Research Square

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Serum Level of *microRNA-429* is as a Promising Diagnostic Biomarker for Cervical Cancer Patients

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Abstract

Backgrounds: Serum microRNAs (miRNAs) are a novel class of diagnostic biomarkers for human cancers. However, the clinical relevance of circulating microRNA-429 (*miR-429*) in cervical cancer patients remains unclear. The purpose of this study was to investigate serum *miR-429* levels in cervical cancer patients, as well as its association with clinical characteristics and diagnostic value.

Methods: Serum *miR-429* expression was investigated in 113 cervical cancer patients and 107 healthy normal controls by quantitative real-time PCR (qRT-PCR). Chi-square test was applied to evaluate the correlation between *miR-429* expression and clinicopathological parameters. Receiver operating characteristic (ROC) curves were constructed to assess the diagnostic significance of serum *miR-429* in cervical cancer.

Results: Serum *miR-429* expression was significantly down-regulated in cervical cancer compared with healthy controls (P=0.000). In addition, serum *miR-429* level was correlated with differentiation (P=0.031), FIGO stage (P=0.030), and lymph node metastasis (P=0.045). ROC curves demonstrated that serum *miR-429* expression could distinguish cervical cancer patients from healthy controls with the sensitivity of 81.4% and the specificity of 69.2%. Additionally, the area under the curve (AUC) was 0.816 and the cut-off value was 0.825.

Conclusion: *MiR-429* is down-regulated in cervical cancer and associated with aggressive clinical characteristics. *MiR-429* may be a potential diagnostic biomarker for cervical cancer.

Background

Cervical cancer, one the most prevalent gynecologic malignant tumors, has become a major health problem for women worldwide, particularly in developing countries [1, 2]. Several factors are confirmed as risk factors for cervical cancer, including infection with human papilloma virus (HPV), smoking, and oral contraceptives [3]. Until now, the first-line treatment regimens for cervical cancer include surgery, chemotherapy, and radiotherapy. Despite of the significantly improvements in the treatments, the outcomes of cervical cancer are still unsatisfactory [4]. Late diagnosis and metastasis may be responsible for the high mortality [5, 6]. It was reported that distant metastasis was the primary cause for treatments failure and cancer-related death in cervical cancer patients [7]. Therefore, identification of potential biomarkers for early detection is extremely important for prognosis in cervical cancer patients.

MicroRNAs (MiRNAs), a group of small non-coding RNA molecules, play regulative roles in gene expression by binding to the 3’-UTR of their target genes [8]. Growing evidences have indicated that miRNAs are involved in almost all cellular processes and their abnormal expression is associated with various human diseases, as well as cancers [9, 10]. Recent studies demonstrate that the differences of miRNAs expression between cancerous and non-cancerous tissues or cells may reveal that miRNAs participate in the carcinogenesis [11–13]. Based on their important roles in tumor progression, a variety of miRNAs are confirmed as diagnostic or prognostic biomarkers for cancers [14].

MicroRNA-429 (*MiR-429*) is a cancer-related miRNA. The abnormal expression of *miR-429* was observed in several cancers, such as gastric cancer, bladder cancer, nasopharyngeal carcinoma [15–17]. Furthermore, a study scheduled by Meng et al. demonstrated that serum levels of *miR-429* was significantly associated with clinical characteristics of epithelial ovarian cancer (EOC) patients, which could serve as a biomarker for diagnosis and prognosis of EOC [18]. The effects of *miR-429* in cervical cancer had also been reported in the previous studies. Wang et al. reported that abnormal expression of *miR-429* could regulate the biological behaviors of cervical cancer cells [19]. However, the diagnostic performances of *miR-429* in cervical caner had been rarely reported.

In the present study, we investigated the expression levels of serum *miR-429* in cervical cancer patients, as well as its association with clinicopathological characteristics of patients. In addition, the diagnostic significance of serum *miR-429* in patients with cervical cancer was estimated in the current study.

