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Original Article

Reduced serum miR-142-3p predicates worse prognosis in patients with cervical cancer

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Abstract: MicroRNAs (miRNAs) are promising for diagnosis and prognosis prediction in many diseases including cancer. The aim of the current study was to investigate the clinical value of serum miR-142-3p in cervical cancer. Real-time PCR was carried out to examine the expression level of serum miR-142-3p in patients with cervical cancer. Then its relationship with clinical features was analyzed. Our results showed that cervical patients with lower tissue miR-142-3p had a shorter long term overall survival time based on the Cancer Genome Atlas (TCGA) cohort. Serum miR-142-3p level was reduced in cervical cancer patients compared to cervical intraepithelial neoplasia (CIN) patients and healthy controls. Serum miR-142-3p had good performance in discriminating cervical cancer patients from CIN patients and healthy controls. In addition, low serum miR-142-3p was positively correlated with advanced clinical stage and lymph node metastasis as well as worse 5 year overall/disease free survival. Furthermore, serum miR-142-3p was an independent risk factor for cervical cancer. In conclusion, low expression of serum miR-142-3p was associated with poor clinical outcome of cervical cancer, indicating it might be a promising novel biomarker for predicting the prognosis of cervical cancer.

Keywords: miR-142-3p, biomarker, cervical cancer, prognosis, serum

Introduction

Despite the successful introduction of cervical screening, cervical cancer remains the third most prevalent malignancy affecting women worldwide [1]. About 265,000 women died from cervical cancer in 2012 [2]. The clinical outcome of cervical cancer is mainly depended on clinical stage. Cervical patients at the advanced stage suffered a higher recurrence rate and worse 5 year five year survival [3]. The identification of biomarkers to predict cancer development and prognosis of patients is very important for individualized therapies [4].

MicroRNAs (miRNAs) are small, non-coding RNAs that can inhibit their target genes at the posttranscriptional level by binding to the target mRNA and typically in the 3'-untranslated region (3'-UTR) [5]. miRNAs play a central role in regulating many biological processes such as proliferation, differentiation, apoptosis, growth and survival [6-8]. Deregulation of miRNAs has been demonstrated to be key regulators of carcinogenesis [9]. As miRNAs are highly stable in the serum sample, they are promising biomarkers for early detection of cancer and prognosis prediction [10, 11]. Liu et al reported that the expression level of miR-196a was significantly upregulated in the serum samples from cervical cancer patients. In addition, higher serum miR-196a was associated with poor prognosis of cervical cancer, indicating miR-196a might act as an oncogene in cervical cancer [12]. Similarly, serum concentration of miR-425-5p in cervical cancer patients was higher in cervical patients compared to those with cervical diseases and healthy controls. More importantly, serum miR-425-5p was an independent prognosis factor for cervical cancer [13].

Our pre-experiment indicated that miR-142-3p was one of the most dysregulated miRNAs in the serum samples of cervical patients (data not shown). In this study, we systematically evaluated the expression of serum miR-142-3p in healthy controls, cervical intraepithelial neo-

