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Decreased miR-452 expression in human cervical cancer and its prognostic significance

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Summary

Objective: MicroRNA-452 (miR-452) was previously reported to be dysregulated in several types of human cancers and involved in tumor progression. However, its role in cervical cancer is still unclear. The aim of this study was to investigate the clinical significance and prognostic value of miR-452 expression in human cervical cancer. *Materials and Methods:* The expressions of miR-452 was detected in 137 pairs of cervical cancer specimens and adjacent non-cancerous tissues using qRT-PCR assay. Then, the association of miR-452 levels with clinicopathological features and prognosis was evaluated. *Results:* MiR-452 expression was significantly downregulated in cervical cancer tissues than those corresponding non-cancerous tissues ($p < 0.001$). Decreased miR-452 expression was linked to lymph node metastasis, vascular invasion, poor tumor differentiation, advanced FIGO Stage, and short overall survival. Multivariate Cox regression analysis confirmed that low level of miR-452 expression predicted poor prognosis of patients with cervical cancer independently. *Conclusions:* The present results suggest that miR-452 downregulation may be involved in cervical cancer formation and progression, and that miR-452 would serve as a novel prognostic biomarker for patients with this disease.

Key words: MicroRNA-452; Cervical cancer; Real-time quantitative RT-PCR; Prognosis.

Introduction

Cervical cancer is the second most frequent malignant gynecological neoplasm worldwide, with more than 0.5 million new cases annually [1]. Despite recent improvements in multimodal therapy including surgery, radiotherapy, and chemotherapy, about 230,000 women die of cervical cancer each year [2]. Accumulating studies have revealed that genetic changes, including the silencing of tumor-suppressor genes and the overexpression of oncogenes, are involved in cervical malignant transformation [3]. However, the molecular pathogenesis of cervical cancer are still largely unknown till now. Therefore, it is urgent to elucidate the regulatory network underlying cervical cancer and develop novel biomarkers for its early diagnosis, accurate assessment, targeted therapy, and prognosis evaluation.

MicroRNAs (miRNAs) are short (about 22 nucleotides in length), highly conserved small non-coding RNA molecules that negatively regulate gene expression by binding to target message RNAs (mRNAs) at their 3′-untranslated region, leading to mRNA degradation or translation suppression [4]. Deregulation of miRNA expression has been identified in many human diseases, including cancers. Emerging evidence suggests that miRNAs act as key regulators in a wide variety of biological processes that contribute to tumorigenesis and development, such as tumor cell differentiation, proliferation, apoptosis, invasion, angiogenesis, and epithelial mesenchymal transition [5-9]. A large number of studies have demonstrated that miRNAs may be promising as diagnostic and prognostic biomarkers of human cancers [10]. In terms of cervical cancer, abnormal expression of several miRNAs and their function has been reported. For example, miR-126 showed decreased expression in cervical cancer tissues, and its downregulation was correlated with lymphatic invasion, distant metastasis, poor tumor differentiation, and advanced clinical stage [11]. Low miR-26b and miR-503 expression levels were unfavourable prognostic factors for both recurrence-free and overall survival in cervical cancer patients [12, 13]. Plasma miR-127 and miR-205 might serve as non-invasive biomarkers for detection of cervical cancer [14]. Ectopic expression of miR-133a inhibited cervical cancer cell proliferation, colony formation, and invasion, promoted cell apoptosis in vitro, and suppressed tumorigenicity in vivo [15]. Overexpression of miR-223 inhibited cervical cancer metastasis by modulating epithelial-mesenchymal transition [16]. Furthermore, miR-126 enhanced chemosensitivity, and miR-21 decreased radiosensitivity of cervical cancer [17, 18]. Thus, miRNAs may be applied for cervical cancer diagnosis and prognosis, and also act as potential novel therapeutic targets.