Methods And Materials

Patients and serum samples

All enrolled individuals gave their informed consents, and the Ethical Committees of the hospital approved this study. A total of 113 cervical cancer patients and 107 healthy women were collected in the study which was carried out in Southwest Hospital, Army Medical University. None of the patients had received chemotherapy, radiotherapy or other treatments before blood collection. In addition, the healthy individuals in the study were without any cancer history.

After fasting for 8–10 h, 5 mL blood samples were collected from each participant in red tiger-top gel separator tubes (Thermo Fisher Scientific Inc., Waltham, MA, USA). All samples were processed within 2–5 h after collection as follow: the serum was separated by centrifugation at 1,200 × g at 4℃ for 20 min and passed through a 13-mm serum filter (Thermo Fisher Scientific Inc.). Serum samples were stored at -80℃ until total RNA isolation.

RNA extraction and quantitative real-time reverse transcription PCR (qRT-PCR)

Total RNA was extracted from serum samples using Trizol reagent (invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Its concentration was measured by Thermo 2000D type ultraviolet spectrophotometer, which was determined by the ratio of absorbance at 260/280 nm. High quality RNA was used for reverse transcription which was performed using Prime-ScriptTM RT Master Mix (Takara) according to the manufacture's instructions. Quantitative real-time PCR experiments were done in 7500 Real-time PCR System (Applied Biosystems) using SYBR Premix Ex Taq reagents (Takara). *U6* served as internal control. The primer sequences were as followed: *miR-429* forward: internal control. The primer sequences were as followed: miR-429 forward: 5'-UAAUACUGUCUGGUAAAACCGU-3', reverse: 5'-CAAGAUCGGAUCUACGGGUUUU-3'; U6 forward: 5'-UUCUCCGAACGUGUCACGUTT-3', reverse:5'

ACGUGACACGUUCGGAGAATT-3'. The relative expression of *miR-429* was calculated by $2^{-\Delta\Delta Ct}$ method and each sample was conducted in triplicate.

Statistical analyses

Statistical analyses were performed using SPSS 18.0. Data were expressed as mean ± SD (Standard Deviation). The difference between two groups was assessed using Student's *t* test. Chi-square test was applied to analyze the correlation between serum *miR-429* expression and clinicopathological factors. A receiver operating characteristic (ROC) curve was constructed to evaluate the diagnostic significance of *miR-429* in cervical cancer patients. $P < 0.05$ was considered to be significant.

Results

The relative expression of serum miR-429 in cervical cancer

QRT-PCR assay results showed that the relative expression of serum *miR-429* in the cervical cancer patients was significantly down-regulated, compared with healthy controls ($P = 0.000$) (Fig. 1).

The association between serum miR-429 level and clinicopathological characteristics

To better understand the potential roles of serum *miR-429* in cervical cancer progression, the relationships between *miR-429* and various clinical features of cervical cancer were evaluated. According to the median value of serum *miR-429*, the cervical cancer patients were divided into low expression (n = 58) and high expression (n = 55) groups. Chi-square test demonstrated that *miR-429* expression was significantly associated with differentiation ($P = 0.031$), FIGO stage ($P = 0.030$), and lymph node metastasis ($P = 0.045$). However, there were no significant association between serum *miR-429* levels and age, tumor size, or HPV (all $P > 0.05$) in cervical cancer patients (Table 1).

Table 1
The relationships between serum *miR-429* and clinicopathological features in patients with cervical cancer

Features	NO. of cases (n = 113)	*MiR-429* expression		χ^2	*P* values
		Low (n = 58)	High (n = 55)		
Age (years)					
< 50	47	23	24	0.184	0.668
≥ 50	66	35	31		
Tumor size (cm)					
< 4	74	36	38	0.616	0.443
≥ 4	39	22	17		
HPV					
Negative	31	13	18	1.508	0.219
Positive	82	45	37		
Differentiation					
Well-moderately	54	22	32	4.640	0.031
Poor	59	36	23		
FIGO stage					
Ib - IIa	42	16	26	4.685	0.030
IIb - IIIa	71	42	29		
Lymph node metastasis					
Positive	61	26	35	4.020	0.045
Negative	52	32	20		

The diagnostic efficacy of serum miR-429 expression in cervical cancer

ROC analysis revealed that *miR-429* could distinguish the cervical cancer patients from the healthy control with the sensitivity of 81.4% and the specificity of 69.2%. Additionally, the area under the curve (AUC) was 0.816 and the cut-off value was 0.825 (Fig. 2).