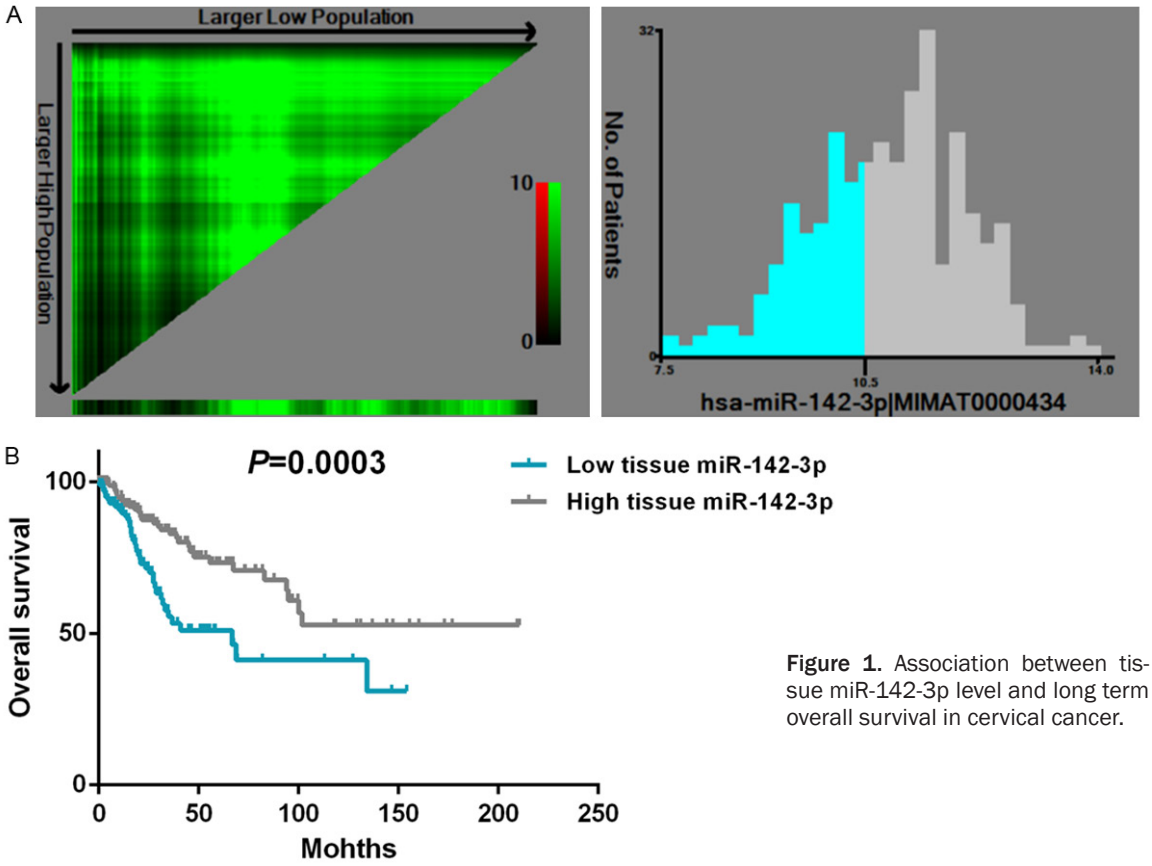

Figure 1. Association between tissue miR-142-3p level and long term overall survival in cervical cancer.

plasia (CIN) patients and cervical cancer patients. Our goal was to determine the potential clinical value of serum miR-142-3p as a biomarker for diagnosis and prognosis of cervical cancer.

Materials and methods

Patients and clinical samples

Patients with cervical cancer or cervical intraepithelial neoplasia were prospectively recruited from the Department of Gynecology. The diagnosis of cervical cancer and CIN was confirmed by histological evaluation. The study design was approved by the Research Ethics Committee of the 2nd Affiliated Hospital of Harbin Medical University and all patients signed an informed consent form for collection of blood samples. None of the patients had previously received chemotherapy, radiation therapy or surgery before blood collection. All of the enrolled cervical patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. Overall survival (OS) was defined as the date of diagnosis to death or last follow-up. The primary endpoint of disease-free survival (DFS) was the evidence of recurrence or metastasis.

