Plasma miR-145 as a novel biomarker for the diagnosis and radiosensitivity prediction of human cervical cancer

Hu Wei, Chen Wen-Ming and Jiao Jun-Bo

Abstract

Objectives: The objective of this study was to evaluate levels of plasma miR-145 in patients with cervical cancer (CC) and investigate its biomarker potential.

Methods: Using qRT-PCR, we compared plasma miR-145 levels in 120 patients with CC, 120 patients with cervical intraepithelial neoplasia (CIN), and 120 healthy volunteers. The association between plasma miR-145 expression and clinicopathological factors, including radiation response, was also analyzed.

Results: Plasma miR-145 levels were lower in CC patient than in CIN patients and healthy controls. Low levels were significantly associated with poor cancer differentiation, lymph node metastasis, HPV, and advanced FIGO stage. CC patients who achieved complete response to radiotherapy had higher plasma miR-145 levels than incomplete responders. ROC analysis confirmed that plasma miR-145 is a candidate biomarker for detecting CC and differentiating complete responders from incomplete responders.

Conclusions: Plasma miR-145 is reduced in CC and is a novel candidate biomarker for diagnosing CC and predicting radiosensitivity.

Introduction

Cervical cancer (CC) is the third most common female malignancy worldwide, with approximately 529,000 new cases and 274,000 deaths per year.[1] Early diagnosis of CC is associated with favorable prognosis.

Department of Oncology, Jining No.1 People's Hospital, Jining, Shandong Province, China

Corresponding author:
Jiao Jun-Bo, Department of Oncology, Jining No.1 People's Hospital, Jining 272011, Shandong Province, China.
Email: sdjnjjb@mail.com

Many CC patients with early stage disease are successfully cured, but the 5-year survival rate for patients diagnosed at FIGO stage IV is as low as 15%.[2] Radiotherapy, one of the main treatment options for CC, is used for over 60% of CC cases. For some early stage patients, radiotherapy is as effective as radical surgery.[3] Unfortunately, CC is often diagnosed at an advanced clinical stage,[4] and some patients with CC present with either intrinsic or acquired radioresistance, resulting in treatment failure and eventually poor outcome. New, effective, reliable, and noninvasive biomarkers for early detection and prediction of radiosensitivity prediction are still urgently needed.

MicroRNAs (miRs) are a class of small noncoding RNAs approximately 20 nucleotides long. MiRs can negatively regulate gene expression by binding to 3′-untranslated regions of target mRNAs.[5] MiRs play important roles in multiple biological processes including cellular proliferation, differentiation, apoptosis, angiogenesis, invasion, and migration.[6] In addition, some miRs may function as either oncogenes or tumor suppressors, and altered expression of miRs has been associated with tumor initiation and progression.[7] Furthermore, miRs are present in plasma and serum, and blood miRs are protected from RNase digestions.[8] These findings suggest that circulating miRs might serve as biomarkers for cancer diagnosis, prognosis, and treatment monitoring.[9] For example, serum miR-21, miR-155, and miR-365 can be used for detection of breast cancer.[10] Low level of serum miR-503 predicted poor overall survival in patients with gastric cancer.[11] Serum miR-4772-3 p expression was associated with response to FOLFOX adjuvant chemotherapy and tumor recurrence in colon cancer patients.[12]

One of the cancer-related miRs is miR-145. MiR-145 is downregulated in osteosarcoma,[13] ovarian cancer,[14] breast cancer,[15] gastric cancer,[16] hepatocellular carcinoma,[17] lung cancer,[18] and colorectal cancer.[19] MiR-145 acts as a tumor suppressor gene in these tumors. Low levels of miR-145 in CC tissues are correlated with lymph node metastasis and advanced clinical stage.[20] Overexpression of miR-145 may suppress CC cell proliferation and invasion and enhance radiosensitivity.[21,22] Recent studies have found decreased blood miR-145 levels in patients with malignant bone tumors and ovarian cancer and have studied the clinical significance of these changes.[23,24] However, plasma miR-145 expression and its potential diagnostic value in CC remains unknown. In the present study, we detected plasma miR-145 levels in CC patients and evaluated its association with clinical parameters. Furthermore, we explored whether plasma miR-145 could serve as a useful biomarker for CC diagnosis and prediction of radiosensitivity.

