Research Square

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MiR-221 represents an innovative bio-marker in cervical carcinoma detection

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Abstract

Background

MiR-221 has been identified to play an important role in tumorigenesis and progression. In the present study, we aimed at to investigate the expression pattern of serum *miR-221* and evaluate its diagnostic value in cervical cancer.

Methods

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure the expression pattern of *miR-221* in cervical cancer patients and healthy controls. The association of *miR-221* with clinicopathological data was analyzed with χ^2 test. Then receiver operating characteristic (ROC) curve was built to evaluate the diagnostic value of serum *miR-221* by calculating the area under the ROC curve (AUC).

Results

The results indicated that the *miR-221* expression level was statistically elevated in cervical cancer patients compared with healthy individuals. The increased *miR-221* expression was significantly associated with lymph node metastasis ($P = 0.026$) and FIGO stage ($P = 0.028$). ROC curve suggested that serum *miR-221* had a high diagnostic value in differentiating cervical cancer patients from healthy controls with AUC of 0.932 (95%CI: 0.903–0.960) corresponding with sensitivity of 77.6% and specificity of 94.8%.

Conclusions

Taken together, the expression level of *miR-221* is increased in cervical carcinoma and it may serve as a promoting bio-marker in the diagnosis of cervical cancer patients.

Background

Cervical cancer is the second most common gynaecological malignancy after breast cancer in the world. Due to the delayed initial screening about 265,000 deaths from cervical cancer, especially in the developing countries, which representing a healthy threaten for women [1, 2]. At present, a series of risk factors have been found to play important roles in the occurrence of cervical cancer, such as life style, hormonal contraceptives, immunosuppression or certain infections, especially human papilloma virus (HPV) [3–7]. However, the morbidity and mortality of cervical cancer are still increasing in the developing countries, and the patients tend to be younger [8]. Screening is the current treatment for early detection of cervical cancer, but those patients who are too young to benefit from screening are usually diagnosed at

advanced stage and has poor prognosis. Consequently, identifying novel invasive bio-markers is crucial for early detection of cervical cancer. In recent years, many researches have found that a lot of miRNAs are involved in the initiation and development of cervical cancer.

MicroRNAs (miRNAs) are a class of small (19–25 nucleotides) noncoding RNA molecules that modulate target gene expression [9]. Accumulating evidences have indicated that miRNAs are involved in diverse biological processes, including cell apoptosis, proliferation, migration, invasion and metabolism [10–12]. Aberrantly expression of miRNAs is observed in various cancers that can function as novel biomarkers for diagnosis and potential therapeutic targets [13, 14]. As a member of miRNAs, *miR-221* was found up-regulated in bladder cancer tissues and influenced T24 cell proliferation and apoptosis [15]. Moreover, the *miR-221* was found that modulate proliferation and invasion of cervical cancer cells, but the clinical significance of it in the diagnosis of cervical cancer was still unclear [16].

In the present study, we mainly explored the serum expression levels of *miR-221* in cervical cancer patients and healthy control and investigated the relationship between *miR-221* expression and clinicopathological characteristics of cervical cancer patients. Then the diagnostic value of *miR-221* was also estimated.

Methods

Patients and specimens

All protocols were approved by the Ethics Committee of the Harrison International Peace Hospital. In total, 125 patients who were diagnosed as cervical cancer before any treatments and 115 healthy control subjects from the Harrison International Peace Hospital, were included in our study. Blood samples from cervical cancer patients and healthy controls were collected during physical examination and then serum was separated and stored at -80℃ for use. The patients with cervical cancer assessment were performed according to histological grade and FIGO staging. The clinical characteristics of the patients were summarized in Table 1. All the participants provided their written informed consent in advance.

Table 1
The relationship between *miR-221* expression and clinicopathological features of cervical cancer patients

Parameters	Cases (n = 125)	*miR-221* expression		χ^2	*P*
		Low	High		
Age				0.385	0.535
< 50	63	27	36		
≥ 50	62	30	32		
Tumor size				0.053	0.818
< 4 cm	65	29	36		
≥ 4 cm	60	28	32		
Histological grade				2.171	0.141
G1-G2	59	31	28		
G3	66	26	40		
Lymph node metastasis				4.936	0.026
Negative	61	34	27		
Positive	64	23	41		
FIGO stage				4.809	0.028
Ib-IIa	59	33	26		
IIb-IIIa	66	24	42		
Differentiation				0.720	0.396
poor	60	25	35		
Moderate + well	65	32	33		

