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Serum MicroRNAs Related with Chemoradiotherapy Resistance in Advanced-Stage Cervical Squamous Cell Carcinoma

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Abstract

OBJECTIVE: To investigate the serum microRNAs as biomarkers in predicting chemoradiotherapy resistance in advanced-stage cervical squamous cell carcinoma (ACSCC) patients. *METHODS:* Serum samples were collected from International Federation of Gynecology and Obstetrics (FIGO) stage IIB to IIIB cervical squamous cell carcinoma patients treated with platinum based Concomitant Chemoradiotherapy (CCRT) in our hospital during September 2013 to November 2015. Twenty well-matched samples (10 resistant and 10 sensitive) were chosen to screen the miRNA expression profile using serum samples pooled with microarrays. miRNAs expressed significantly different between two groups were further verified in 131 patients (29 resistant and 102 sensitive) serum samples with TaqMan Real-time PCR. The AUC was used to evaluate the accuracy of the biomarkers for prediction. *RESULTS:* MiR-136-5, miR-152-3p and miR-206 were expressed significantly different between sensitive and resistant groups. Results of 131 patients verification showed that the levels of miR-206 in sensitive samples and resistant samples were 2.715 ± 0.2115 and 14.64 ± 1.184, respectively, which was significantly different ($P < .0001$), while miR-136-5p and miR-152-3p could not be tested without pre-amplification reactions. Univariate analysis revealed that miR-206 expression was significantly associated with patients' DFS. Multivariate analysis demonstrated that miR-206 expression, tumor differentiation and pelvic lymph nodes metastasis were the independent prognostic factors associated with DFS in this cohort ($P = .008$, 0.000, 0.000, respectively). The probability of the prognostic accuracy of miR-206 expression in predicting chemoradiotherapy sensitivity of ACSCC patients was 91.3% (79.3% sensitivity and 92.2% specificity). *CONCLUSION:* Serum miR-206 is a powerful tool in predicting chemoradiotherapy sensitivity in ACSCC patients.

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Introduction

Cervical carcinoma is the second most common gynecological malignancy worldwide. In China, a developing country, cervical carcinoma occupies the first place of three female reproductive malignancies, among which, patients of advanced stage (≥IIB) account for 60% to 70% [1]. Based on several large randomized controlled clinical trials, National Comprehensive Cancer Network had recommended Concomitant Chemoradiotherapy (CCRT) to be the standard treatment for advanced-stage patients [2–8]. Although the administration of CCRT can significantly improve patient survival, local recurrence is still common due to CCRT resistance. In clinical practice, the independent factors influencing the prognosis of IIB-IIIB stage cervical squamous cell carcinoma patients received chemoradiotherapy are as follows: pathological subtype, clinical stage, lymph node status, treatment interval [9]. Nevertheless, patients with same clinicopathological factors received CCRT may have totally different prognosis [10,11]. It shows that, besides the clinicopathological factors, chemoradiotherapy sensitivity of cervical tumor is a key factor in treatment. The influencing factors to CCRT sensitivity such as tumor oxygen conditions and cell proliferation ability were recognized by early studies. However, these only could roughly estimate the clinical response to treatment [9]. With the progress of the molecular biology, tumor internal chemoradiotherapy sensitivity draws more and more attention. The advantages such as objective and quantifiable of the molecular biological factors bring hope to predict accurately the chemoradiotherapy sensitivity. Some molecular biological factors related to chemoradiation sensitivity have been found, such as ERCC1 [12,13], Cox-2 [14,15], and microRNAs (miRNAs) [16]. Among these molecular factors, miRNA is closely related to tumor radiation and chemotherapy sensitivity and shows good prospects of research and clinical application [16]. MiRNAs are a class of endogenous, 20–22 nucleotides, non-coding small single-stranded RNA molecules that can induce mRNA degradation, translational repression, or both via pairing with partially complementary sites in the 3′-untranslated region (UTR) of the targeted genes [17]. It is estimated that 30% of all genes are regulated by miRNAs [18]. MiRNAs had been demonstrated to be potential tumor markers and targets for cancer therapy [19]. For example, down-regulation of miRNA, let-7, in lung cancer was found to be associated with poor prognosis [20]. Overexpression of miR-200c contributed to chemotherapy resistance in esophageal cancers [21]. MiR-200a was related with metastatic potential of tumor cells in human cervical cancer [22]. In recent years, the theory of circulating microRNA has attracted much attention. Blood specimens are easy to obtain and convenient to clinical application. And the circulating miRNAs constitute a novel class of non-invasive biomarkers which show good stability under a variety of physical and chemical conditions. The mechanism may be correlated with some chemical modifications such as the protection of exosomes, microRNA–protein complex and methylation, etc. [16]. Studies on the roles of serum miRNAs in the tumor development, metastasis, prognosis, treatment reaction have been developing rapidly. Wang et al. firstly identified that serum miR-486-5p could be used to stratify the patients with higher recurrence risk before hepatic resection and potentially guide more effective surveillance strategies for them [23]. Zhong et al. proved that the serum levels of miR-21 expression were significantly higher in the breast cancer patients than in the healthy control group and high miR-21 expression was significantly correlated to advanced clinical stage and lymph node metastasis in breast cancer patients [24]. Studies on the serum miRNAs in cervical carcinoma are also carried out gradually. Sun et al. showed that serum concentrations of miR-425-5p in cervical cancer patients were significantly higher compared with benign cervical disease and healthy controls. Moreover, the up-regulation of serum miR-425-5p occurred more frequently in cervical cancer patients with high TNM stage and positive lymph node metastasis [25]. This study aims to identify serum microRNAs related to CCRT resistance in advanced-stage cervical squamous cell carcinoma (ACSCC) patients, and lays the foundation for further study of serum tumor makers for CCRT resistance and potential targets for therapy.

