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Expression of microRNA-142-3p in cervical cancer and its correlation with prognosis

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Abstract. – OBJECTIVE: The aim of current study was to assess the association between miR-142-3p expression and the prognosis of patients with cervical cancer.

PATIENTS AND METHODS: Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to assess the expression level of miR-142-3p in cervical cancer tissues and matched normal tissues. Then, statistical analysis was performed to determine the associations of miR-142-3p expression with the clinical features and the prognosis of cervical cancer.

RESULTS: Significantly lower levels of miR-142-3p was observed in the cervical cancer tissue, compared with the adjacent normal cervical tissue from the same patient ($p < 0.05$). Low miR-142-3p expression level was significantly associated with advanced FIGO stage ($p = 0.002$), lymph node metastasis ($p = 0.005$), and depth of cervical invasion ($p = 0.006$). Furthermore, Kaplan-Meier survival analysis demonstrated that patients with low miR-142-3 expression had poorer overall survival and progression-free survival ($p < 0.001$, respectively). The univariate proportional hazard model suggested that FIGO stage, lymph node metastasis, depth of cervical invasion and miR-142-3p expression level were prognostic predictors. Finally, Multivariate survival analysis also confirmed that miR-142-3p could be an independent prognostic marker for both overall survival and progression-free survival.

CONCLUSIONS: MiR-142-3p may be a potential novel biomarker that predicts prognosis in cervical cancer.

Key Words:
MiR-142-3p, Prognosis, Cervical cancer.

Introduction

Cervical carcinoma is one of the most common malignant tumors and fourth most frequent cause of cancer death in the female reproductive system[1,2]. Despite significant improvements in screening, diagnosis, and therapy of cervical cancer over the past decade, the prognosis of advanced patients generally remains poor, because tumor recurrence and metastasis frequently occur in patients with advanced cancer[3-5]. Therefore, the identification of new accurate biological markers for diagnosis and prognosis are urgently required.

MicroRNAs (miRNAs) are small, endogenous, noncoding RNAs of approximately 22 nt that regulate the expression of target mRNA by binding to 3'-untranslated regions[6]. Due to their widespread regulation on protein-coding genes, miRNAs regulates many important biological processes, such as tumorigenesis, inflammation, and development[7,8]. Growing evidences show that miRNAs usually promotes or suppress tumor progression, metastasis depending on the cell type or their target genes[9,10]. For instance, Li et al[11] showed that miR-138 functioned as a tumor suppressive effect in cervical cancer. They confirmed that miR-138 suppressed proliferation of cervical cancer cells by targeting c-Met. On the other hand, Xu et al[12] reported that miR-21 served as a tumor promoter in cervical cancer. They further showed that miR-21 could promote the proliferation, migration, and invasion in cervical cancer cells via inhibiting the PTEN expression. The above finding highlighted the important role of miRNAs in tumors including cervical cancer.

Recently, it was reported that miR-142-3p was significantly downregulated in several tumors, such as hepatocellular carcinoma[13], acute myeloid leukemia[14], and colon cancer[15]. However, clinical significance and prognostic value of miR-142-3p in cervical cancer have not been reported previously. Thus, the aim of this work was to investigate the clinical significance of miR-142-3p in cervical cancer.

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Patients and Methods

Patients and Tissue Samples

This study was conducted on a total of 173 cervical cancer samples, which were histopathologically and clinically diagnosed at the Qilu Hospital of Shandong University from 2008 to 2012. Patients with cervical cancer were treated with radical hysterectomy, but none of them received chemotherapy. Medical records were obtained to review patient data including age, tumor size, Histologic grade, FIGO stage, lymph node metastasis, depth of cervical invasion. All samples were confirmed by a senior pathologist and were staged according to the Federation International of Gynecology and Obstetrics (FIGO) staging system for cervical cancer. The characteristics of all patients are listed in Table I. Prior patient's consent and approval from the Institute Research Ethics Committee were obtained.