RNA extraction and quantitative real-time RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from total RNA using the Reverse Transcription Kit (Applied Biosystems). To quantify mature *miR-221* expression, quantitative real-time RT-PCR (qRT-PCR) was performed using an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Inc., Foster City, CA, USA). *U6* was used as an endogenous control for normalization. The primer sequences of *miR-221* were designed as follows: forward, AGCUACAUUGUCUGCUGGGUUUC; reverse, GAAACCCAGTCTCAA TGTAGCTCCGGAAACCCAGTCTCAATGTAGCT [17]. The primers for *U6*:

forward, 5′-CTCGCTTCGGCAGCACA-3′ and reverse 5′-AACGCTTCACGAATTTGCGT-3′ [18]. The relative expression of *miR-221* was calculated and normalized using the $2^{-\Delta\Delta CT}$ method relative to *U6*.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Data were expressed as the mean ± standard deviation (SD) with at least three independent experiments. Student's t-test was used to analyze differences between tumor and normal groups, and χ^2 test was used to analyze the correlation between *miR-221* expression and clinicopathological factors of cervical cancer patients. Receiver-operating characteristic (ROC) curve and the area under the ROC curve (AUC) were applied to assess the serum *miR-221* diagnosis value in cervical cancer. Differences were considered statistically significant when *P*-value less than 0.05 (*$P < 0.05$ and **$P < 0.01$).

Results

The expression of *miR-221* was increased in cervical cancer

We used qRT-PCR analysis to measure the serum *miR-221* expression in cervical cancer patients and healthy individuals. As shown in Fig. 1, the expression of serum *miR-221* was strongly up-regulated in cervical cancer patients compared to healthy individuals ($P < 0.01$).

Correlation of *miR-221* expression with clinicopathological characteristics of cervical cancer patients

To assess to association between *miR-221* expression and the clinicopathological parameters, the 125 patients were divided into high-expression and low-expression groups according to the average level of *miR-221*. Our finding showed that high expression of *miR-221* was significantly related to lymph node metastasis ($P = 0.026$) and FIGO stage ($P = 0.028$), but no relationship was found with other clinicopathological features, including age, tumor size, histological grade and differentiation (all $P > 0.05$, Table 1).

Diagnostic potential of miR-221 in cervical cancer

To investigate the correlations between the *miR-221* dysexpression and diagnosis in cervical cancer patients, we used ROC curve analysis. As shown in Fig. 2, the result showed that *miR-221* had a relatively high accuracy in differentiating cervical cancer patients from healthy individuals based on AUC of 0.932 (95%CI: 0.903–0.960) with the sensitivity of 77.6% and specificity of 94.8% at the optimal cut-off value of 2.155.

Discussion

Cervical cancer is one of the most common malignancies in women all over the world. Among the risk factors, the infection of HPV, especially the high risk type HPV virus, is the main cause of cervical cancer,

and with the longer of carcinogenic infection lasting, showing the greater of risk [19, 20]. Most women with early-stage cervical tumors can be cured. Although benefit from the development of early detection and diagnostic technique, cervical cancer having a good prognosis, there are still many patients appeared tissues infiltration and metastasis and the morbidity and mortality of this cancer are still increasing in the developing countries [8]. Thus, the accurate bio-markers are meaningful for the diagnosis and prognosis of cervical cancer.

To date, molecular markers have been identified to be involved in the tumorigenesis, development and progression of cancers, including cervical cancer [21]. For instance, Azizmohammadi S et al. demonstrated that *miRNA-145* and *miR-9* were both up-regulated in cervical cancer and may be as potential prognostic markers in patients suffering from cervical cancer [22]. SHI et al. suggested that secreted protein acidic and rich in cysteine (SPARC) may be a potential therapeutic option for cervical cancer patients [23]. Liliana Alvarado-Ruiz et al. found that normal cervical cancer from women without cervical lesions expression *HOXA9* but controlling *HOXA9* expression appears to be a necessary step during cervical cancer development [24]. These data suggested the crucial roles of cancer related molecules in cervical cancers. In the present study, we aimed at to identify a novel accurate diagnostic biomarker for patients with cervical cancer.

Upregulation of *miR-221* has been reported in various cancers. Yilmaz SS et al. showed that *miR-221* was upregulated in larynx cancer plasma samples and plasma *miR-221* may be a potential diagnostic/prognostic marker in larynx cancer [25]. Eissa S et al. indicated that the relative level of *miR-221* expression in breast cancer tissues was higher than that in noncancerous tissues and it may be a potential biomarker and molecular therapeutic target for breast cancer [26]. Yang et al. revealed that *miR-221* played a crucial role in the occurrence and the progression of human osteosarcoma and may function as a promising marker for screening individuals with osteosarcoma [27]. In study of Li et al., serum *miR-221* expression levels has been indicated that significantly higher in patients with cutaneous malignant melanoma (CMM) and it has prognostic value in CMM patients [28]. Previous study has found the aberrant expression of *miR-221* in the progression of cervical cancer [16]. But clinical diagnostic value of *miR-221* in cervical cancer has not been investigated. In the present study, we sought to assess serum *miR-221* expression pattern as well as its diagnostic role in patients with cervical cancer.