Materials and Methods

Patients and Samples

From September 2013 to November 2015, we collected serum samples from ACSCC patients with International Federation of Gynecology and Obstetrics (FIGO) stage IIB to IIIB treated with platinum based concomitant chemoradiotherapy at the Department of Gynecologic Oncology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences (CAMS), Beijing, China. The inclusion criteria were as follows: (1) Patients diagnosed with cervical squamous cell carcinoma pathologically; (2) age 18 to 70; (3) FIGO stage (2009 Edition) IIB to IIIB. Patients with any other cancer or a history of chemo/radiotherapy were excluded. A total of 131 patients were involved in this study; the median age was 52 (29-69) years old. Serum samples were collected before treatment and stored at −80°Cafter centrifugation (2800×*g*, 10 min) until further processing. All these patients received the standard, whole pelvic irradiation (45 Gy) in 25 fractions and an additional parametrial boost of 10–15 Gy in 5–7 fractions. Following the external beam radiation therapy (EBRT), patients received brachytherapy (21-28Gy) administered as 4–5 fractions to point A. The patients underwent concurrent chemotherapy of weekly cisplatin (40 mg/m^2). This study was approved by ethics committee approval from cancer hospital CAMS, and all the participants signed written informed consent forms.

Clinical Evaluation

During the treatment period and every 3 months after treatment, regular inspection was conducted. During the follow-up visit, gynecological examination, serum levels of SCC-Ag, chest x-ray, enhanced computed tomography and/or enhanced magnetic resonance imaging, cervical cytology, and cervical biopsy (if necessary) were performed. Patients with progressive disease during treatment or those who suffered recurrence within 12 months of completing therapy were divided into resistant group. Patient with no recurrence or with recurrence beyond 12 months were sensitive group. The last follow-up was August 1, 2016. Until then, 29 patients were divided into resistant group. The median recurrence time of the resistant group was 5.4 months (n = 29). There were 5 cases with cancer processing during treatment and 24 cases with recurrence within 12 months. The follow-up time of 102 sensitive patients was 20 to 27 months. Among them, only two patients were with tumor recurrence (beyond 12 months: at 13 months and 14 months).

TaqMan Real-Time PCR MicroRNA Array

To explore whether serum miRNAs expression were associated with CCRT resistance of ACSCC patients, we first compared the expression profile of miRNAs in 10 resistant serum samples and 10 sensitive serum samples using the TaqMan Real-time PCR microRNA Array (Applied Biosystems, CA, USA). Five serum samples from each group were pooled together, respectively. Total RNAs from pooled serum samples was isolated using mirVana PARIS kit (Ambion) according to the manufacturer's protocol. Cel-miR-39-3p was used as a control for normalization. The final concentration of cel-miR-39-3p was 80 fmol/μL [26].

RNA concentrations were measured using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Megaplex RT reactions by using 150 ng of total RNA extracted from serum samples and pre-amplification reactions were performed according to the manufactures' protocols (Applied Biosystems, CA, USA). TaqMan Real-time PCR microRNA Arrays were performed on the ABI 7900HT Instrument (Applied Biosystems, CA, USA). All reactions were performed according to the standard manufactures' protocols. Analysis of the data was performed by using the SDS 2.0.1 software (settings: automatic baseline, threshold 0.2) and Data Assist v2.0 software (Applied Biosystems, CA USA). The fold changes in miRNA expression were calculated using the $2^{-\Delta\Delta Ct}$ method [25].

MiRNA-Specific Quantitative Real-Time RT-PCR

Serum samples from 131 ACSCC patients were analyzed using miRNA specific quantitative real-time RT-PCR. MiRNA was isolated using a mirVana PARIS kit (Ambion). RT reactions were run according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). Cel-miR-39-3p was used as a control for normalization. The final concentration of cel-miR-39-3p was 80fmol/μL. Real-time PCR was performed using the Step-One Plus Real-time system (Applied Biosystems, Foster City, CA, USA), and fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method [27]. The mean miRNA level from three real-time quantitative PCR experiments was calculated for each case.