RNA Isolation, Quantitative Real-Time RCR (qRT-PCR)

Total RNA was extracted from surgical specimens using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The purity of RNA was detected by Spectrophotometer 260/280. Then 1 ug of RNA was reverse transcribed to cDNA. Real-time PCR was performed in triplicate using QuantiFast SYBR Green PCR kit (Suzhou, Jiangsu, China) with a Bio-Rad C1000 Thermal Cycler. The primers selected were as follows: miR-142-3p, forward primer, 5'-CGCCGTGTAGTGTTTCCTAC-3', reverse primer: 5'-CAGTGCAGGGTCCGAGGT-3'; A common universal reverse primer and U6 was used as an endogenous standard. For quantitation, the comparative ΔCt method was used.

Statistical Analysis

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) or Prism 5.0 (GraphPad Software, La Jolla, CA, USA) software. The difference between two groups was analyzed by students' *t*-test. The statistical significance of the correlations between miR-142-3p expression and the clinicopathologic features were analyzed using the Chi-square test. Survival time was calculated from the date of CRC diagnosis to the date of death or last follow-up. For survival analysis, Kaplan-Meier curve was generated with the log-rank test. Moreover, the prognostic significance of miR-142-3p was valued by Cox regression analysis. The difference was considered to be statistically significant when the *p*-value was less than 0.05.

Results

Expression Level of miR-142-3p is Down-regulated in Cervical Cancer Patients

To determine whether miR-142-3p expression was changed in human cervical cancer, RT-qPCR

Table I. Association between miR-142-3p and clinicopathological parameters of cervical cancer.

Clinicopathological features	Total	miR-142-3p expression		*p*
		Low	High	
Age				0.769
< 45	65	31	34	
≥45	108	54	54	
Tu mor size (cm)				0.518
< 4.0	63	33	30	
≥ 4.0	110	52	58	
Histologic grade				0.917
G1 + G2	76	37	39	
G3	97	48	49	
FIGO stage				0.002
Ib-IIa	88	33	55	
IIb-IIIa	85	52	33	
Lymph node metastasis				0.005
No	121	51	70	
Yes	52	34	18	
Depth of cervical invasion				0.006
< 2/3	130	56	74	
≥ 2/3	43	29	14	

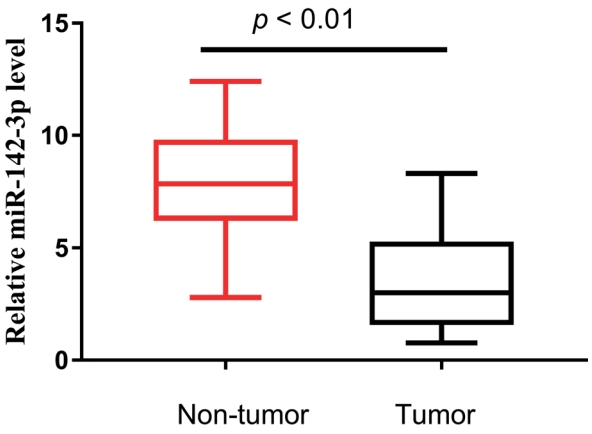

Figure 1. Expression of miR-142-3p is decreased in cervical cancer tissues compared with normal cervical tissues ($p < 0.001$).

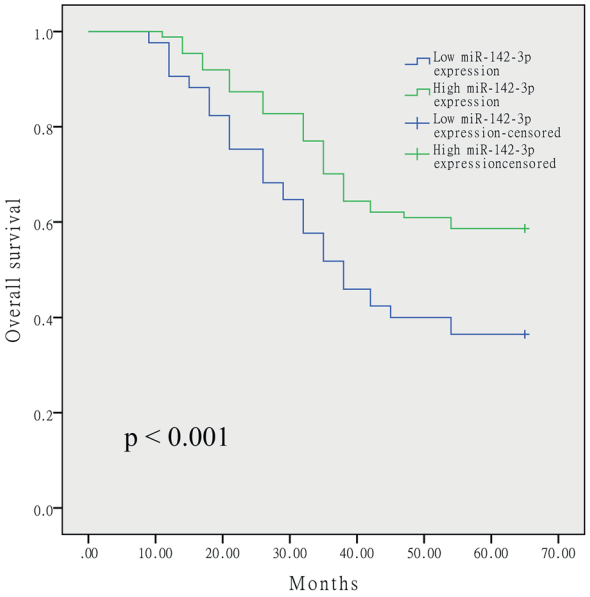

Figure 2. The 5-year overall survival rate of cervical cancer patients with high-miR-142-3p expression was significantly higher than that of those patients with low-miR-142-3p expression ($p < 0.001$).

