the results of multivariate analysis indicated that miR-329-3p expression was an independent prognostic factor for overall survival time [HR, 2.821; 95% CI, 1.343-5.671; $p = 0.004$] in patients with CC.

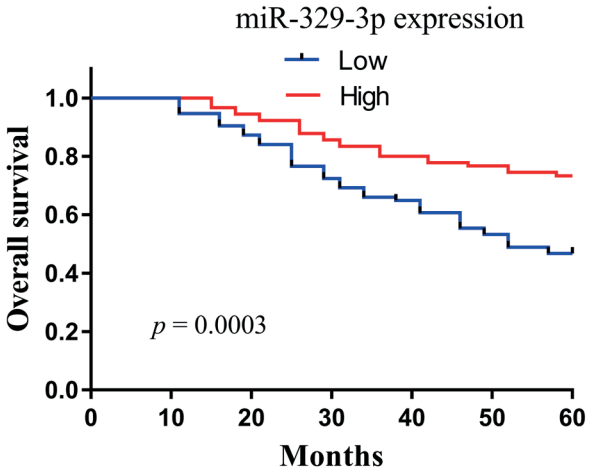

Figure 2. MiR-329-3p expression determined by Real-time PCR. MiR-329-3p expression was significantly higher in CC tissues compared with normal cervical tissues ($p < 0.01$).

Table III. Univariate and multivariate analysis of overall survival in CC patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p	HR	95% CI	p
Age	1.321	0.677-1.974	0.423	–	–	
Tumor size	1.789	0.782-2.213	0.277	–	–	–
Histological type	1.219	0.519-1.833	0.179	–	–	–
Tumor differentiation	1.472	0.683-2.733	0.338	–	–	–
Lymph node metastasis	3.782	1.832-6.423	0.002	3.127	1.423-5.219	0.004
Distant metastasis	3.313	1.523-7.892	0.001	2.783	1.201-6.872	0.001
FIGO stage	2.452	1.178-4.388	0.007	2.021	1.044-3.672	0.011
MiR-329-3p expression	3.231	1.672-6.896	0.001	2.821	1.343-5.671	0.004

Discussion

Cervical cancer contributes to an estimated 530,000 new cases and 275,000 deaths per year[22]. The clinical outcome of patients with CC depends mainly on the clinical stage[23]. The reliable identification of CC progression has important clinical implications for its prevention and treatment. Many genes are involved in CC development and progression and more and more markers were identified to be predictive of CC aggressiveness[24,25]. However, up to now, most of these markers had not been proven to be sufficiently effective.

MiRNAs exhibit an abnormal expression and function in various types of malignancies. MiR-329-3p has been reported to serve as a tumor suppressor in a variety of human tumors. For instance, Yang et al[26] found that miR-329 was down-regulated in glioblastoma and the overexpression of miR-329 in neuroblastoma cell lines inhibited the proliferation and migration by targeting KDM1A 3'-UTR. Wang et al[27] reported that miR-329 served as a tumor suppressor in pancreatic cancer by targeting GRB2. Furthermore, down-regulation of miR-329 was associated with poor prognosis of pancreatic cancer patients. Xiao et al[28] revealed that over-expression of miR-329 inhibited glioma proliferation by modulating E2F1 expression and Akt pathway. Moreover, Zhou et al[19] suggested that miR-329 was down-regulated in hepatocellular carcinoma and patients with low expression of miR-329 have significantly poorer prognosis, miR-329 up-regulation could promote cell invasion by regulating BRD4. Besides, Li et al[21] found that miR-329-3p expression was significantly down-regulated in CC tissues. *In vitro* assay indicated that forced expression of miR-329-3p suppresses cell proliferation, migration, and invasion in CC by targeting MAPK1. Those results indicated that miR-329-3p was important in controlling ovarian carcinogenesis. However, previous study only reported the effect of miR-329-3p in tumor proliferation and metastasis. The clinical value of miR-329-3p in CC patients has not been investigated.

In the present study, we detected the expression levels of miR-329-3p in CC tissues and matched normal tissues. Our data also confirmed down-regulated expression of miR-329-3p in CC tissues. Furthermore, we found that miR-329-3p expression was associated with lymph node metastasis, FIGO stage and distant metastasis, indicating that miR-329-3p might be involved in the metastasis of CC. Then, by Kaplan-Meier method, we showed that patients with low miR-329-3p expression had shorter mean months of overall survival than did patients with high miR-329-3p expression. More importantly, univariate and multivariable Cox regression analyses identified that down-regulated miR-329-3p might act as an independent prognostic factor for CC patients. However, further studied are needed to explore the potential mechanisms by which miR-329-3p influenced the outcome of CC patients.

Conclusions

This is the first work highlighting the clinical significance of miR-329-3p in CC, and it might serve as a potential prognosis marker for CC patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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