Survival Analysis

Univariate Cox proportional hazards regression analysis were done to evaluate the association of miRNA or clinical parameters to disease-free survival (DFS). The *P* values were calculated using the Wald test. Multivariate Cox proportional hazards regression analysis were done to evaluate the independent prognostic value of the miRNA signature. The Kaplan–Meier estimator was used to evaluate the median survival time of the DFS that was based on miRNA expression signature. The *P* value of the Kaplan–Meier analysis was calculated with the log-rank test. Disease-free survival was defined as the time interval from the first date of treatment to the time of initially detected recurrence/progression or censored on the last follow-up.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 16 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism v5.0 (Graphpad Software Inc.). Statistical descriptions were used to describe the clinical pathological features, and the Student's *t* test was used to analyze the measurement data. A two-sided *P* value of less than .05 was considered statistically significant. Logistic regression analysis was performed to analyze various combinations of clinical parameters and miRNA. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to determine the feasibility. The Youden's Index was used to identify the optimal cut-off point. As defined, the corresponding sensitivity and specificity was shown.

Results

Screening Phase

To explore whether miRNAs were associated with CCRT resistance in ACSCC patients, as shown in Figure 1, we first compared the expression of miRNAs in 10 serum samples from resistant group and 10 serum samples from sensitive group by using TaqMan MicroRNA

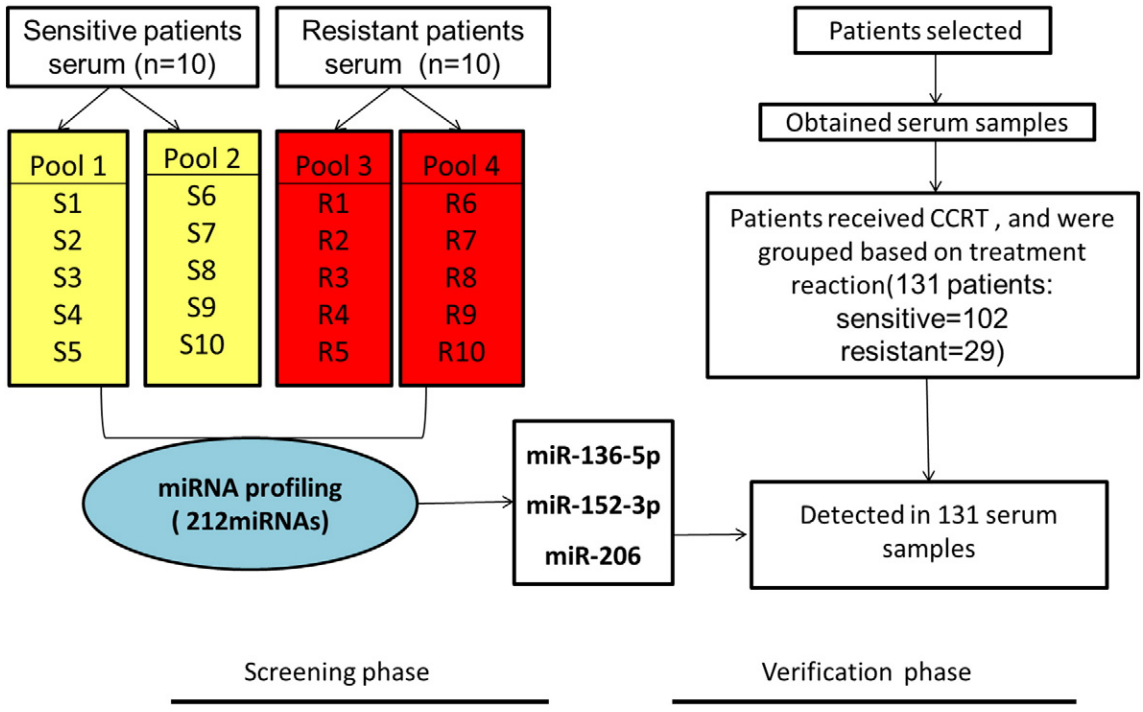

Figure 1. Flow chart of the screening and verifying processes. TaqMan Real-time PCR microRNA Array (Card A) (Applied Biosystems, CA) representing 212 mature miRNAs was used to identify differentially expressed miRNAs from 20 serum samples (10 from sensitive group vs. 10 from resistant group). Take miRNA expression in sensitive group as standard, a total of 62 miRNAs were up-regulated expression while 150 miRNAs were down-regulated expression in resistant group. Among these miRNAs, 3 miRNAs expressed statistically significantly different between two groups. MiR-136 was down-regulated expression in resistant group while miR-152 and miR-206 was up-regulated expression. Three candidate miRNAs were further validated in 131 independent serum samples.

array. Of the 20 patients, the differences between two groups in different clinicopathological factors according to clinical stages, age, histological grade, tumor size, serum Scc-Ag level had no significance ($P > .05$, Table 1). Analysis of microarray data showed that a total of 212 microRNAs expressed differently at the standard that the fold change was more than 2.0 or less than 0.5. Take miRNA expression in sensitive group as standard, a total of 62 miRNAs were up-regulated expression while 150 miRNAs were down-regulated expression in resistant group (Supplementary tables 1 and 2). Among these miRNAs, three miRNAs expressed statistically significantly different between two groups: miRNA-136-5p was down-regulated expression in resistant group while miR-152–3p and miR-206 were up-regulated expression ($P < .05$, Table 2).