About 4 mL peripheral blood sample was drawn from all the participants and serum was immediately separated from the clot by centrifugation at 1300 g for 20 min at room temperature. The supernatant serum was stored at -80°C until further use.

miRNA isolation and real-time RT-PCR

Total RNA was isolated from 300 μl serum samples using a miRVana PARIS Kit (Ambion, Austin, TX, USA) according to the manufacturer's instruction. The first strand cDNA was synthesized using miScript II RT kit (Qiagen). Then the miScript SYBR Green PCR kit (Qiagen) was used for the amplification of cDNA. The reactions were performed on ABI PRISM 7900 HT real-time RT-PCR system (Applied Biosystems, Foster City, CA) under following conditions: 15 min at 95°C for 1 cycle, 15 s at 94°C, 30 s at 55°C, and 30 s at 70°C for 40 cycles. Relative expression of serum miR-142-3p was normal-

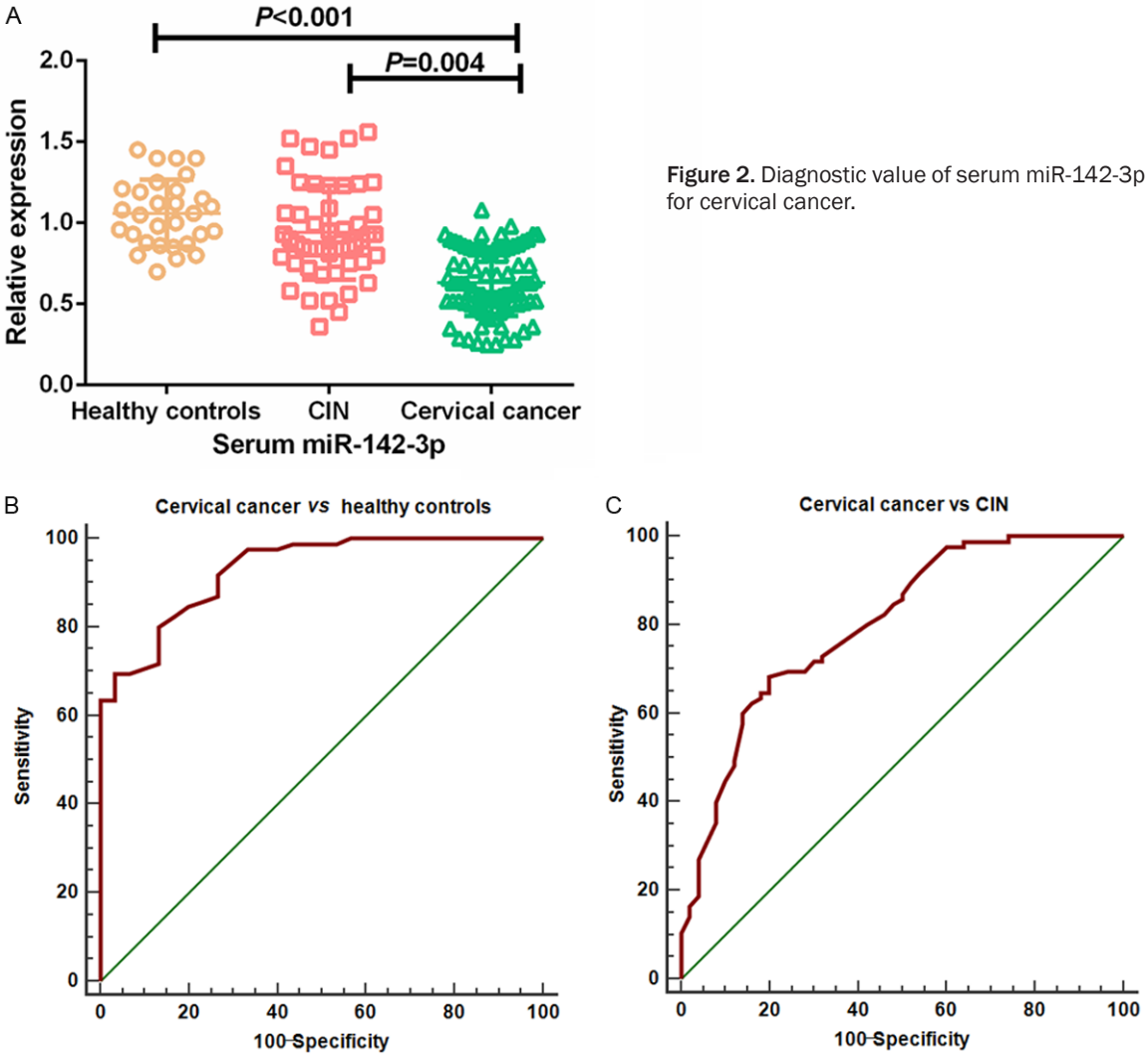

Figure 2. Diagnostic value of serum miR-142-3p for cervical cancer.