was performed using 173 paired cervical cancer tissues and adjacent normal tissues. As shown in Figure 1, the results showed that miR-142-3p was significantly down-regulated in cervical cancer tissues compared to normal adjacent cervical tissues ($p < 0.001$).

Correlation of miR-142-3p Expression in Cervical Cancer with Clinicopathological Features

Next, We analyzed the correlation between the miR-142-3p expression and various clinicopathological factors of the cervical cancer patients. Patients with above average miR-142-3p expression were assigned to the high expression group, and patients with below average miR-142-3p expression were assigned to the low expression group. As shown in Table II, the data showed that low miR-142-3p expression level was significantly associated with advanced FIGO stage ($p = 0.002$), lymph node metastasis ($p = 0.005$), and depth of cervical invasion ($p = 0.006$). However, there was no significant difference in age, tumor size and histologic grade (all $p > 0.05$).

Low miR-142-3p Expressions Correlated with Poor Prognosis in Cervical Cancer

Survival data were obtained from 173 cervical cancer patients with median follow-up time of 21 months ranging from 3 to 60 months. From the Kaplan-Meier survival curves, we found that patients with low expression of miR-142-3p had poorer overall survival ($p < 0.001$) and progression-free survival ($p < 0.001$) compared with those with the high miR-142-3p group.

Table II. Univariate analysis of prognostic parameters in patients with cervical cancer by Cox regression analysis.

Variable	Overall survival		Progression-free survival	
	RR	p	RR	p
Age ≥ 45 *vs.* < 45	1.318	0.327	1.126	0.342
Tumor size (cm) < 4.0 *vs.* ≥ 4.0	1.126	0.311	0.922	0.351
Histologic grade G1 + G2 *vs.* G3	1.149	0.216	1.028	0.237
FIGO stage Ib-IIa vs. IIb-IIIa	2.879	0.006	2.673	0.014
Lymph node metastasis No *vs.* Yes	3.236	0.004	2.894	0.008
Depth of cervical invasion < 2/3 *vs.* ≥ 2/3	3.563	0.003	2.955	0.007
miR-142-3p expression Low *vs.* High	3.236	0.002	2.762	0.004

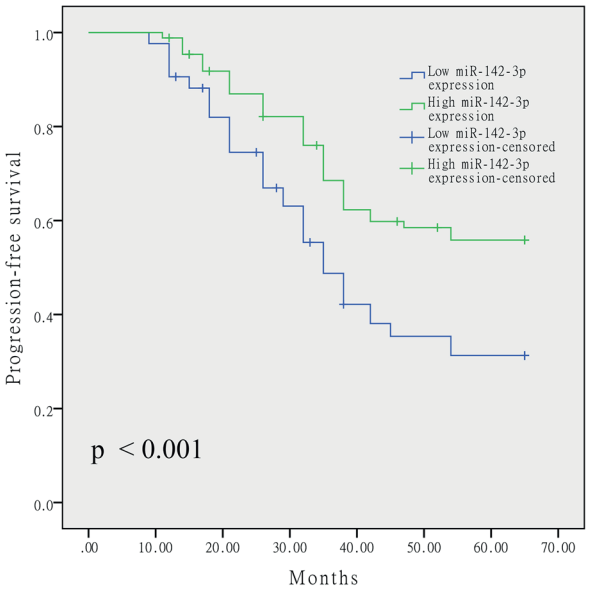

Figure 3. The 5-year progression-free survival rate of cervical cancer patients with high-miR-142-3p expression was significantly higher than that of those patients with low-miR-142-3p expression ($p < 0.001$).