These three miRNAs were chosen into the verifying stage. MiRNA-specific quantitative real-time RT-PCR was used to test the levels of miR-136-5p, miR-152–3p and miR-206 in 131 patients. The results demonstrated that miR-136-5p, miR-152–3p could not be tested by the method without pre-amplification reactions. Only miR-206 could be detected that having a 15–35 Ct value. In addition, miR-206 demonstrated the most obvious difference in fold change between two groups in the microarray assay (Table 2). With regard to all of the points stated, we chose miR-206 to further validate its probable relationship with CCRT resistance.

Serum miRNA-206 in ACSCC Patients

A total of 131 patients were involved in the verification study. The clinicopathological information of these patients was summarized in Table 3. Expression of miR-206 in sensitive group was 2.715 ± 0.2115 while 14.64 ± 1.184 in resistant group and the difference was statistically significant ($P < .0001$). The scatter diagram of miR-206 in all patients was shown in Figure 2. We divided the 131 patients into 2 groups based on the median value of the expression level of miR-206. The expression of miR-206 was significantly associated with DFS ($P < .0001$). The Kaplan–Meier curves of DFS according miR-206 was shown in Figure 3. The Multivariate Cox proportional hazard regression analysis revealed that miR-206 was the independent prognostic factor associated with DFS (Table 4). To further understand the significance of miR-206 in the prognosis of ACSCC patients, whether the expression was significantly associated with the clinicopathological features were analyzed. As shown in Table 5, univariate analysis showed that the expression of miR-206 was considerably associated with FIGO stage and tumor size ($P < .005$).

Serum miR-206 for Predicting Sensitivity in CCRT of ACSCC Patients

Multivariate analysis revealed that miR-206 was the independent prognostic factor associated DFS in ACSCC patients. Then, the discriminative power of miR-206 in predicting the outcome before CCRT was verified. According to the DFS, the patients were stratified into two subgroups, including a resistant group and sensitive group. To evaluate the prognostic value, the ROC curve was used to analyze the sensitivity and specificity. As shown in Figure 4. The ROC curve of miR-206 showed an AUC of 91.3% (79.3% sensitivity and 92.2% specificity) (Figure 4*A*). In order to further confirming the potential role of miR-206 in predicting the sensitivity of CCRT in ACSCC patients, the prognostic values of multiple commonly used clinicopathological features were analyzed with univariate and multivariate analysis. Multivariate Cox proportional hazard regression analysis revealed that tumor differentiation and pelvic lymph nodes metastasis were the independent prognostic factors associated with CCRT resistance of ACSCC patients (Table 4). The ROC curve of tumor differentiation and pelvic lymph nodes metastasis had an AUC of 76.5% (82.8% sensitivity and 57.8% specificity) (Figure 4*B*). The results demonstrated that miR-206 was a much more powerful tool in predicting CCRT sensitivity in ACSCC patients.

Table 1. Clinicopathological Parameters of 20 ACSCC Patients

			Sensitive Group (n = 10)	*P* Value
Mean age at diagnosis(years)		49	47	.67
FIGO stage	IIB	2	3	.78
	IIIB	8	7	
Histological grade	Well/moderately differentiated	3	4	.98
	Poorly differentiated	7	6	
Tumor size	≤4 cm	3	3	.91
	>4 cm	7	7	
SCC-Ag(ng/ml)	>1.5	6	5	.75
	≤1.5	4	5	

Table 2. MiRNAs Expressed Differently in Resistant Group Compared With in Sensitive Group

microRNA	Up/down Regulated	Fold Change	*P* Value
hsa-miR-152	↑	5.0654	.0156
hsa-miR-206	↑	24.01	.0256
hsa-miR-136	↓	0.0232	.001

Table 3. Clinicopathological Features in 131 ACSCC Patients Received CCRT

Parameters	Patients with ACSCC (n = 131)
Age (years)	
≤52	80 (61%)
>52	51 (39%)
FIGO stage	
IIB	68 (51.9%)
IIIB	63 (48.1%)
Differentiation	
Well	7 (5.3%)
Moderately	99 (75.6%)
Poorly	25 (19.1%)
Tumor size	
≤4 cm	56 (42.7%)
>4 cm	75 (57.3%)
SCC-Ag(ng/ml)	
>1.5	87 (66.4%)
≤1.5	44 (33.6%)
Tumor types	
Cauliflower-like	40 (30.5%)
Ulceration	21 (16.0%)
Endogenous	70 (53.5%)
Pelvic lymph nodes metastasis based on image examination	
Negative	71 (54.2%)
Positive	56 (42.7%)
Suspicious	4 (3.1%)
Treatment interval(days)	
≥49	67 (51.1%)
<49	64 (48.9%)
Recurrence/local uncontrolled (<12 months)	
Yes	29 (22.1%)
No	102 (77.8%)
miR-206	
CCRT sensitive(n = 102)	2.715 ± 0.2115
CCRT resistant(n = 29)	14.64 ± 1.184

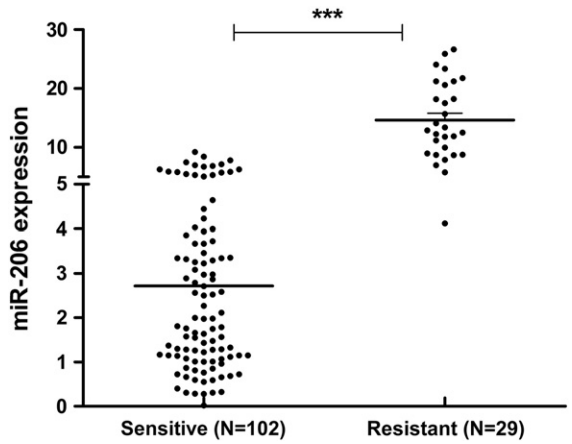

Figure 2. The scatter diagrams of serum miR-206 in 131 ACSCC patients. Resistant group includes 29 cases, sensitive group includes 102 cases.