One of the cancer-related miRNAs is miR-452. Recently, aberrant miR-452 expression has been reported in glioma

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Table 1. — *Association of miR-452 expression with different clinicopathological features of human cervical cancer.*

Clinicopathological features	No. of cases	miR-452 expression High (n, %)	Low (n, %)	*p*
Age (years)				
≤ 50	69	37 (53.6%)	32 (46.4%)	0.496
> 50	68	32 (47.1%)	36 (52.9%)	
Tumor size (cm)				
< 4	75	40 (53.3%)	35 (46.7%)	0.484
≥ 4	62	29 (46.8%)	33 (53.2%)	
Histological grades				
Well/moderate	72	44 (61.1%)	28 (38.9%)	0.002
Poor	65	25 (38.5%)	40 (61.5%)	
Lymph node metastasis				
Positive	40	10 (25.0%)	30 (75.0%)	<0.001
Negative	97	59 (60.8%)	38 (39.2%)	
Vascular invasion				
Positive	70	27 (38.6%)	43 (61.4%)	0.006
Negative	67	42 (62.7%)	25 (37.3%)	
Histological type				
Adenocarcinoma	25	12 (48.0%)	13 (52.0%)	0.828
Squamous cell carcinoma	112	57 (50.9%)	55 (49.1%)	
FIGO stage				
I	58	47 (81.0%)	11 (19.0%)	<0.001
II	79	22 (27.8%)	57 (72.2%)	

[19], non-small cell lung cancer (NSCLC) [20], bladder cancer [21], prostate cancer [22], urothelial carcinoma [23], and hepatocellular carcinoma [24]. In these malignancies, miR-452 acts as a potential oncogene or a candidate tumor suppressor in different cellular contexts. However, the expression and clinical significance of miR-452 in human cervical cancer has not been investigated. Therefore, in the current study, the authors examined miR-452 expression in cervical cancer specimens and paired adjacent non-cancerous tissues, and analyzed the correlation between miR-452 expression and clinicopathological factors, as well as patient's survival.

Materials and Methods

Fresh cervical cancer specimens and matched adjacent noncancerous tissues were collected from 137 patients who underwent surgery at The Second Affiliated Hospital of Zhengzhou University (Zhengzhou 450003, China) between January 2007 and December 2010. The diagnosis of all samples were confirmed by pathologists. All specimens were frozen in liquid nitrogen immediately after surgical resection and stored at -80°C until use. None of the patients received preoperative chemotherapy or radiotherapy. Clinicopathological information is summarized in Table 1. Tumor differentiation was graded following WHO criteria. Tumor stage was classified according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). Overall survival was calculated from the date of initial surgical operation to death or last follow-up. This study was approved by the Research Ethics Committee of Zhengzhou University. Written informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

Total RNA was extracted from clinical specimens by using Trizol reagent according to the manufacturer's instructions. RNA concentration was measured using a spectrophotometer. Complementary DNA (cDNA) was synthesized from isolated RNA using a microRNA reverse transcription kit. Real-time PCR was performed with a microRNA assay kit real-time PCR detection system. Quantitative PCR was conducted at 95°C for ten minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. U6 small nuclear RNA was used as an internal control. All reactions were run in triplicates. The cycle threshold (Ct) values were recorded, and the relative amount of miR-452 to U6 was calculated using the equation 2^{-DCt}, where DCT = ($CT_{miR-452}$ - CT_{U6}). The fold change of miR-452 in cervical cancer relative to the matched adjacent noncancerous tissues was determined by the 2^{-DDCt} method.

All statistical analyses were performed using the SPSS statistical software, version 16.0. Data are presented as the mean ± standard deviation (SD). Comparisons were performed using Student's *t*-test and Chi square test. Survival curves were constructed with the Kaplan-Meier method and compared by log-rank tests. The multivariate analyses were evaluated with Cox proportional hazards models. $P < 0.05$ was considered statistically significant.

Results

MiR-452 expression was detected by using qRT-PCR and normalized to U6. Figure 1A shows decreased miR-452 expression in cervical cancer specimens compared to matched adjacent noncancerous tissues. Figure 1B shows that the relative level of miR-452 in cervical cancer specimens was significantly lower than that in corresponding noncancerous tissues (mean ± SD, 6.31 ± 0.95 *vs*. 13.69 ± 2.08, $p < 0.001$).

The median miR-452 expression level was used as a cut-off value to divide all 137 patients into two groups: high miR-452 expression group (n = 69) and low miR-452 expression group (n = 68). As shown in Table 1, the authors found that low miR-452 expression was significantly associated with lymph node metastasis ($p < 0.001$), vascular invasion ($p = 0.006$), poor tumor differentiation ($p = 0.002$), and advanced FIGO stage ($p < 0.001$). However, there were no significance between miR-452 expression and other clinicopathological factors, such as age, tumor size, and histological type.