Discussion

Cervical cancer represents one of the commonly diagnosed cancers in women worldwide [20]. Despite of the improved therapeutic regimens, the prognosis of patients with advanced cervical cancer remains poor [21]. It was reported that the therapeutic efficacy for cervical cancer patients mainly depended on the clinical stage when diagnosed [3]. Therefore, molecular biomarkers for early diagnosis were fatally important for outcomes in cervical cancer patients.

Accumulating evidences have demonstrated that miRNAs play critical roles in initiation and development of cancers. It was reported that about 52.5% miRNAs were located at fragile sites in genome, which were more vulnerable to mutate or influenced by environmental changes [22]. Aberrant miRNA expression and mutations have been shown to contribute to cancer initiation and development [23, 24]. In the previous studies, a variety of miRNAs were proved to take part in the carcinogenesis of cervical cancer. For instance, Zhang et al. reported that the levels of *miR-664* in cervical cancer tissues were down-regulated and correlated with clinical progression [25]. Wang et al. proved that abnormal expression of *miR-328* could regulate cervical cancer cell proliferation, cell cycle, and colony formation in vitro [26]. The study carried out by Li et al. demonstrated that *miR-138* could inhibit cervical cancer cell proliferation via regulating c-Met, which might be a potential target for cervical cancer therapy [27]. All of the related data suggested that miRNAs played important roles in cervical cancer progression. In the present study, we investigated the diagnostic value of *miR-429* in cervical cancer.

In this study, we examined the expression level of serum *miR-429* in cervical cancer and healthy controls using qRT-PCR assay. Results showed that serum *miR-429* level was significantly lower in cervical cancer patients than that in healthy controls. Moreover, down-regulated *miR-429* level was closely associated with poor differentiation, advanced FIGO stage, and positive lymph node metastasis in the patients. The results might reveal that *miR-429* served as a tumor suppressor gene in cervical cancer, which could inhibit the aggressive tumor progression. Wang et al. had proved that the expression levels of *miR-429* in cervical cancer could regulate cell elongation, migration, stress fiber formation, and invasion through targeting ZEB1 and CRKL, thus participate in the cervical cancer progression [19]. However, the mechanisms for *miR-429* regulating cervical cancer were not investigated in the present study, which was needed to be identified in the further researches.

MiR-429 was a cancer-related miRNA, which could serve as a diagnostic and prognostic biomarker in various cancers. The research conducted by Li et al. indicated that the expression levels of *miR-429* were up-regulated in colorectal cancer tissues, and the elevated expression levels were significantly correlated with malignant tumor progression, suggesting its predictive roles in colorectal cancer prognosis [28]. Zhu et al. reported that serum levels of *miR-429* were obviously different between non-small cell lung cancer patients and healthy individuals, which could act as a diagnostic biomarker for the cancer [29]. In this study, we estimated the diagnostic value of *miR-429* in cervical cancer. ROC analysis showed that *miR-429* was able to distinguish cervical cancer patients form healthy controls with high sensitivity and specificity. All these findings implicated that *miR-429* might be a potential biomarker for diagnosis in cervical cancer.

Conclusion

In conclusion, *miR-429* serves as a tumor suppressor gene in cervical cancer, which can inhibit malignant tumor progression. *MiR-429* may be a potential diagnostic biomarker for cervical cancer. With wide applications, *miR-429* may significantly improve the managements of cervical cancer.