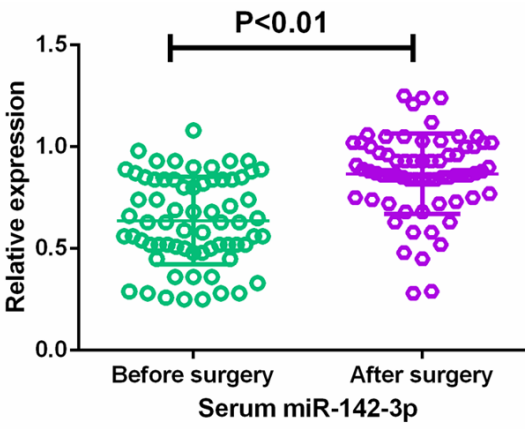

Figure 3. Serum miR-142-3p was significantly upregulated following surgery.

ized to U6 snRNA and the fold change in miR-142-3p expression in different experimental groups was expressed as $2^{-\Delta\Delta Ct}$. All the samples were run in triplicate.

Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). A $P<0.05$ was considered statistically significant. Nonparametric Kruskal-Wallis test was used to compare the differences in serum miR-142-3p expression among experimental groups. The diagnostic value of serum miR-142-3p was evaluated by receiver operating curves (ROCs). Association between serum miR-142-3p and clinicopathological parameters was analyzed by Chi-squared test. Cox proportional hazards regression was performed to explore the independent prognostic factors. For the TCGA database

Table 1. Association between serum miR-142-3p expression level and the clinical features of cervical cancer

Parameters	Cases	Serum miR-142-3p expression		*P*
		Low	High	
Age				0.935
<50	46	22	24	
≥50	39	19	20	
Tumor size				0.213
<4	37	15	22	
≥4	48	26	22	
FIGO Stage				0.007
I-II	50	18	32	
III-IV	35	23	12	
Lymph node metastasis				0.036
No	59	24	35	
Yes	26	17	9	
Distant metastasis				0.242
No	76	35	41	
Yes	9	6	3	
Cell type				0.766
Squamous	78	38	40	
Non-squamous	7	3	4	
Histological grade				0.249
Well/moderate	57	25	32	
Poor	28	16	12	

analysis, the expression level of miR-142-3p was log2-transformed (http://cancergenome.nih.gov/). X tile software was used to find out the best cutoff point to divide the cervical cancer patients from TCGA database into high and low miR-142-3p expression group [14]. All the survival curves were constructed with the Kaplan-Meier method and compared by log-rank tests.

Results

Correlation between tissue miR-142-3p expression and long term overall survival

X tile software was used to find out the best cutoff point for the cervical cancer patients cohort in the TCGA database. A total of 192 cancer patients were in the high tissue miR-142-3p expression group (gray color) while 113 patients were in the low tissue miR-142-3p expression group (blue color) (**Figure 1A**, **1B**). The results showed the cervical cancer patients in the low tissue miR-142-3p expression group suffered poorer long term overall survival than the patients in the high tissue miR-142-3p expression group (*P*=0.0003) (**Figure 1C**).

Serum miR-142-3p was downregulated in cervical cancer and its diagnostic value

We first compared the serum miR-142-3p levels among 85 cervical cancer patients, 50 CIN patients and 30 healthy controls using real-time PCR. Our data showed that the expression level of serum miR-142-3p was remarkably downregulated in patients with cervical cancer when compared to CIN patients (*P*=0.004) and healthy volunteers (*P*<0.001). However, no significant difference was found between CIN patients and healthy controls (*P*>0.05) (**Figure 2A**).