In the univariate survival analysis, FIGO stage ($p = 0.006$ and $p = 0.014$), lymph node metastasis ($p = 0.004$ and $p = 0.008$), Depth of cervical invasion ($p = 0.003$ and $p = 0.007$) and miR-142-3p expression ($p = 0.002$ and $p = 0.004$) were significant adverse prognostic factors for overall survival and progression-free survival, respectively (Table II). Notably, multivariate analysis results revealed that miR-142-3p expression ($p = 0.001$ and $p = 0.002$) was an independent prognostic factor for overall survival and progression-free survival (Table III).

Discussion

MiRNA has been identified as important developmental regulators, and their aberrant expression may play a critical role in clinical outcome[16]. Growing miRNAs were confirmed as independent prognostic biomarkers for cervical cancer, such as miR-335[17], miR-664[18], and miR-1246[19]. A previous study showed that miR-142-3p expression levels in cervical cancer tissues were significantly downregulated, and over-expression of miR-142-3p inhibits cell proliferation and invasion of cervical cancer cells by targeting FZD7, suggesting that miR-142-3p may serve as a tumor suppressor cervical cancer. In the present, we focus the prognostic value of miR-142-3p in patients with cervical cancer. Firstly, we detected miR-142-3p expression in 173 cervical cancer tissues and normal cervical tissues. We found that MiR-142-3p expression is significantly down-regulated in cervical cancer tissues compared with normal controls. Next, we analyzed the association between miR-142-3p expression and clinicopathologic variables. The data showed that low miR-142-3p expression level was significantly associated with advanced FIGO stage, lymph node metastasis, and depth of cervical invasion, suggesting miR-142-3p may play a positive effect in the progression of cervical cancer. Moreover, to explore the effect of miR-142-3p in the prognosis of cervical cancer, we performed the Kaplan-Meier method. Our findings showed that low miR-142-3p levels in cervical cancer were associated with shorter overall survival and progression-free survival. At last, the univariate and multivariate analysis revealed that low expression of plasma miR-142-3p was an independent predictor of overall survival and progression-free survival. Taken together, our findings revealed that decreased miR-142-3p could be a promising biomarker for predicting poor survival in cervical patients.

MiR-142-3p has been reported to play critical effect in various tumors. For instance, Wu et al[13] reported that overexpression of miR-142-3p suppressed colony formation, migration, and invasion in hepatocellular carcinoma cell by regulating RAC1. Shen et al[15] found that the miR-142-3p was markedly decreased in colon cancer specimens, and its over-expression could inhibited the

Table III. Multivariate analysis of prognostic parameters in patients with cervical cancer by Cox regression analysis.

Variable	Overall survival		Progression-free survival	
	RR	p	RR	p
FIGO stage Ib-IIa *vs.* IIb-IIIa	2.562	0.003	2.267	0.016
Lymph node metastasis No *vs.* Yes	2.983	0.002	2.633	0.005
Depth of cervical invasion < 2/3 *vs.* ≥ 2/3	3.125	0.001	2.673	0.005
miR-142-3p expression Low *vs.* High	2.842	0.001	2.531	0.002

growth of colon cancer cells by downregulation of CD133, Lgr5, and ABCG2. Schwickert et al[20] showed that miR-142-3p suppressed breast cancer cell invasiveness by targeting of WASL. More important, a previous study by Cao et al[21] showed that miR-142-3p inhibits breast, lung and cervical cell proliferation by directly regulating CDC25C expression. All these results revealed the critical effect of miR-142-3p in tumors. Thus, we hypothesized that miR-142-3p may be associated with the prognosis of cervical cancer patients. As our above results, we conformed our thought. However, further studies were needed to elucidate the mechanisms underlying miR-142-3p regulating cervical cancer pathogenesis.

Conclusions

We observed that miR-142-3p performs an essential function in cervical cancer pathogenesis and can serve as a novel prognostic marker of cervical cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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