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Southwest Hospital, Army Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Data availability

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

K. Z., T. M. design of the work; Z. H., X. W. the acquisition, analysis, Y. P., Y. C. interpretation of data; Y. D. the creation of new software used in the work; Z. R., Z. W. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

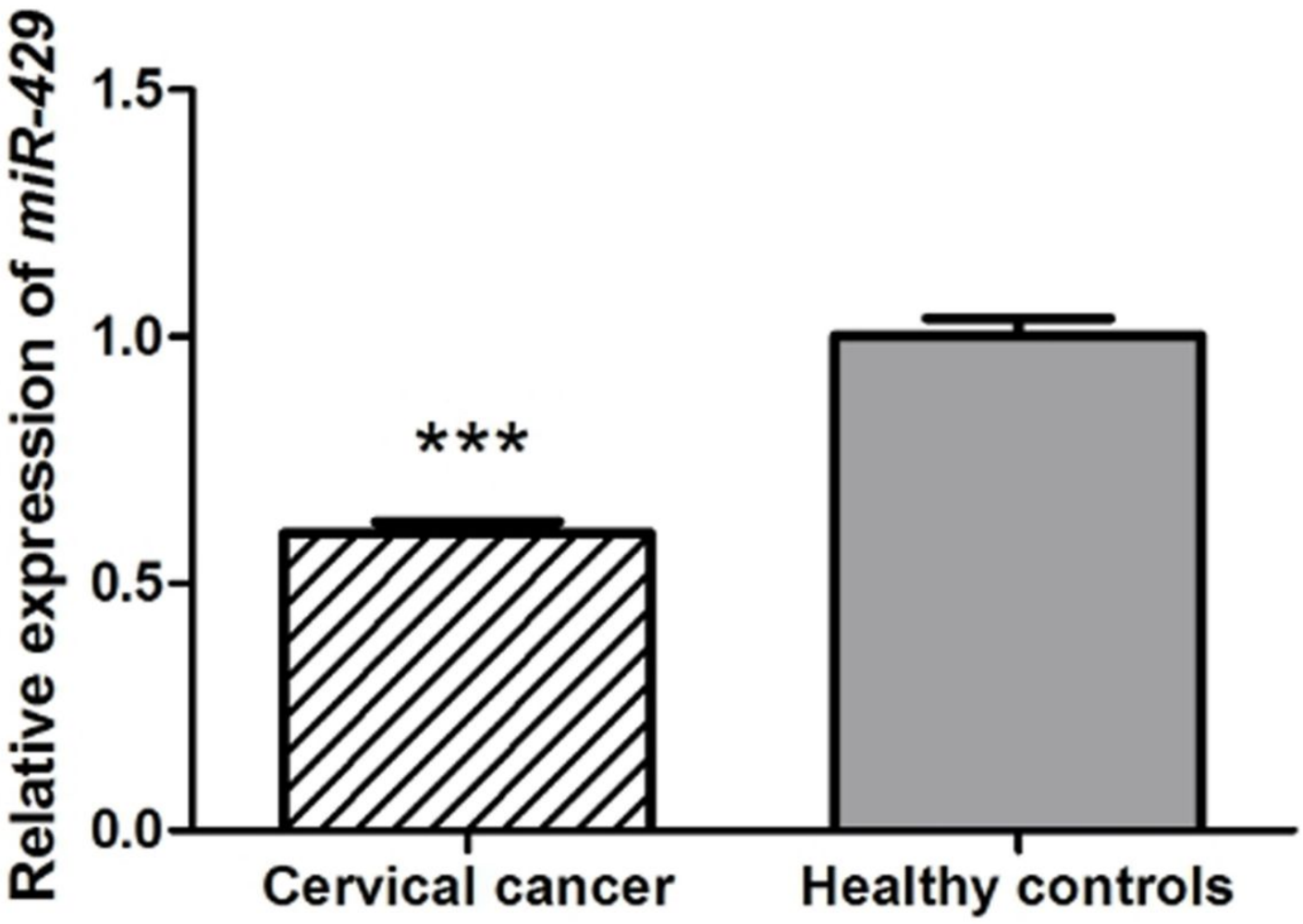


Figure 1

Expression level of serum miR-429 in cervical cancer and healthy controls. The expression level of serum miR-429 was significantly lower in cervical cancer than thant in healthy controls. ***: indicated P<0.001.