We then evaluated the diagnostic value of serum miR-142-3p for cervical cancer. Our ROC analysis showed that serum miR-142-3p not only was able to discriminate cervical cancer patients from healthy controls (AUC=0.88, specificity =0.92, sensitivity =0.80) (**Figure 2B**), but also differentiate cervical cancer from CIN (AUC=0.79, specificity =0.85, sensitivity =0.72) (**Figure 2C**).

Serum miR-142-3p was upregulated in cervical cancer patients who received surgery

Sixty-eight cervical cancer patients received surgery therapy and we compared the serum miR-142-3p levels before and after surgery. Our results showed that serum miR-142-3p was significantly upregulated following surgery (*P*< 0.01), indicating serum miR-142-3p was a sensitive marker for monitoring therapeutic responses (**Figure 3**).

The association between serum miR-142-3p and clinicopathological parameters of cervical cancer

The median value of serum miR-142-3p was used to divide the patient cohort into two groups. The Chi-squared results showed that a higher percentage of cervical patients in the low serum miR-142-3p group suffering from advanced clinical stage (*P*=0.007) and positive lymph node metastasis (*P*=0.036). However, serum miR-142-3p levels were not correlated with age, tumor size, distant metastasis, cell type and histological grade (**Table 1**).

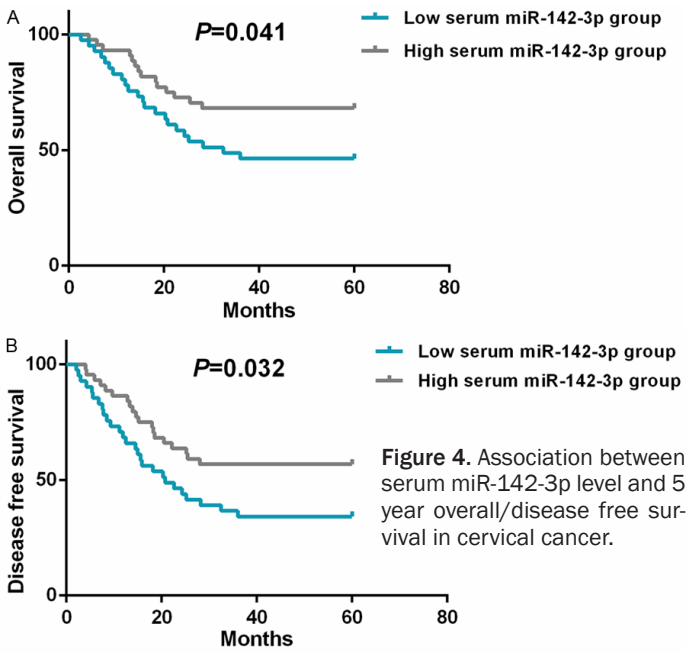

Figure 4. Association between serum miR-142-3p level and 5 year overall/disease free survival in cervical cancer.

Table 2. Multivariate analysis of 5-year overall/disease free survival in patients with cervical cancer

Variable	HR	95% CI	*P*
Overall survival			
FIGO stage (III-IV *vs.* I-II)	3.84	1.86-7.78	0.007
Serum miR-142-3p (low *vs.* high)	3.05	1.52-6.56	0.021
Disease free survival			
FIGO stage (III-IV *vs.* I-II)	4.28	1.92-8.49	0.005
Serum miR-142-3p (low *vs.* high)	2.91	1..42-6.24	0.028

Decreased serum miR-142-3p was associated with poor prognosis of cervical cancer

Our survival analysis demonstrated that the cervical patients in the low serum miR-142-3p group had a significant worse 5 year overall (*P*=0.041) and disease free survival rate (*P*=0.032) than those in the high serum miR-142-3p group (**Figure 4A**, **4B**). The multivariate analysis revealed that serum miR-142-3p was independent prognostic risk factor for both overall survival (HR=3.05, 95% CI=1.52-6.56, *P*=0.021) and disease free survival (HR=2.91, 95% CI=1.42-6.24, *P*=0.028) (**Table 2**).