Table 4. Univariate and Multivariate Cox Proportional Hazards Regression Analysis of miR-206 and Clinical Parameters in Relation to Resistance of ACSCC

Variable	DFS			
	Univariate Analysis		Multivariate Analysis	
	HR (95%CI)	*P* Value	HR (95%CI)	*P* Value
miR-206	9.750 (2.269-41.899)	.002	9.125(2.71-42.121)	.008
Age	0.997 (0.945-1.051)	.899		
FIGO stage	2.860 (1.109-7.371)	.030		
Tumor differentiation	3.701 (1.614-8.484)	.002	4.369(2.106-9.063)	.000
SCC-Ag	1.019 (1.002-1.037)	.029		
Tumor types	1.191 (0.828-1.713)	.345		
Tumor size	3.460 (1.164-10.287)	.026		
Pelvic lymph nodes metastasis	3.770 (1.873-7.590)	.000	3.453(1.765-6.758)	.000
Treatment interval	1.033 (0.985-1.083)	.018		

Discussion

An important factor influencing the prognosis of advanced stage cervical carcinoma is the chemoradiotherapy resistance. Therefore, prediction of chemoradiotherapy sensitivity has become an issue which is needed to be studied further. For the past few years, miRNAs constitute a class of non-invasive biomarkers due to the high stability. For cervical cancer patients, serum samples are much easier to get than cancer tissues and easily accessible serum based miRNAs may provide a clue in monitoring of cervical carcinoma.

In this study, we screen the differentially expressed miRNAs in the serum of ACSCC patients with different treatment outcomes, focusing on the miRNA profiling before treatment. Notably, a number of serum miRNAs differentially expressed between the CCRT sensitive and resistant patients, including miR-136-5p, miR-152–3p and miR-206. The preliminary results demonstrated that miR-136-5p, miR-152–3p could not be tested by MiRNA-specific quantitative real-time RT-PCR without pre-amplification reactions. Only miR-206 could be detected that having a 15–35 Ct value. So we can see that the abundance of serum miR-206 was much higher than miR-136-5p and miR-152-3p. In addition, the results of miRNA array showed that miR-206 was the most differential expressed miRNA between the two groups (Table 1). With regard to all of the points stated, we chose miR-206 to further validate its probable relationship with CCRT resistance.

Previous studies have reported the important role of miR-206 in many cancers. Tian et al. reported that miR-206 may be implicated in aggressive progression of melanoma and the serum level of miR-206 may be a noninvasive prognostic biomarker for the patients with melanoma [28]. MiR-206 was also reported as a potential diagnostic marker for rhabdomyosarcoma [29]. Tan et al. identified 8 miRNAs including miR-206 could provide high diagnostic accuracy for HCC (AUC = 0.887) [30]. In breast cancer, miR-206 expression is decreased in ERa-positive patients, restoration of miR-206 in estrogen-dependent breast cancer cells inhibits cell growth and that reduced expression levels of miR-206 is associated with breast cancer metastasis [31,32]. As to the

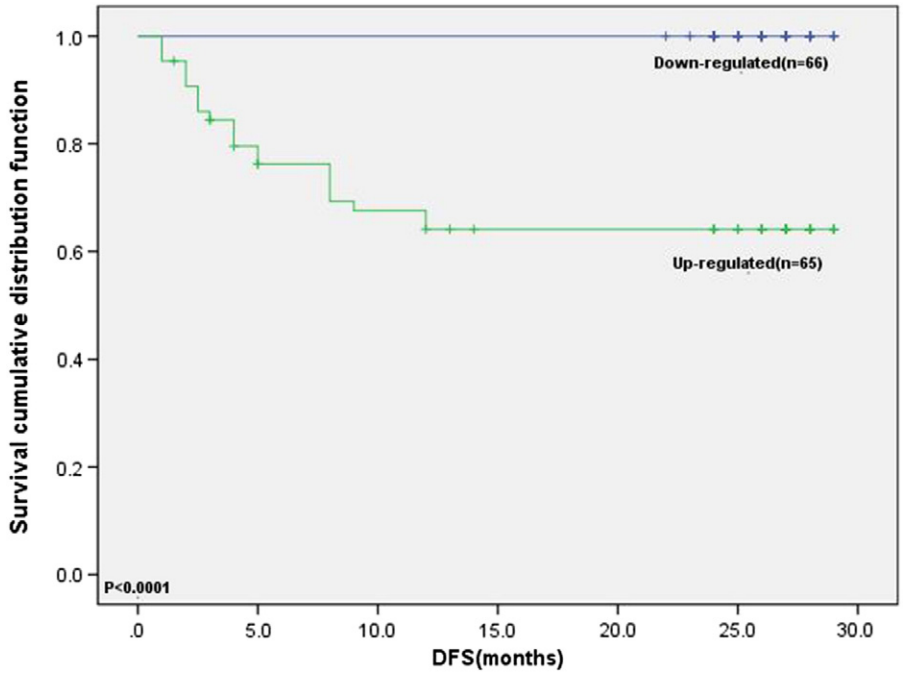

Figure 3. The level of serum miR-206 was associated with disease free survival.