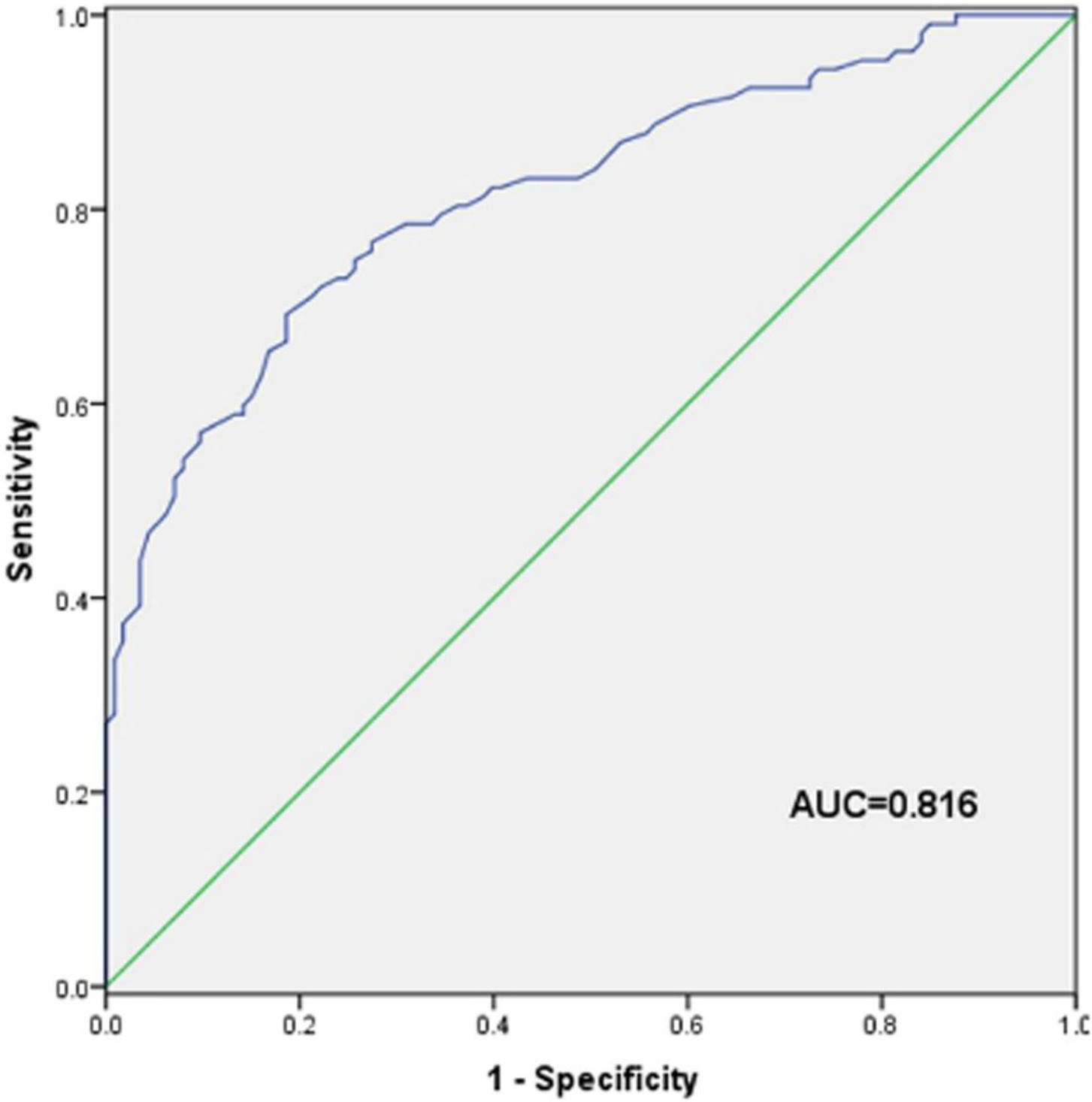


Figure 2

ROC curve was constructed to evaluate the diagnostic value of miR-429 in cervical caner patients. Analysis results demonstrated that miR-429 could discriminate between cervical cancer patients and healthy individuals at the cut-off value of 0.825, with the sensitivity of 81.4% and the specificity of 69.2%. The AUC value was 0.816.