Discussion

TCGA database consist of the genetic information and clinical data of more than 30 types of cancers, which are very useful for exploring clinical outcome related markers. In the present study, we first demonstrated that cervical cancer patients with lower tissue miR-142-3p levels had poorer long term overall survival based on the TCGA cohort. Then serum miR-142-3p was found to be significantly downregulated in cervical cancer patients and able to distinguish the cervical patients from CIN patients and healthy controls. In addition, serum miR-142-3p level was very sensitive to surgery therapy. Furthermore, low serum miR-142-3p was demonstrated to be associated with poor prognosis of cervical cancer. These data indicate that miR-142-3p downregulation in cervical cancer promote its progression. Similarly, Deng et al reported that ectopic expression of miR-142-3p suppressed the proliferation and invasion capacity of cervical cancer cells, while miR-142-3p downregulation promoted their invasive capability, indicating miR-142-3p might play a tumor suppressive role in cervical cancer. They also demonstrated that Frizzled7 receptor was a downstream target of miR-142-3p [15]. A limitation of current study was the small sample size, large multicenter cohort should be carried out to further determine the clinical significance of serum miR-142-3p in cervical cancer. In addition, combination of miR-142-3p and other biomarkers might improve the diagnostic and prognostic performance. The molecular mechanisms accounting for the tumor suppressive role of miR-142-3p in cervical cancer needed to be elucidated.

miR-142-3p has been reported to function as a tumor suppressor in various types of cancers. miR-142-3p was overexpressed in non-small-cell lung carcinoma (NSCLC) tissues and cell lines. Upregulation of miR-142-3p enhanced proliferation and induced apoptosis of NSCLC cells, and HMGB1 was proved to be a direct target of miR-142-3p [16]. Both miR-142-3p and miR-142-5p were significantly reduced in hepatocellular carcinoma (HCC) and their expression levels were closely correlated with disease progression. Overexpression of miR-142 inhibited the migration and invasive capacity of HCC cells by influencing cell motility associated

pathways [17]. The expression of miR-142-3p was decreased in follicular thyroid adenomas and carcinomas compared to the controls. Ectopic overexpression of miR-142-3p inhibited the proliferation capacity of thyroid cancer cell lines and two genes (ASH1L and MLL1) were demonstrated to be downstream targets of miR-142-3p, suggesting miR-142-3p was a tumor suppressor in thyroid cancer [18].

Some miRNAs might act as tumor suppressors in some types of cancers, while play an oncogenic role in other types of cancers [19]. Therefore it is no wonder that miR-142-3p could promote tumor progression in some studies. The expression level of miR-142-3p was significantly overexpressed in nasopharyngeal carcinoma (NPC) tissues and cell lines. In addition, miR-142-3p inhibition suppressed the proliferation capacity of cancer cells both i*n vitro* and *in vivo*, indicating that miR-142-3p was a tumor promoter in NPC [20]. Similarly, miR-142-3p levels were increased in renal cell carcinoma (RCC) tissues compared to adjacent normal tissues. Knockdown of miR-142-3p inhibited the proliferation and migration capacity of RCC cells, while promoted their apoptosis [21]. These data indicate that the role of miR-142-3p is dependent on the tumor type and its function might be associated with the concrete microenvironment.

Taken together, serum miR-142-3p is decreased in patients with cervical cancer and has good diagnostic performance. Decreased serum miR-142-3p is a predictor of poor prognosis in cervical cancer, supporting that miR-142-3p might be a promising biomarker for this malignancy.

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Disclosure of conflict of interest

None.

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