Table 5. Correlation between the MicRNA-206 expression and clinical parameters of 131 patients

Parameter	*P* Value
Age	.663
FIGO stage	**.006**
Tumor differentiation	.303
SCC-Ag	.091
Tumor types	.436
Tumor size	**.027**
Pelvic lymph nodes metastasis	.069
Treatment interval	.526

mechanism of miR-206 in cancer progression, it was reported that miR-206 directly targets 3'UTR of CCND2 and represses cell growth by down-regulating CCND2 in gastric cancer [33]. And it was reported by Singh A [34] that miRNA-206 was related with nuclear factor erythroid-2 related factor 2 (NRF2), controlling the pentose phosphate pathway (PPP) and the tricarboxylic acid (TCA) cycle and reprogramming glucose metabolism. In primary tumor samples, the expression of miR-206 was inversely correlated with PPP gene expression, and increased expression of NRF2-dependent genes was associated with poor prognosis. However, the function and mechanism of serum miR-206 as a circulating biomarker in cervical cancer progression is still remained unknown. After our further verification, we found that serum miR-206 showed a powerful discrimination potential in identifying the sensitivity of CCRT in ACSCC patients. The miR-206 expression level was significantly different between two groups: up-regulated in resistant group with almost 7-fold change than sensitive group. We also observe a relatively high prediction accuracy (AUC = 91.3%, 79.3% sensitivity and 92.2% specificity) by using miR-206 independently. And our results also showed that the up-regulation of serum miR-206 occurred more frequently in cervical cancer patients with high FIGO stage and tumor size, which may be related with the CCRT resistance.

In clinical practice, the independent factors, such as pathological subtype, clinical stage, lymph node status and treatment interval, also influence the prognosis to IIB-IIIB stage cervical squamous cell carcinoma patients received chemoradiotherapy [9]. In the present study, multivariate analysis showed that miR-206, tumor differentiation, pelvic lymph nodes metastasis were the independent factors of the prognosis. Then, we compared the accuracy of miR-206 along with clinicopathological factors. And the AUC of miR-206 alone was higher than the combination of tumor differentiation and pelvic lymph nodes metastasis which yielded an AUC of 76.5% (82.8% sensitivity and 57.8% specificity). Consequently, our study indicated that serum miR-206 as a relative high-abundance miRNA which is easy to detect in serum can serve as a biomarker to predict the response to chemoradiotherapy in cervical cancer, which has great significance in individualized treatment and improving prognosis. Our study had some potential limitations: (1) Prospective studies are required to confirm the correlation between serum miR-206 level and patient outcome; (2) The underlying mechanism of secretion of miR-206 was not demonstrated. In addition, our study lacked an independent, large validation cohort, which is needed to further appreciate the significance of results reported in our study.

Conclusion

In summary, we identified that miR-206 expression was up-regulated in chemoradiotherapy resistant patients and miR-206 expression was an independent prognostic factor of ACSCC. Our study demonstrates that miR-206 has considerable clinical value being a potential noninvasive biomarker for predicting the sensitivity to CCRT of ACSCC patients. And this study is the first report showing that serum miRNA could apply to predict the CCRT sensitivity in cervical carcinoma patients as a biomarker.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tranon.2017.03.005.