In this study, we measured the expression of *miR-221* and the association between *miR-221* with clinicopathological features in cervical cancer patients. The result of qRT-PCR revealed the expression of *miR-221* in cervical cancer was increased compared with healthy control, which suggests that *miR-221* may therefore function as a tumor oncogene. The elevated expression of *miR-221* was correlated with lymph node metastasis and FIGO stage, which suggested that *miR-221* was involved in the development of cervical cancer. Furthermore, the diagnostic value of *miR-221* has also been investigated in our study using ROC curve analysis. The high AUC value, sensitivity and specificity values suggested that *miR-221* may be a valuable diagnosis biomarker for cervical cancer detection.

Conclusions

In conclusion, serum *miR-221* was significantly up-regulated in cervical cancer in this study. Moreover, the elevated *miR-221* expression might play as a non-invasive diagnostic bio-marker for detection of cervical cancer patients from healthy individuals. Further studies with larger sample sizes are still needed to enhance the accuracy and potential of *miR-221* in cervical cancer.

Abbreviations

Quantitative real-time polymerase chain reaction (qRT-PCR)

receiver operating characteristic (ROC)

area under the ROC curve (AUC)

human papilloma virus (HPV)

MicroRNAs (miRNAs)

standard deviation (SD)

cutaneous malignant melanoma (CMM)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Harrison International Peace Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

S.C. design of the work; L.Z. the acquisition, analysis, S.C. interpretation of data; L.Z. the creation of new software used in the work; S.C. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