Materials and methods

Patients and samples

This study was approved by the ethics committee of Jining No.1 People's Hospital (No 2010037) and written informed consent was obtained from each participant. A total of 120 patients were enrolled in the study. All patients had pathologically confirmed CC and were treated with radiotherapy at Jining No.1 People's Hospital between January 2010 and September 2015. None of the patients had previously undergone chemotherapy, radiation therapy, or immunotherapy. Peripheral blood (10 ml) samples were obtained at diagnosis before any treatment. The samples were collected in PAXgene blood RNA tubes (PreAnalytiX QIAGEN/ BD) and centrifuged at 3000 rpm for 15 min at 4°C. The plasma was immediately frozen and stored at −80°C until use. Plasma samples from 120 cervical intraepithelial neoplasia (CIN) patients and 120 healthy

Table 1. Correlations of plasma miR-145 levels and clinical characteristics of cervical cancer patients. NS = not significant.

Characteristics	Patients	miR-145 level	P value
Age (yr)			
<50	59	0.34 ± 0.04	
≥50	61	0.31 ± 0.03	NS
Histological grades			
Well/Moderate	64	0.41 ± 0.05	
Poor	56	0.26 ± 0.03	0.027
Histological type			
Adenocarcinoma	19	0.30 ± 0.03	
Squamous cell carcinoma	101	0.35 ± 0.04	NS
Tumor size (cm)			
<4	66	0.37 ± 0.04	
≥4	54	0.29 ± 0.02	NS
Lymph node metastasis			
Positive	47	0.22 ± 0.03	
Negative	73	0.45 ± 0.06	0.009
FIGO stage			
I–II	77	0.49 ± 0.06	
III	43	0.16 ± 0.02	0.002
HPV infection			
Positive	81	0.25 ± 0.03	
Negative	39	0.42 ± 0.05	0.016
Tumor response			
Complete responder	68	0.47 ± 0.05	
Incomplete responder	52	0.19 ± 0.02	0.005

volunteers were used as controls. The clinical characteristics of CC patients were summarized in Table 1. These patients were classified into two groups according to the response to radiotherapy: a complete response group and an incomplete response group (including partial response, stable disease, and progressive response) as previously described.[25,26]

MiR isolation and real-time RT-PCR

Total RNA was extracted from 400 μL of plasma samples using a miRVana PARIS Kit (Ambion, Austin, TX, USA). Reverse-transcription was carried out with the TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, CA, USA). The reaction mixtures were incubated at 16°C for 30 min, followed by 42°C for 30 min, then 85°C for 5 min before being held at 4°C. Quantitative PCR was performed on an ABI7500 fast real-time PCR system (Applied Biosystems) in a final volume of 20 μL with the human TaqMan MicroRNA Assay Kit (Applied Biosystems). PCR cycling conditions included 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The expression of miR-145 was normalized to RNU6B as previously described,[27,28] and relative expression of miR-145 was calculated using the $2^{-\Delta\Delta Ct}$ method.[29,30] The primer sequences used were: miR-145, 5′-AAG CTT CAG AGG GTT TCC GGT ACTT-3′ (forward) and 5′-CTC GAG AGC

CTC ACA GGG ATG TTA TG-3′ (reverse); RNU6B, 5′-CGC TTC GGC AGC ACA TAT AC-3′ (forward) and 5′-TTC ACG AAT TTG CGT GTC AT-3′ (reverse).