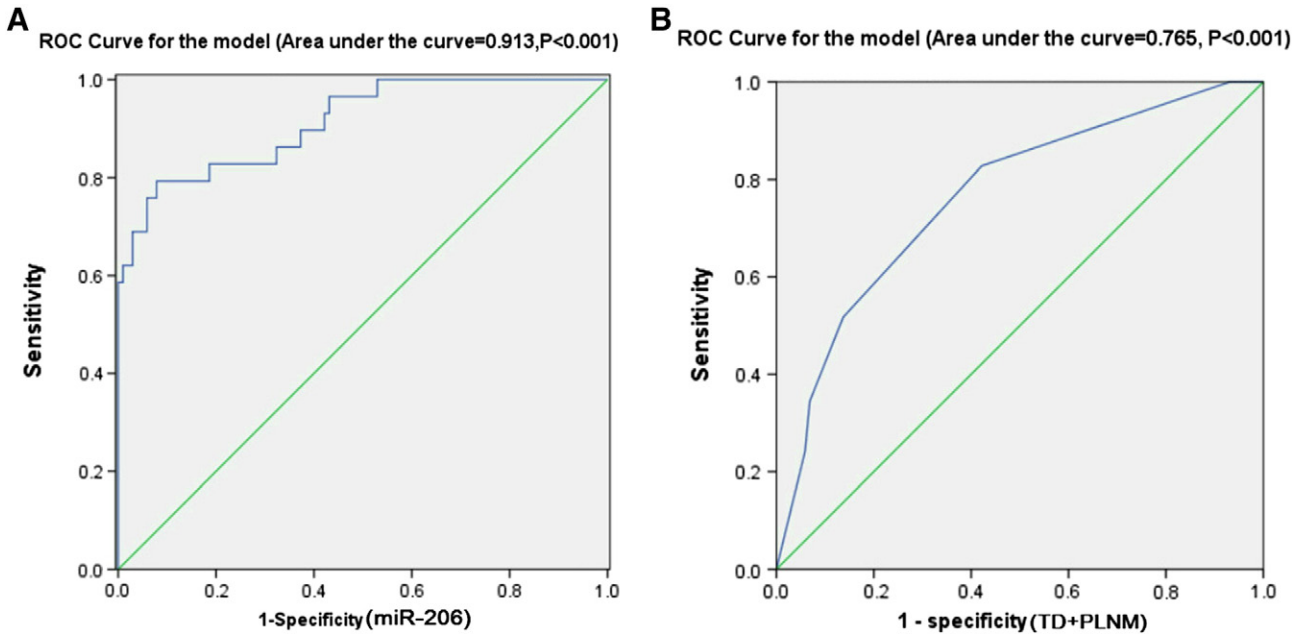

Figure 4. ROC analysis for predicting CCRT sensitivity of ACSCC patients: (A) ROC curve for miR-206 yielded area under the curve (AUC) of 91.3%, the sensitivity of 79.3% and specificity of 92.2% in predicting chemoradiotherapy sensitivity; (B) ROC curve for tumor differentiation (TD) and pelvic lymph nodes metastasis (PLNM) yielded an AUC of 76.5%, the sensitivity of 82.8% and specificity of 57.8% in predicting chemoradiotherapy sensitivity.

Disclosure of Potential Conflicts of Interest

We declare that no potential conflicts of interest were disclosed.

Acknowledgments

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References

[1] International Agency for Research on Cancer (2012). Cervical Cancer Estimated Incidence, Mortality and Prevalence Worldwide in 2012. World Health Organization; 2012.

[2] Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, Rotman M, Gershenson DM, and Mutch DG (1999). Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. *N Engl J Med* **340**(15), 1137–1143.

[3] Rose PG, Bundy BN, Watkins EB, Thigpen JT, Deppe G, Maiman MA, Clarke-Pearson DL, and Insalaco S (1999). Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. *N Engl J Med* **340**(15), 1144–1153.

[4] Keys HM, Bundy BN, Stehman FB, Muderspach LI, Chafe WE, Suggs 3rd CL, Walker JL, and Gersell D (1999). Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. *N Engl J Med* **340**(15), 1154–1161.

[5] Whitney CW, Sause W, Bundy BN, Malfetano JH, Hannigan EV, Fowler Jr WC, Clarke-Pearson DL, and Liao SY (1999). Randomized comparison of fluorouracil plus cisplatin versus hydroxyurea as an adjunct to radiation therapy in stage IIB-IVA carcinoma of the cervix with negative para-aortic lymph nodes: a Gynecologic Oncology Group and Southwest Oncology Group study. *J Clin Oncol* **17**(5), 1339–1348.

[6] Peters 3rd WA, Liu PY, Barrett 2nd RJ, Stock RJ, Monk BJ, Berek JS, Souhami L, Grigsby P, Gordon Jr W, and Alberts DS (2000). Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. *J Clin Oncol* **18**(8), 1606–1613.

[7] Wiebe E, Denny L, and Thomas G (2012). FIGO Cancer Report 2012. Cancer of the cervix uteri. *Int J Gynaecol Obstet* **119**(Suppl. 2), S100–S109.

[8] Koh WJ, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Chan J, Cho KR, Cohn D, Crispens MA, DuPont N, et al (2013). Cervical cancer. *J Natl Compr Canc Netw* **11**(3), 320–343.

[9] Grigiene R, Valuckas KP, Aleknavicius E, Kurtinaitis J, and Letautiene SR (2007). The value of prognostic factors for uterine cervical cancer patients treated with irradiation along. *BMC Cancer* **7**, 234.

[10] Yokoi E, Mabuchi S, Takahashi R, Matsumoto Y, Kuroda H, Kozasa K, and Kimura T (2016). Impact of histological subtype on survival in patients with locally advanced cervical cancer that were treated with definitive radiotherapy: adenocarcinoma/adenosquamous carcinoma versus squamous cell carcinoma. *J Gynecol Oncol* e19.

[11] Vordermark D (2016). Radiotherapy of Cervical Cancer. *Oncol Res Treat* **39**, 516–520.

[12] Postel-Vinay S and Soria JC (2017). ERCC1 as Predictor of Platinum Benefit in Non-Small-Cell Lung Cancer. *J Clin Oncol* **35**, 384–386.

[13] Strippoli A, Rossi S, Martini M, Basso M, D'Argento E, Schinzari G, Barile R, Cassano A, and Barone C (2016). ERCC1 expression affects outcome in metastatic pancreatic carcinoma treated with FOLFIRINOX: A single institution analysis. *Oncotarget* **7**, 35159–35168.