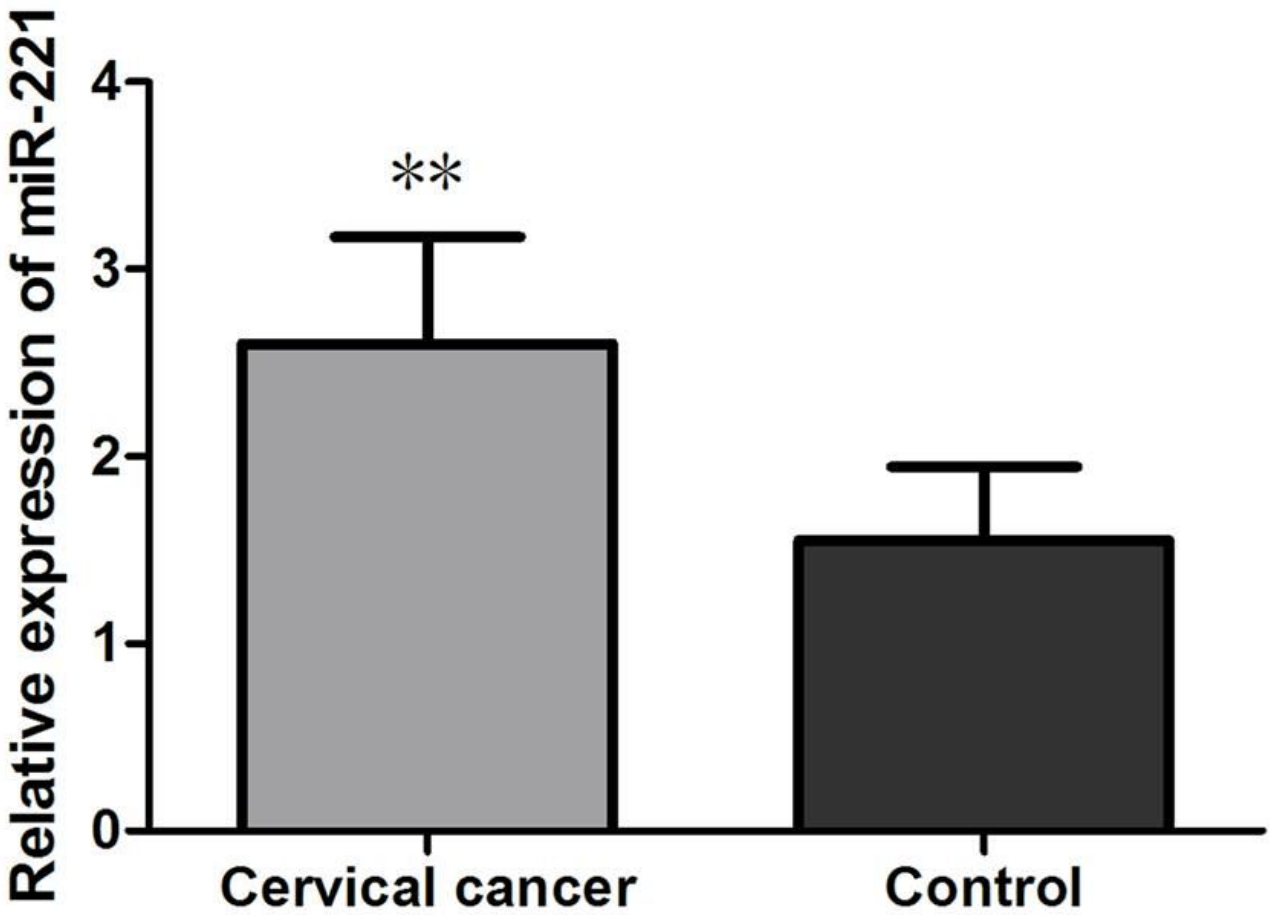

Figure 1

Relative miR-221 expression in cervical cancer patients and healthy controls. The serum expression of miR-221 in cervical cancer patients was significantly higher than that in healthy individuals ($P < 0.01$).

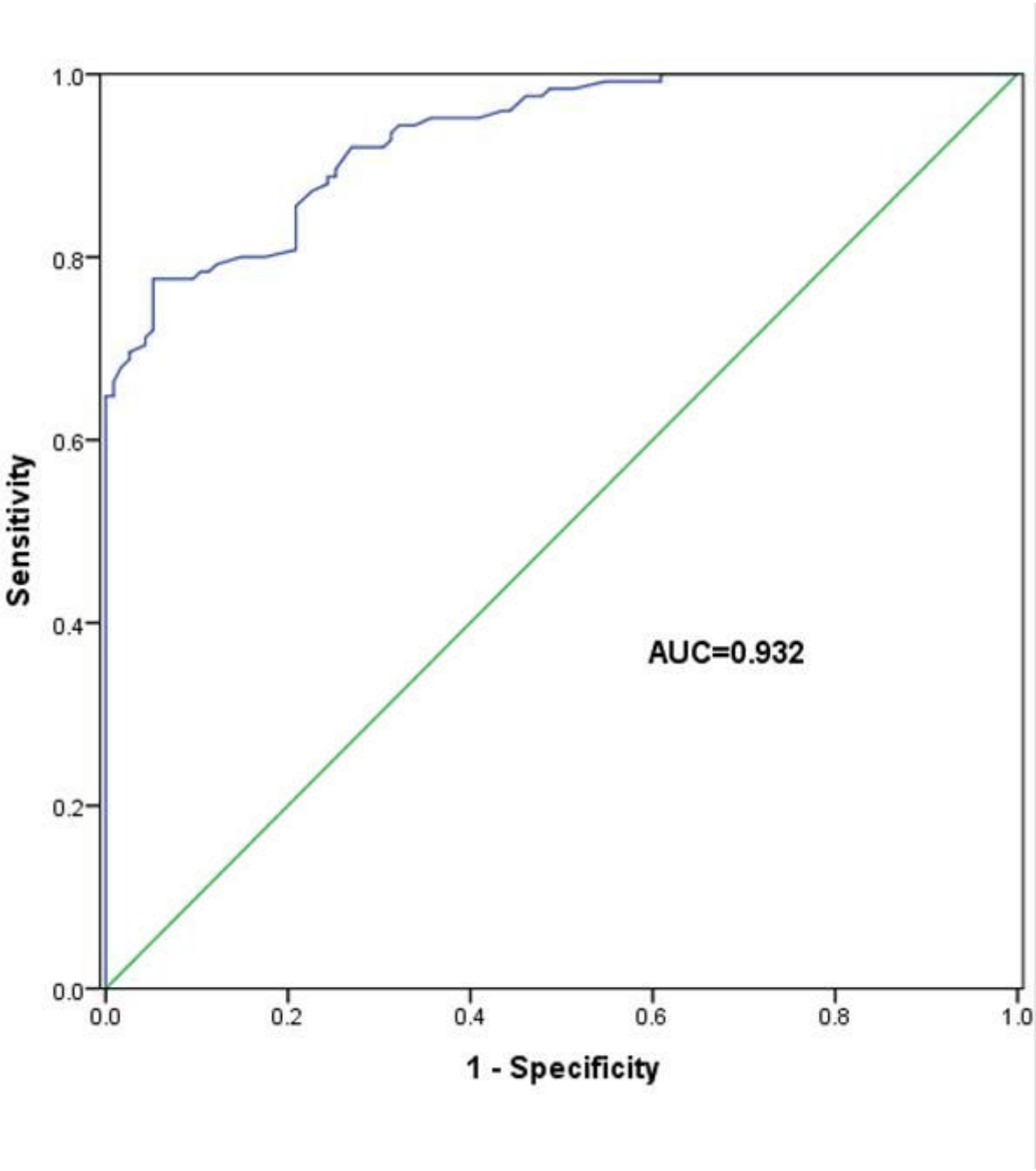

Figure 2

The diagnostic significance of miR-221 analyzed by establishing ROC curve.