Finally, the authors evaluated the prognostic significance of miR-452 expression in patients with cervical cancer. As shown in Figure 2, patients in high miR-452 expression group had longer overall survival than those in low miR-452 expression group ($p < 0.001$). Univariate Cox regression analysis demonstrated that lymph node metastasis ($p = 0.005$), vascular invasion ($p = 0.031$), and advanced FIGO stage ($p < 0.001$) were also significantly associated with shorter overall survival (Table 2). Multivariate analyses

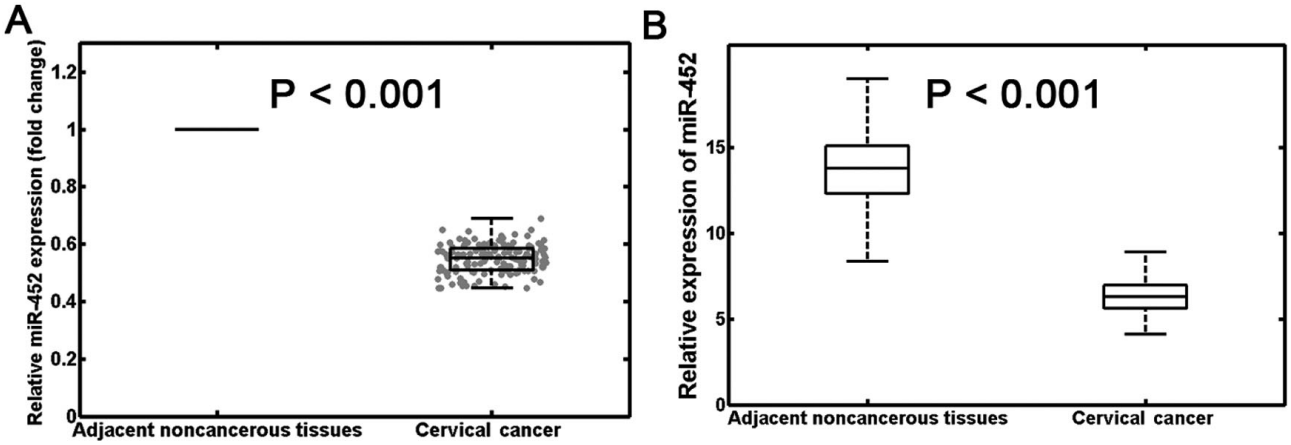

Figure 1. — The relative expression levels of miR-452 in cervical cancer tissues and adjacent non-neoplastic tissues. (A) The fold change of miR-452 in cervical cancer relative to the matched adjacent non-neoplastic tissues. (B) miR-452 expression is significantly lower in cervical cancer tissues than in the corresponding adjacent normal tissues. miR-452 levels were calculated by the 2^{-DCt} method and normalized to U6 small nuclear RNA.

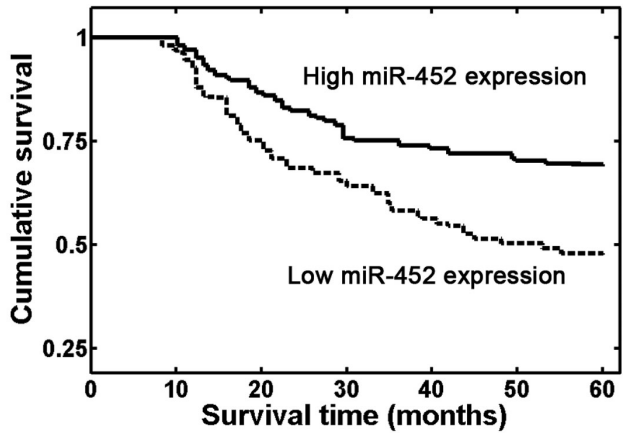

Figure 2. — Cervical cancer patients with low miR-452 expression has a significantly shorter overall survival than those with high miR-452 expression ($p < 0.001$, log-rank test).