Statistics

All statistical calculations were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA), and *P* values below 0.05 were considered statistically significant. The differences in miR-145 expression were analyzed using a Mann-Whitney unpaired test. Receiver operating characteristic (ROC) curves and area under the ROC curve (AUC) were used to assess the diagnostic value of miR-145.

Results

Decreased levels of plasma miR-145 in patients with CC

The levels of plasma miR-145 in CC patients, CIN patients, and healthy volunteers were examined using qRT-PCR. Levels of miR-145 were significantly lower in
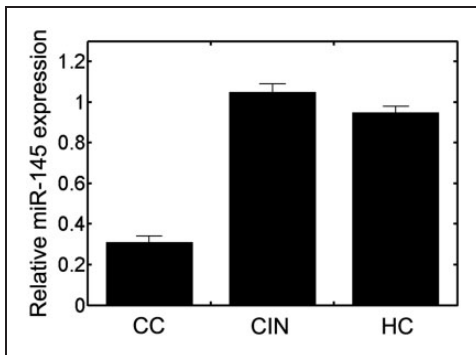
Figure 1. Plasma miR-145 levels in cervical cancer (CC) patients (n = 120) were significantly lower than those in cervical intraepithelial neoplasia (CIN) patients (n = 120) and healthy controls (HC; n = 120). The relative expression of miR-145 was determined by qRT-PCR with the $2^{-\triangle\triangle Ct}$ method.

plasma from patients with CC than in plasma from CIN patients and healthy controls (Figure 1; both $P < 0.01$). No significant difference was observed in the plasma miR-145 expression between CIN patients and healthy controls.

ROC curve analysis indicated that the plasma levels of miR–145 differentiated CC from healthy controls, with an AUC of 0.848 (95% CI: 0.802–0.894; sensitivity: 81.7%, specificity: 63.3%; Figure 2(a)). We also evaluated the diagnostic value of plasma miR-145 for differentiating CC patients from CIN patients. As shown in Figure 2(b), the AUC was 0.828 (95% CI: 0.779–0.878), with a sensitivity of 91.7% and specificity of 54.2%.

Low level of plasma miR-145 correlates with CC progression and radioresistance

We examined the association of plasma miR-145 level with clinicopathological characteristics of the 120 CC patients. Low plasma miR-145 level was significantly associated with poor cancer differentiation ($P = 0.027$), lymph node metastasis ($P = 0.009$), HPV infection ($P = 0.016$), and advanced FIGO stage ($P = 0.002$). In addition, the expression level of miR-145 was significantly higher in the plasma samples of the complete response group than in those of the incomplete response group ($P = 0.005$, Table 1). ROC curve analysis revealed that plasma miR-145 distinguished complete responders from incomplete responders with an AUC of 0.801 (95% CI: 0.724–0.878; sensitivity: 64.7%, specificity: 84.6%; Figure 2(c)).