[14] Simonsson M, Björner S, Markkula A, Nodin B, Jirström K, Rose C, Borgquist S, Ingvar C, and Jernström H (2017). The prognostic impact of COX-2 expression in breast cancer depends on oral contraceptive history, preoperative NSAID use, and tumor size. *Int J Cancer* **140**, 163–175.

[15] Matsuo T, Miyata Y, Mitsunari K, Yasuda T, Ohba K, and Sakai H (2017). Pathological significance and prognostic implications of heme oxygenase 1 expression in non-muscle-invasive bladder cancer: Correlation with cell proliferation, angiogenesis, lymphangiogenesis and expression of VEGFs and COX-2. *Oncol Lett* **13**, 275–280.

[16] Redova M, Sana J, and Slaby O (2013 Mar). Circulating miRNAs as new blood-based biomarkers for solid cancers. *Future Oncol* **9**(3), 387–402.

[17] Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**(2), 281–297.

[18] Lewis BP, Burge CB, and Bartel DP (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* **120**, 15–20.

[19] Lui WO, Pourmand N, Patterson BK, and Fire A (2007). Patterns of known and novel small RNAs in human cervical cancer. *Cancer Res* **67**(13), 6031–6043.

[20] Takamizawa J, Konishi H, Yanagisawa K, Tomida S, and Osada H (2004). Reduced expression of the let-7 MicroRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* **64**, 3753–3756.

[21] Hamano R, Miyata H, Yamasaki M, Kurokawa Y, Hara J, Moon JH, Nakajima K, Takiguchi S, Fujiwara Y, Mori M, et al (2011). Overexpression of miR-200c induces chemoresistance in esophageal cancers mediated through activation of the Akt signaling pathway. *Clin Cancer Res* **17**, 3029–3038.

[22] Hu X, Schwarz JK, Lewis Jr JS, Huettner PC, Rader JS, Deasy JO, Grigsby PW, and Wang X (2001). A microRNA expression signature for cervical cancer prognosis. *Cancer Res* **70**, 1441–1448.

[23] Wang L, Liu M, Zhu H, Rong W, Wu F, An S, Liu F, Feng L, Wu J, and Xu N (2015). Identification of recurrence-related serum microRNAs in hepatocellular carcinoma following hepatectomy. *Cancer Biology & Therapy* **16**(10), 1445–1452.

[24] Zhong Q, Zhou WM, and Ma ZH (2015). Expression of serum microRNA-21 in breast cancer patients and its association with clinicopathoIogical features. *Chin J Exp Surg* **32**(1).

[25] Sun L, Jiang R, Li J, Wang B, Ma C, Lv Y, and Mu N (2016). MicroRNA-425-5p is a potential prognostic biomarker for cervical cancer. *Ann Clin Biochem.*

[26] Qi R, Weiland M, Gao XH, Zhou L, and Mi QS (2012). Identification of endogenous normalizers for serum microRNAs by microarray profiling: U6 small nuclear RNA is not a reliable normalizer. *Hepatology* **55**, 1640–1642 [author reply 2-3; PMID:22213067] http://dx.doi.org/10.1002/hep.25558.

[27] Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the2-ΔΔCt method Method. *Methods* **25**, 402–408 [PMID:11846609] http://dx.doi.org/10.1006/meth.2001.1262.

[28] Tian R, Liu T, Qiao L, Gao M, and Li J (2015). Decreased serum microRNA-206 level predicts unfavorable prognosis in patients with melanoma. *Int J Clin Exp Pathol* **8**(3), 3097–3103 [eCollection 2015].

[29] Miyachi M, Tsuchiya K, Yoshida H, Yagyu S, Kikuchi K, Misawa A, Iehara T, and Hosoi H (2010). Circulating muscle-specific microRNA, miR-206, as a potential diagnostic marker for rhabdomyosarcoma. *Biochem Biophys Res Commun* **400**, 89–93.

[30] Tan Y, Ge G, Pan T, Wen D, Chen L, Yu X, Zhou X, and Gan J (2014). A Serum MicroRNA Panel as Potential Biomarkers for Hepatocellular Carcinoma Related with Hepatitis B Virus. *PLoS One* , 9(9).

[31] Kondo N, Toyama T, Sugiura H, Fujii Y, and Yamashita H (2008). MiR-206 Expression is down-regulated in estrogen receptor alpha-positive human breast cancer. *Cancer Res* **68**, 5004–5008.

[32] Negrini M and Calin G (2008). A breast cancer metastasis: a microRNA story. *Breast Cancer Res* **10**, 203–211.

[33] Zhang L, Liu X, Jin H, Guo X, Xia L, Chen Z, Bai M, Liu J, Shang X, Wu K, et al (2013 May 10). miR-206 inhibits gastric cancer proliferation in part by repressing cyclinD2. *Cancer Lett* **332**(1), 94–101.

[34] Singh A, Happel C, Manna SK, Acquaah-Mensah G, Carrerero J, Kumar S, Nasipuri P, Krausz KW, Wakabayashi N, Dewi R, et al (2013). Transcription factor NRF2 regulates miR-1and miR-206 to drive tumorigenesis. *J Clin Invest* **123**(7), 2921–2934. http://dx.doi.org/10.1172/JCI66353.