Table 2. — *Cox regression analysis of factors associated with overall survival in 137 patients with cervical cancer.*

Variables	Univariate log-rank test (p)	Cox multivariable analysis (P)	Relative risk (RR)
Age at diagnosis (years)			
≤ 50 *vs.* > 50	0.39	—	—
Histological grades			
Well/moderate vs. poor	0.45	—	—
Tumor size			
< 4cm *vs.* ≥ 4cm	0.08	—	—
Lymph node metastasis			
Positive *vs.* negative	0.005	0.006	5.26
Vascular invasion			
Positive *vs.* negative	0.031	0.092	1.07
Histological type			
Adenocarcinoma *vs.* SCC	0.68	—	—
FIGO stage			
I *vs.* II	< 0.001	0.001	6.35
MiR-452 expression			
High *vs.* low	< 0.001	0.008	4.83

using the Cox proportional hazards regression model revealed that miR-452 expression (relative risk [RR] 4.83; $p = 0.008$), lymph node metastasis (RR 5.26; $p = 0.006$), and FIGO grade (RR 6.35; $p = 0.001$) were independent prognostic factors for overall survival of cervical cancer patients.

Discussion

Up to now, the exact mechanisms underlying cervical cancer are not fully understood. Identification of genetic alterations would be important for the screening, diagnosis, and treatment of cervical cancer. Accumulating evidence has suggested the important roles of miRNAs in tumor formation and progression. In the present study, the authors revealed that the expression of miR-452 was significantly reduced in cervical cancer tissues, and reduced miR-452 expression was significantly associated with lymph node metastasis, vascular invasion, poor tumor differentiation, and advanced FIGO stage. Patients with low level of miR-452 showed shorter overall survival than those with high miR-452 level. Thus, loss of miR-452 expression might be involved in cervical cancer development and serve as a novel biomarker for poor prognosis.

Previous research has reported miR-452 downregulation in many human malignancies, and its function as a tumor suppressor by targeting a number of oncogenes. In non-small cell lung cancer, low miR-452 expression was associated with advanced tumor stage and lymph node metastasis [20]. In vitro, upregulated miR-452 inhibited the invasive capability of NSCLC cells by targeting oncogene BMI1. In prostate cancer, miR-452 inhibited proliferation, migration, and invasion of tumor cells by direct or indirect regulation of pathways related to the cell cycle and cellular adhesion [22]. High miR-452 methylation correlated with high PSA level, high T-stage, high Gleason score, and short recurrence-free survival time in prostate cancer patients treated by radical prostatectomy. In addition, miR-452 was downregulated in human gliomas, especially, high-grade, undifferentiated gliomas [19]. Upregulation of miR-452 suppressed glioma stem-like traits and tumorigenesis, both in vitro and in vivo. Further, overexpression of miR-452 could significantly increase the sensitivity of MCF-7 breast cancer cells to Adriamycin and enhance cell apoptotic via targeting insulin-like growth factor-1 receptor (IGF-1R) [25].

In contrast to the antitumor properties mentioned above, miR-452 may act as a potential oncogene in bladder cancer and hepatocellular carcinoma [21, 23, 24]. Veerla *et al.* confirmed miR-452 upregulation in bladder urothelial carcinomas and its correlation with lymph nodes metastasis and shorter overall survival [23]. Zheng *et al.* reported that miR-452 expression levels increased in hepatocellular carcinoma tissues and cell lines [24]. Overexpression of miR-452 can promote cell proliferation and invasion, and inhibit apotosis of hepatocellular carcinoma cell lines, through targeting cyclin-dependent kinase inhibitor 1B (CDKN1B). Therefore, miR-452 plays dual functions in cancer pathogenesis and progression, and the role of miR-452 should be tumor specific and possibly dependent on its targets in different cancer types.

Undoubtedly, miRNAs exert important functions in carcinogenesis by regulation of target gene expression [26]. In human cancers, some highly expressed miRNAs could function as oncogenes by repressing tumor suppressor genes, whereas low-expressed miRNAs could function as tumor suppressors by negatively regulating oncogenes [27]. Although the present study showed decreased miR-452 expression in cervical cancer and its clinical significance, the underlying mechanisms have not been well characterized. Therefore, identification of miR-452 function and its downstream genes in cervical cancer cells would be an important facet in future investigations.

In summary, the present research showed miR-452 downregulation in cervical cancer and its correlation with aggressive clinicopathological features. Decreased miR-452 expression might be an independent biomarker for poor prognosis. Large scale prospective studies are needed to confirm the present conclusion and clarify the mechanisms.

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