Discussion

There is a growing body of evidence showing that miRs are stable in human body fluids and their expression levels may be clinically relevant. In this study, levels of miR-145 were lower in plasma from CC patients than
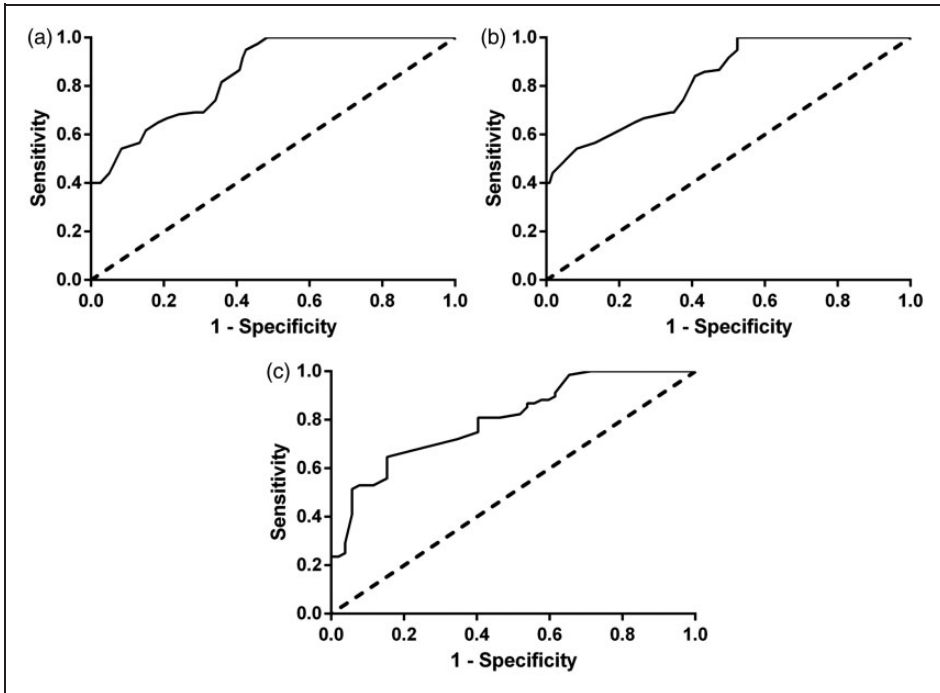
Figure 2. ROC curve analysis of plasma miR-145 for discriminating (a) cervical cancer (n = 120) from healthy controls (n = 120), (b) cervical cancer from cervical intraepithelial neoplasia (n = 120), and (c) complete responders (n = 68) from incomplete responders (n = 52).

in plasma from CIN patients and healthy controls. Low plasma miR-145 expression was correlated with poor differentiation, lymph node metastasis, HPV infection, advanced clinical stage, and poor tumor response to radiotherapy. Moreover, plasma miR-145 level could differentiate CC from CIN and healthy controls and distinguish complete responders from incomplete responders. To our knowledge, this is the first study to investigate circulating miR-145 levels and explore the clinical significance of this biomarker in CC patients.

Decreased circulating miR-145 has been previously reported in patients with other malignancies. Gao et al.[24] revealed that miR-145 expression in the plasma of patients with malignant bone tumors was significantly lower than in plasma of patients with benign bone tumors or healthy

subjects. Liang et al.[23] demonstrated that serum miR-145 is decreased in ovarian cancer patients and serum miR-145 level could be used to distinguish ovarian cancer patients from healthy controls. Low levels of serum miR-145 predicted poor overall survival. Thus, circulating miR-145 may be a useful marker for cancer diagnosis and prognosis, and the potential clinical significance of blood miR-145 in other cancers should be studied further.

The efficacy of radiation therapy is often limited by radioresistance. Reliable markers that can predict radiosensitivity before treatment begins are critically needed. MiR-145 can target the DNA damage repair-associated gene HLTF, which is involved in radioresistance,[21] and ectopic expression of miR-145 significantly sensitized prostate cancer cells to radiation.[31] Overexpression of

miR-145 in CC cells enhanced radiosensitivity in vitro and in vivo.[21] In our study, CC patients who achieved complete response to radiotherapy had higher plasma miR-145 levels than those who failed to achieve a complete response. ROC curve analysis confirmed that plasma miR-145 is a valuable biomarker for differentiating complete responders from incomplete responders. Therefore, plasma miR-145 is a candidate biomarker for prediction of radiosensitivity of cancers.

We are aware of some limitations in our work. First, this was a retrospective study. Second, our study investigated miR-145 only. Since miRs acts as a network rather than individually,[32] clinical significance of other circulating miRs in CC should be evaluated.

In summary, this study showed that plasma miR-145 is decreased in CC and associated with aggressive clinicopathological features. More importantly, plasma miR-145 is a candidate biomarker for diagnosis of CC and prediction of radiosensitivity. Prospective studies with larger sample size should be conducted to confirm these conclusions.

Declaration of conflicting interest

Funding

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